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Research Article

Synthesis of a carbon-14 labeled 1-(indole-6-carbonyl-D-phenylglycinyl)-4-(1-methylpiperidin-4-YL)piperazine-[carbonyl-^{14}C], LY517717-[^{14}C], a factor Xa inhibitor

Fengjiun Kuo*, Dean K. Clodfelter, Tamara R. Priest and Donald L. K. Kau
Lilly Research Laboratories, A Division of Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285, USA

Summary

Human Factor Xa is a trypsin-like serine protease, which serves a critical role in blood coagulation events. LY517717 is currently under clinical investigation as a Factor Xa inhibitor. To support the ADME studies, LY517717 was synthesized using D-phenylglycine with a carbon-14 labeled carboxyl moiety. This key component, D-phenylglycine-[carboxyl-^{14}C], was synthesized by a Strecker synthesis on benzaldehyde with potassium [^{14}C]cyanide, followed by a resolution of DL-phenyl-glycine methyl ester-[carbonyl-^{14}C] with (+)-tartaric acid in the presence of benzaldehyde.

Key Words: factor Xa; D-phenylglycine-[^{14}C] synthesis; LY517717-[^{14}C]

Introduction

Human factor Xa is a trypsin-like serine protease, which serves a critical role in blood coagulation events. Competitive inhibitors of factor Xa have demonstrated potent anticoagulant activities *in vitro* and anti-thrombotic efficacy in a pre-clinical animal model *in vivo*. During the past 10 years, research efforts have been increasing to identify small molecule inhibitors of this coagulation enzyme as novel therapies for thrombotic disorders.[1] LY517717, 1-(indole-6-carbonyl-D-phenylglycinyl)-4-(1-methylpiperidin-4-yl)piperazine, is a Factor Xa inhibitor which is currently under clinical investigation. To support its ADME studies, a carbon-14 labelled isotopomer was required.

*Correspondence to: F. Kuo, Eli Lilly and Company, Lilly Corporate Center, DC1543, Indianapolis, IN 46285, USA. E-mail: KUO_FENGJIUN@lilly.com

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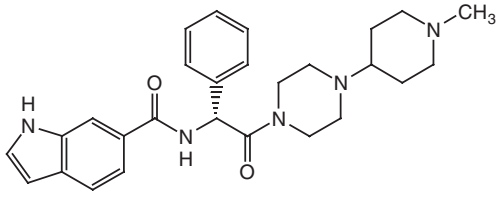
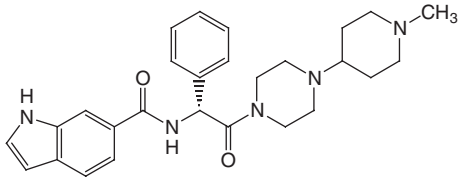
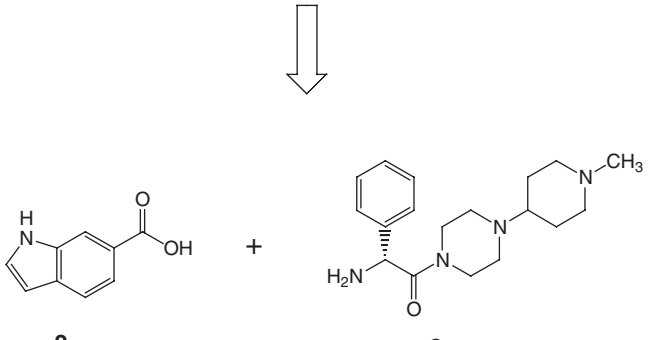


Discussion

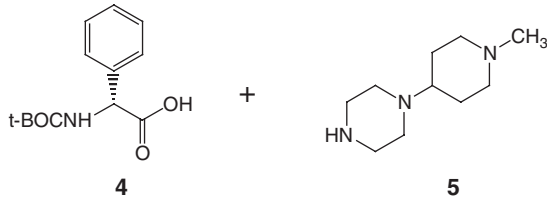
Retro-synthetic analysis of LY517717 (as below) indicated two possible sites to introduce the carbon-14, either on indole-6-carboxylic acid **2** or on D-phenylglycine **4**.



