Synthesis of [^{11}C]GSK1482160 as a new PET agent for targeting P2X$_7$ receptor

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Abstract—The authentic standards GSK1482160 and its isomer, as well as the radiolabeling precursors desmethyl-GSK1482160 and Boc-protected desmethyl-GSK1482160 were synthesized from *L*-pyroglutamic acid, methyl *L*-pyroglutamate and 2-chloro-3-(trifluoromethyl)benzylamine with overall chemical yield 27-28% in 3 steps, 58% in 4 steps, 76% in 1 step and 33% in 2 steps, respectively. [^{11}C]GSK1482160 was prepared from either desmethyl-GSK1482160 or Boc-protected desmethyl-GSK1482160 with [^{11}C]CH_3OTf through *N*-[^{11}C]methylation and isolated by HPLC combined with SPE in 40-50% and 30-40% radiochemical yield, respectively, based on [^{11}C]CO_2 and decay corrected to end of bombardment (EOB). The radiochemical purity was >99%, and the specific activity at EOB was 370-1110 GBq/μmol with a total synthesis time of ~40-minutes from EOB.

Keywords: [^{11}C]GSK1482160; Radiosynthesis; Positron emission tomography (PET); P2X$_7$ receptor; Neuroinflammation.

The purinergic receptor P2X ligand-gated ion chanel type 7 (P2X$_7$ receptor) is an adenosine triphosphate (ATP)-gated ion-channel. It is found in the immune, peripheral, and central nervous systems; implicated in ATP-mediated cell death, regulation of receptor trafficking and inflammation; and associated with various cancer, neurological and cardiovascular disorders.[1-4] Neuroinflammation is considered to play an important role in a variety of neuropathologies, such as Alzheimer's disease, Parkinson's disease and traumatic brain injury.[5-7] P2X$_7$ receptor antagonists have been developed as potential pharmaceuticals for the treatment of various diseases including neuroinflammation.[8-10]

We are interested in biomedical imaging to detect and quantify neuroinflammation, with positron emission tomography (PET) offering the best opportunity for success with development and validation of suitable targeted radioligands. However, an ideal PET radiopharmaceutical is still missing.[11,12] Like others, we have used [^{11}C]PBR28-PET (Figure 1) to image neuroinflammation, targeting the 18-kDa translocator protein (TSPO) formerly named the peripheral benzodiazepine receptor (PBR).[13,14] However, the limitations[15,16] of [^{11}C]PBR28-PET such as low receptor binding, high inter-subject variability in binding affinity, and nonspecific binding in the human brain, due to TSPO polymorphism, have motivated us to search for new molecular targets and PET radioligands.

Increasing evidence suggests the P2X$_7$ receptor as an interesting neuroinflammation-associated molecular target.[12,17] Recently the synthesis and initial preclinical evaluation of a P2X$_7$ receptor radioligand [^{11}C]A-740003 (racemic compounds, Figure 1) have been reported, but it showed little uptake in brain in healthy male Wistar rats.[12] We have instead initially focused on GSK1482160 (a chiral compound, (*S*)-*N*-(2-chloro-3-(trifluoromethyl)benzyl)-1-methyl-5-oxopyrrolidine-2-carboxamide, **3**). Originally developed by GlaxoSmithKline, GSK1482160 is a potent P2X$_7$ antagonist with excellent biological activity {PIC$_{50}$ 8.5

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(IC_{50} 3 nM) for human $P2X_7$, and PIC_{50} 6.6 for rat $P2X_7$}.[18-20] This compound readily crosses the blood-brain barrier (BBB), and has been evaluated as a therapeutic agent in a Phase 1 human study,[18-20] making it an attractive candidate for possible translation to a PET diagnostic agent.

Here we report the synthesis of [^{11}C]GSK1482160 {(*S*)-*N*-(2-chloro-3-(trifluoromethyl)benzyl)-1-[^{11}C]methyl-5-oxopyrrolidine-2-carboxamide, [^{11}C]**3**} as a new candidate PET agent for imaging of $P2X_7$ receptor.

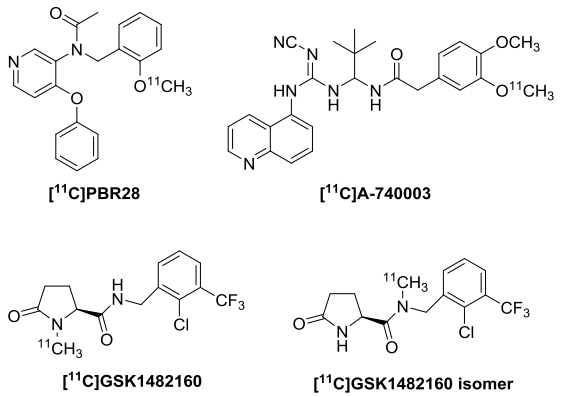
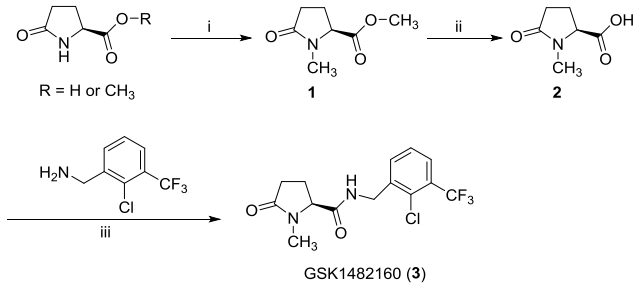