LY517717



2 **3**



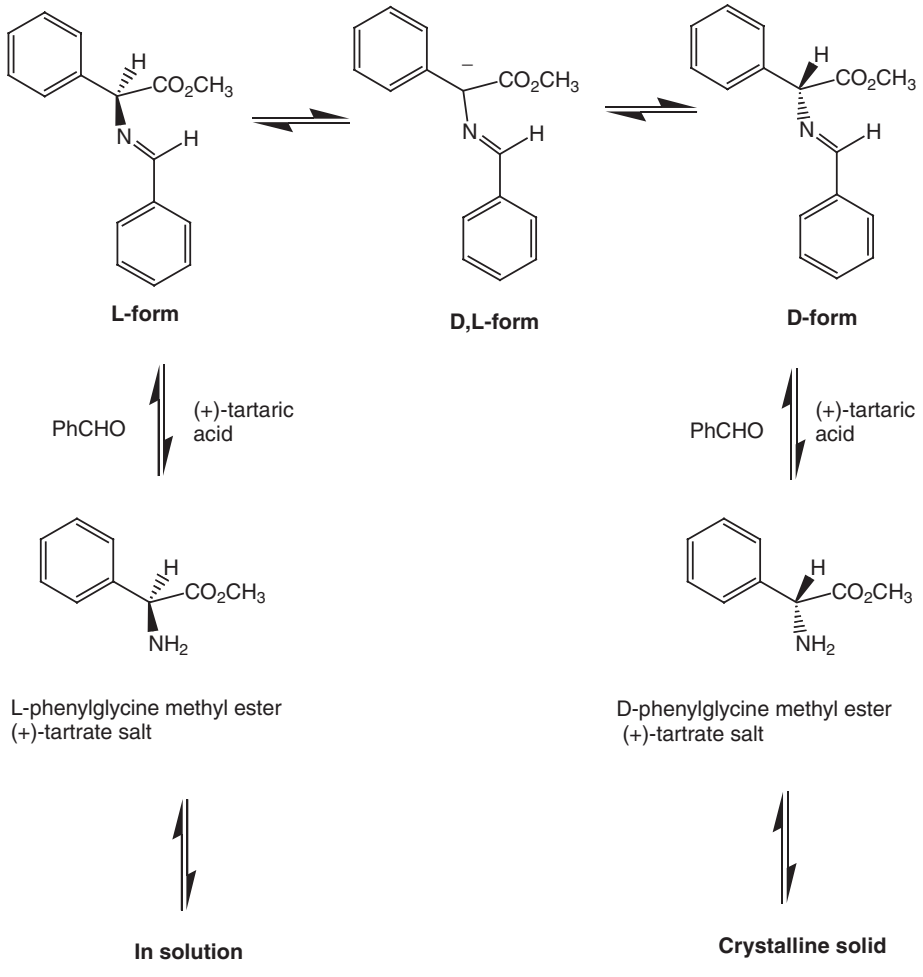
4 **5**

Indole-6-carboxylic acid-[carboxyl-^{14}C] (**2-[^{14}C]**) should be readily available by the reaction of 6-bromoindole with potassium [^{14}C]cyanide in the presence of CuI, followed by simple hydrolysis; alternatively, *N*-Boc-D-phenylglycine-[carboxyl-^{14}C] (**4-[^{14}C]**) could be easily prepared by the Strecker synthesis of

benzaldehyde with potassium [^{14}C]cyanide, followed by resolution of its methyl ester. Although indole-6-carboxylic acid-[carboxyl-^{14}C] (**2-[^{14}C]**) might appear to be easier to synthesize, considering the potential metabolic stability *in vivo*, LY517717-[^{14}C] labeled in the phenylglycine moiety was synthesized.

A modified Strecker synthesis[2] on benzaldehyde with potassium [^{14}C]cyanide gave D,L-phenylglycine-[carboxyl-^{14}C] in good yield (about 70%). This racemic phenylglycine could be resolved directly or as a *N*-protected derivative enzymatically.[3] It also has been reported[4] that the ester of D,L-phenylglycine could be resolved by (+)-tartaric acid in the presence of benzaldehyde, where the L-salt in the solution was racemized during the process, and the D-salt of (+)-tartrate was separated as a crystalline solid upon cooling (Scheme 1).

The combining resolution and racemization undergoes a second-order asymmetric transformation to give the desired D-phenylglycinate (+)-tartrate



Scheme 1.

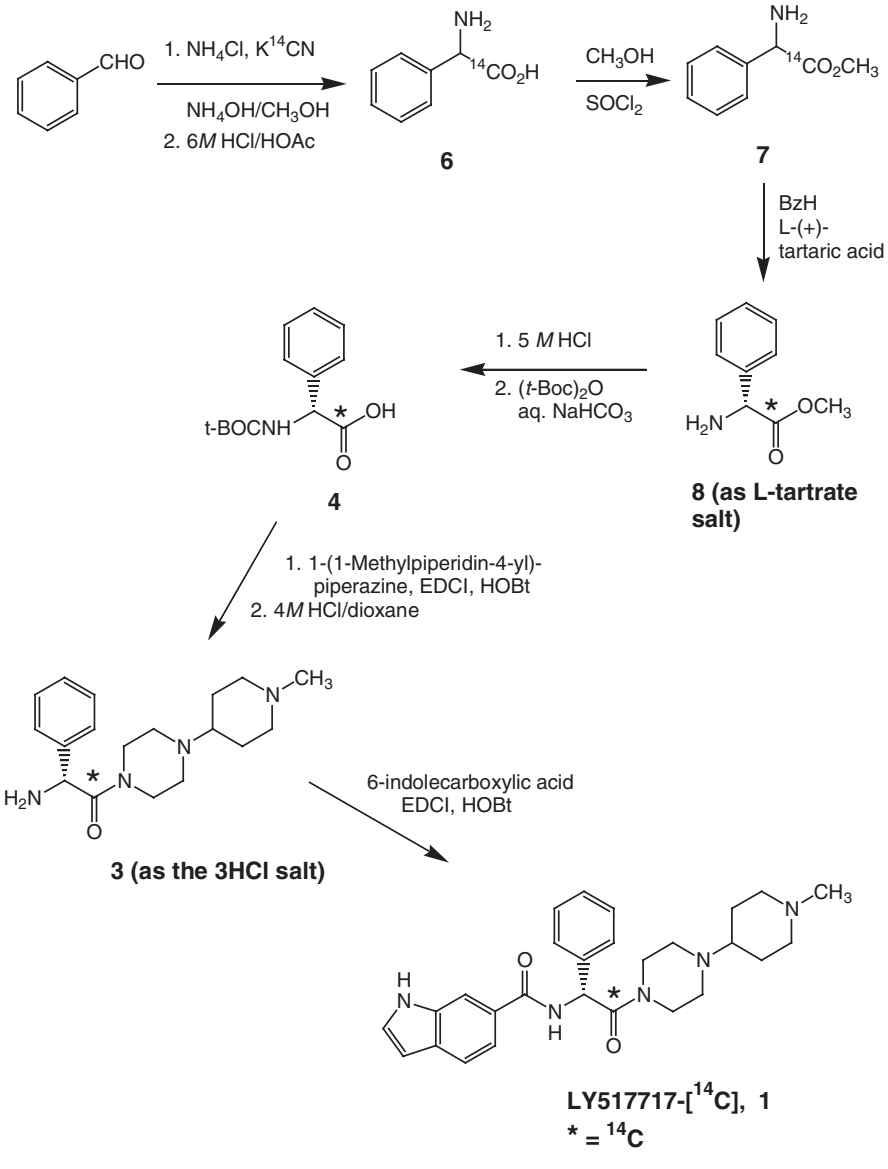
in high yield, presumably via a Schiff's base intermediate, where the benzylic hydrogen in the glycinate moiety was very acidic, and an equilibration occurred during the process.[4] Thus, racemic phenylglycine-[carboxyl-^{14}C], **6**, from the Strecker synthesis was converted to a methyl ester, **7**, which was then resolved by refluxing in ethanol in the presence of one equivalent of L-(+)-tartaric acid and benzaldehyde. The resulting clear solution was cooled to room temperature with stirring, and the white salt, methyl D-phenylglycinate-[carbonyl-^{14}C] L-(+)-tartrate salt, **8**, was collected by filtration. The filtrate was concentrated and the resolution process was repeated. A very high yield (93.8%, 97–98% ee) of the D-salt **8** was obtained from racemic DL-phenylglycine-[carboxyl-^{14}C] (**6**) after three cycles. Hydrolysis of **8** by refluxing in 5 M HCl gave D-phenylglycine-[carboxyl-^{14}C] HCl salt, where the amino group was then protected with $(Boc)_2O$[5] to give *N*-Boc-D-phenylglycine-[carboxy-^{14}C], (**4**). Coupling of **4** with 1-(1-methylpiperidin-**4**-yl)piperazine, **5**, using EDCI/HOBt[6] reagent, followed by removing of *N*-Boc protecting group gave the glycinamide, **3**. Further coupling it with indole-6-carboxylic acid gave the desired product LY517717-[carbonyl-^{14}C], **1**, after re-crystallization from acetonitrile (Scheme 2).