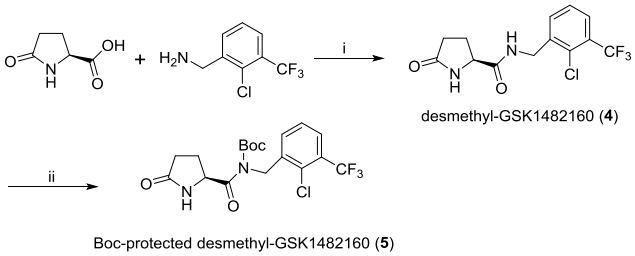
Figure 1. Chemical structures of [^{11}C]PBR28, [^{11}C]A-740003, [^{11}C]GSK1482160, and [^{11}C]GSK1482160 isomer

The reference standard GSK1482160 was synthesized as shown in Scheme 1.[18,19] *L*-Pyroglutamic acid or methyl *L*-pyroglutamate was reacted with methyl iodide (CH_3I) and NaH in *N*,*N*-dimethylformamide (DMF) to convert into methyl (*S*)-1-methyl-5-oxopyrrolidine-2-carboxylate (**1**) in 41-43% yield. Subsequently, compound **1** was de-esterified with NaOH to give (*S*)-1-methyl-5-oxopyrrolidine-2-carboxylic acid (**2**) in 82% yield. Then compound **2** underwent a coupling reaction with 2-chloro-3-(trifluoromethyl)benzylamine, using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) or 1-ethyl-3-(3-dimethylaminopropyl)carbodimide (EDAC) as catalyst, affording standard compound **3** in 80% yield.

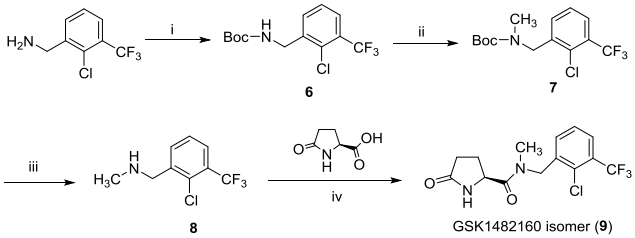


Scheme 1. Synthesis of GSK1482160. Reagents, conditions and yields: (i) NaH, CH_3I, DMF, 0 °C to RT, 5 h; 41-43%. (ii) 2 M NaOH, MeOH, reflux, 3 h; 82%. (iii) EEDQ, CH_2Cl_2, RT, 15 h; 80%.

The radiolabeling precursors desmethyl-GSK1482160 {(*S*)-*N*-(2-chloro-3-(trifluoromethyl)benzyl)-5-oxopyrrolidine-2-carboxamide, **4**} and Boc-protected desmethyl-GSK1482160 (*tert*-butyl (*S*)-(2-chloro-3-(trifluoromethyl)benzyl)(5-oxopyrrolidine-2-carbonyl)carbamate, **5**), were synthesized as indicated in Scheme 2. The precursor desmethyl-GSK1482160 was prepared from *L*-pyroglutamic acid and 2-chloro-3-(trifluoromethyl)benzylamine, with EDAC and 1-hydrobenzotrizole (HOBt) as catalysts,[21] in 76% yield. The alternative radiochemistry precursor, Boc-protected desmethyl-GSK1482160, was prepared from desmethyl-GSK1482160 with di-*tert*-butyl-dicarbonate {$(Boc)_2O$} using base catalyst 4-dimethylaminopyridine (DMAP)[22] in acetonitrile in 44% yield.



Scheme 2. Synthesis of desmethyl-GSK1482160 and Boc-protected desmethyl-GSK1482160. Reagents, conditions and yields: (i) EDAC, HOBt, DCM, RT, 15 h; 76%; (ii) $(Boc)_2O$, Et_3N, DMAP, CH_3CN, RT, 15 h; 44%.



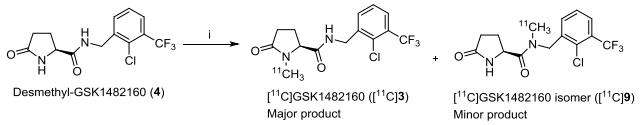
Scheme 3. Synthesis of GSK1482160 isomer. Reagents, conditions and yields: (i) $(Boc)_2O$, THF/H_2O; 90%. (ii) NaH, CH_3I, THF; 91%. (iii) THA, CH_2Cl_2, RT, 3 h; 91%. (iv) EEDQ, CH_2Cl_2, RT, 15 h; 78%.