Conclusions

A general and simple method has been developed for the synthesis of a carbon-14 labeled LY517717, a Factor Xa Inhibitor. The key component, D-phenylglycine-[carboxyl-^{14}C] was synthesized by Strecker synthesis, followed by combining resolution and racemization stages in a second-order asymmetric transformation to give the desired amino acid intermediate, methyl D-phenylglycinate-[carboxyl-^{14}C] (+)-tartrate salt.

Experimental

Potassium [^{14}C]cyanide was purchased from American Radiolabeled Chemicals, Inc. ^{1}H-NMR spectra were obtained on a General Electric QE-400 nuclear magnetic resonance spectrometer at 400 MHz. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane. Direct chemical ionization mass spectra (DCI-MS) and electron impact mass spectra (EI-MS) were recorded on a Nermag R30-10 triple stage quadrupole mass spectrometer. Flash chromatography was performed as described by Still *et al.*,[7] using E.M. Science silica gel 60 (230-400 mesh). Unless otherwise noted, the organic extracts were dried over anhydrous sodium sulfate. Radiochemical purity (RCP) was assessed by autoradiography employing E. Merck silica gel F-254 TLC plates and Kodak BB-5 X-ray film. The radioactive lane was divided, suspended in methanol, and after sonication, the mixture was diluted with Aquassure™ scintillation cocktail (DuPont NEN) and counted. As a further check of the radiochemical purity, the sample was subjected



Scheme 2.

to radio-HPLC; 30 s samples of the eluent were collected, diluted with Aquassure™ and counted.

Synthesis of D,L-*Phenylglycine-[carboxyl-^{14}C],* ***6***

A mixture of 400 mg of ammonium chloride, 200 mg of potassium cyanide and 200 mCi of potassium [^{14}C]cyanide (55 mCi/mmol) in 8 ml of concentrated ammonium hydroxide was stirred in an ice bath for about 10 min. Some of the solid was insoluble and floated on the surface of the solution. Removing the

ice bath and stirring the solution at room temperature for 15 min did not change the situation – some of the solid still remained. The solution was re-stirred in an ice bath, and a solution of 0.8 ml of benzaldehyde in 8 ml of anhydrous methanol was added dropwise during a period of 10 min. The solution was stirred overnight (about 15.5 h). The temperature rose to 15°C overnight. The solution was stirred at room temperature for another hour before it was concentrated *in vacuo*. The residue was taken into 11 ml of glacial acetic acid and 11 ml of concentrated HCl. The resulting solution was refluxed in a 120°C oil bath for 6 h. After cooling to room temperature, the solution was concentrated *in vacuo* to give a residue as a yellowish solid. This crude solid was suspended in 20 ml of water which was adjusted to pH 6.5 with 5 M NaOH at room temperature. To the solution was added 10 ml of ethanol, and the resulting mixture was stirred for 1 h at room temperature. The solid was filtered and rinsed with ethanol. Some yellowish gummy solid remained in the flask. About 10 ml of ethanol was added to the flask to dissolve the gummy material. Some white solid was left behind. The white solid was combined with the previous crop, filtered and rinsed with ethanol. The solid was then suspended in ethanol, transferred to a 100 ml flask and concentrated to give a white solid (713 mg). Further drying *in vacuo* gave the desired crude D,L-phenylglycine-[carboxyl-^{14}C], **6**, as a white solid (709 mg, 4.63 mmol, 69.9%).

Synthesis of Methyl D-*Phenylglycinate-[carbonyl-^{14}C]* L-*(+)-Tartrate,* ***8***

The crude D,L-phenylglycine-[carboxyl-^{14}C], **6** (709 mg, 4.63 mmol), was suspended in 15 ml of anhydrous methanol, and 2 ml of thionyl chloride was added dropwise at 0°C. The solution was refluxed in an oil bath for about 6 h. After cooling to room temperature, the solution was concentrated *in vacuo*. The residue was dissolved in 10 ml of water and 10 ml of ethyl acetate, and the resulting solution was adjusted to pH 7.3 with a saturated sodium bicarbonate solution. This solution was extracted with ethyl acetate four times. The combined extracts were washed with water and concentrated under reduced pressure. The residue was further dried *in vacuo* to give a crude methyl ester, **7**, as a pale brown liquid (760 mg, 4.55 mmol), which solidified in the refrigerator. This crude methyl ester **7** was dissolved in 10 ml of ethanol, to which, 680 mg of L-(+)-tartaric acid was added. The mixture was stirred in an 80°C oil bath as a white slurry. To the slurry was added dropwise 0.65 ml of benzaldehyde. The solid dissolved quickly to give a clear yellowish solution. A lot of white solid formed after the solution was stirred at room temperature for about 3 h. More ethanol (6 ml) was added to the solution to give smooth stirring. After about 52 h, the solid was filtered and rinsed with 25 ml of ethanol. Further drying the solid *in vacuo* gave a first crop of product, methyl *D*-phenyl-glycinate-[carbonyl-^{14}C] L-(+)-tartrate salt **8**, as a white solid (996 mg, 3.14 mmol, 97.4% ee). This salt was used in the next step without further

purification. The filtrate contained some white salt that was filtered to give a second crop of product, **8**, (150 mg, 0.473 mmol, 98% ee). The filtrate was concentrated under reduced pressure to give a yellowish residue, which was stirred with ethanol and filtered to give a third crop of product (210 mg, 0.66 mmol, 97% ee), co-eluted with cold reference on HPLC. Chiral HPLC conditions: Chiracel OD-H column, 4.6×250 mm.UV at 235 nm, mobile phase: 15:85 EtOH/hexanes with 0.2% of diethylamine. Flow rate = 0.5 ml/min at room temperature.

*Synthesis of N-Boc-*D*-phenylglycine-[carboxyl-^{14}C], **4***

The tartrate salt, **8**, (550 mg, 1.73 mmol, from the first crop) was refluxed in 15 ml of 5 N HCl in a 120°C oil bath for 3.5 h. The solution was concentrated to give a residue which was taken into ethanol and re-concentrated to remove most of the residual acid to give D-phenylglycine-[carboxyl-^{14}C] HCl salt as a white solid. The solid was dissolved in a solution of 20 ml of water and 20 ml of dioxane, to which 2.5 g of sodium bicarbonate was added slowly, followed by 0.8 g of *di-tert*-butyl dicarbonate [$(Boc)_2O$] as a solid in one portion. More water and dioxane (5 ml each) were added to rinse the flask. After about 16 h, the solution was diluted with water and extracted three times with ethyl ether. The aqueous layer was adjusted to pH 3.4 with solid citric acid in the presence of 20 ml of ethyl acetate, and the layers were separated. The organic layer was washed with water twice, concentrated with ethanol and dried *in vacuo* to give the desired *N-tert*-boc-D-phenylglycine-[carboxyl-^{14}C], **4**, as a colorless, viscous oil (399 mg, 1.59 mmol, 92%). TLC (SiO_2, EtOAc): co-eluted with cold reference. This crude product was used in the next step without further purification.

*Synthesis of 1-(*D*-phenylglycinyl)-4-(1-methylpiperidin-4-yl)-piperazine-[carbonyl-^{14}C] trihydrochloride, **3***

The crude *N*-Boc-D-phenylglycine-[carboxyl-^{14}C], **4**, (399 mg, 1.59 mmol) was dissolved in 20 ml of anhydrous THF at room temperature to give a clear solution, to which 460 mg of EDCI, 320 mg of HOBt and 1 ml of triethylamine were added with stirring. After stirring for 10 min, 1-(1-methylpiperidin-4-yl)piperazine (575 mg) was added, and the solution was stirred at room temperature overnight (about 26 h). Some of the starting material remained. Additional EDCI (200 mg), HOBt (100 mg) and **5** (110 mg) along with 5 ml of THF were added. After stirring for five additional h, the reaction was quenched with saturated sodium bicarbonate solution. To the solution was added ethyl acetate with stirring to give two clear layers. The layers were separated and the aqueous layer was extracted once with ethyl acetate. The combined organic layers were concentrated *in vacuo* to give a crude residue. The residue was dissolved in ethyl acetate, and the solution was washed with

saturated sodium bicarbonate, water (three times) and concentrated *in vacuo*. The residue was taken into ethanol and concentrated. This process was repeated to remove the residual water and gave a colorless, viscous oil, which was further dried *in vacuo* to give a crude intermediate as a white foam (396 mg, 0.95 mmol). The white foam was then taken into 3 ml of anhydrous methanol. The solution was filtered to remove insoluble particles. The filtrate was concentrated *in vacuo* to give a viscous oil. The viscous oil was taken into 4 ml of anhydrous methanol and cooled in an ice bath. To the solution was quickly added 8 ml of 4 M HCl (in dioxane) and the mixture stirred. A white precipitate formed gradually. After stirring for about 5.5 h, the solution was diluted with about 50 ml of ethyl acetate while stirring in an ice bath. The white precipitate was filtered, rinsed with ethyl acetate and dried. A total of 249 mg of white solid was obtained as a first crop of the desired 1-(D-phenylglycinyl)-4-(1-methylpiperidin-4-yl)piperazine-[carbonyl-^{14}C] trihydrochloride, **3**, along with some gummy material, which was separated from the white solid and dissolved in methanol. The methanol solution was concentrated to about 2 ml in volume, then diluted with about 50 ml of ethyl acetate with stirring at room temperature to give a white precipitate. After storing in the refrigerator over the weekend, the solution was filtered and the solid was rinsed (ethyl acetate) and dried *in vacuo* to give a second crop of product, **3**, (159 mg) as a white solid.

TLC (SiO_2, MeOH with NH_4OH): co-eluted with non-labelled sample.

ES-MS, $[M+H]^+ = 317$. ^{1}H-NMR of the non-labelled sample (CD_3OD) δ 7.53 (5 H, bs), 5.61 (1 H, bs), 3.3–3.7 (9 H, m), 3.13 (4 H, m), 2.9 (3 H, s), 2.42 (2 H, m), 2.1 (2 H, m).

*Synthesis of 1-(indole-6-carbonyl-*D*-phenylglycinyl)-4-(1-methylpiperidin-4-yl)-piperazine-[carbonyl-^{14}C], LY517717-[^{14}C]*

The trihydrochloride salt, **3**, (169 mg, 0.4 mmol from the first crop) was dissolved in 10 ml of anhydrous THF, to which was added EDCI (115 mg), HOBt (85 mg) and 1 ml of triethylamine. After stirring at room temperature for 5 min, 6-indolecarboxylic acid (82 mg) was added, and the resulting mixture was stirred at room temperature overnight (about 22.5 h). The reaction was quenched with a saturated sodium bicarbonate solution followed by the addition of ethyl acetate while stirring to give a clear, two-layered solution. The layers were separated. The organic solution was washed with a saturated sodium bicarbonate solution (twice), water (three times) and filtered through a pad of anhydrous potassium carbonate. The filtrate was concentrated to give a first crop of crude product **1** (137 mg) as a white solid. The combined aqueous layers were extracted with ethyl acetate. The extract was dried over anhydrous sodium sulfate, filtered and concentrated to give a second crop of product **1** (57 mg) as a white solid. These two crops of

crude product were re-crystallized from acetonitrile separately then combined to give 80 mg of product as a white powder. HPLC indicated that the product was contaminated with a single non-radioactive impurity about 1.6%. Trying to remove the impurity by repeated re-crystallization was unsuccessful. Co-injection of 6-indolecarboxylic acid with the crude product in an HPLC analysis showed that the impurity co-eluted with 6-indolecarboxylic acid. It was reasonable to assume that the impurity was 6-indolecarboxylic acid, which formed a salt with the product (or existed as a potassium salt) and thus could not be removed by re-crystallization. The solid and mother liquor from the re-crystallizations were combined in methylene chloride. The solution was washed with saturated sodium bicarbonate, dried over anhydrous sodium sulfate, filtered and concentrated to give a residue as a white solid.

The solid was re-crystallized from CH_3CN to give the desired product, **1**, (50 mg) as a white solid. 98.8% Radiochemical purity with 99% ee.

HPLC conditions: Zorbax RX-C8, 4.6×250 mm, U.V. at 235 nm, at room temperature. Flow rate = 1 ml/min. Buffer: 10 mM NaOAc, pH 4.35 with glacial acetic acid. Gradient: starting with buffer/MeOH at 75/25 for 1 min, then increasing to 15/85 during the next 44 min.

Chiral HPLC conditions: Chiracel OD-H, 4.6×250 mm, UV at 220 nm at room temperature. Flow rate = 1 ml/min. Eluent: 15:85 EtOH/hexanes with 0.2% of diethylamine.

^{1}H-NMR of non-labelled sample (CD_3OD), δ 7.85 (1 H, s), 7.5 (1 H, d), 7.2–7.5 (8 H, m), 6.4 (1 H, s), 6.2 (1 H, s), 3.3–3.7 (4 H, m), 3.78 (2 H, bd), 2.3–2.6 (3 H, m), 2.15 (3 H, s), 1.6–2.2 (6 H, m), 1.38 (2 H, m). ES-MS, $[M+H]^+ = 460$.

Analytical Data for LY517717-[^{14}C]

	LY517717-[^{14} C]
Specific activity in μCi/mg	54.8
Radiochemical purity (%) HPLC	98.80
Identity TLC-autoradiography	Co-elutes with standard
ES-MS: $[M+H]^+$, m/z	459/461
Optical purity (%)[b]	99

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