As a reference compound, the GSK1482160 isomer (*S*)-*N*-(2-chloro-3-(trifluoromethyl)benzyl)-*N*-methyl-5-oxopyrrolidine-2-carboxamide (**9**) was synthesized as outlined in Scheme 3. 2-Chloro-3-(trifluoromethyl)benzylamine was reacted with $(Boc)_2O$ in THF and water (1:1) without catalyst to give Boc-protected *tert*-butyl (2-chloro-3-(trifluoromethyl)benzyl)carbamate (**6**) in 90% yield. Subsequently compound **6** was methylated with CH_3I and NaH to afford *tert*-butyl (2-chloro-3-(trifluoromethyl)benzyl)(methyl)carbamate (**7**) in 91% yield. Compound **7** was deprotected with trifluoroacetic acid (TFA) in CH_2Cl_2 to obtain 1-(2-chloro-3-(trifluoromethyl)phenyl)-*N*-methylmethanamine (**8**) in 91% yield. Then compound **8** was reacted with *L*-pyroglutamic acid using similar synthetic method for standard compound **3** to produce GSK1482160 isomer in 78% yield. The ^{1}H NMR spectra of compound **9** showed it contained two epimers, since the methyl

group is located at the side chain amide. This steric factor does not apply to compound **5**. Compound **9** was formed by the coupling reaction from two different reaction directions, which gave two epimers. The reaction mechanism for synthesis of compound **5** is the substitution reaction of amide; in addition, Boc is a bulky group, thus compound **5** was formed through a preferential reaction direction, and no epimers were seen in its ^{1}H NMR spectra.

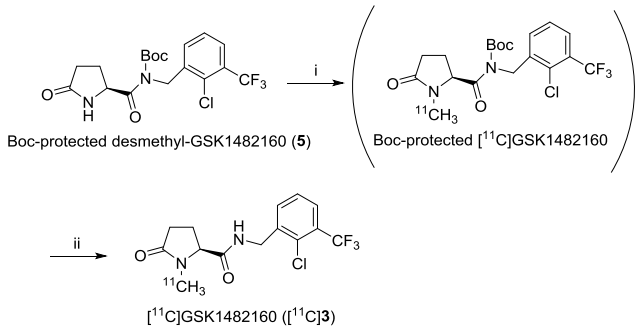
Synthesis of [^{11}C]GSK1482160 via the desmethyl-GSK1482160 precursor is presented in Scheme 4. Precursor **4** underwent *N*-[^{11}C]methylation[23-26] using the reactive [^{11}C]methylating agent [^{11}C]methyl triflate ([^{11}C]CH_3OTf)[27,28] in acetonitrile at 80 °C under basic conditions (NaH) The product was isolated by semi-preparative reverse-phase (RP) high performance liquid chromatography (HPLC) with a C-18 column, and then concentrated by solid-phase extraction (SPE) with a disposable C-18 Plus Sep-Pak cartridge[29-31] to produce the corresponding pure radiolabeled compound [^{11}C]**3** in 40-50% radiochemical yield, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2. This is a 1-pot-1-step radiosynthesis.

The desmethyl-GSK1482160 precursor contains both cyclic amide and side chain amide. Although the cyclic amide is more easily deprotonated and methylated than side chain amide, a minor by-product [^{11}C]GSK1482160 isomer (*S*)-*N*-(2-chloro-3-(trifluoromethyl)benzyl)-*N*-[^{11}C]methyl-5-oxopyrrolidine-2-carboxamide ([^{11}C]**9**) was formed as well. The [^{11}C]GSK1482160 and [^{11}C]GSK1482160 isomer were formed in a ~10:1 ratio from desmethyl-GSK1482160. Different solid bases, such as NaOH, KOH, K_2CO_3, Cs_2CO_3 and NaH, were tested in the radiolabeling reaction, and NaH proved to be the best base for the *N*-[^{11}C]methylation of cyclic amide.



Scheme 4. Synthesis of [^{11}C]GSK1482160 via precursor desmethyl-GSK1482160. Reagents, conditions and yields: (i) [^{11}C]CH_3OTf, CH_3CN, NaH, 80 °C, 3 min; 40-50%.

The synthesis of [^{11}C]GSK1482160 via Boc-protected desmethyl-GSK1482160 is outlined in Scheme 5. The precursor **5** was labeled by [^{11}C]CH_3OTf through *N*-[^{11}C]methylation in dimethyl sulfoxide (DMSO) at 100 °C under basic condition (K_2CO_3) to give a radiolabeled intermediate Boc-protected [^{11}C]GSK1482160. This was followed by a quick deprotection reaction with 1 M HCl, without isolation, to provide the target tracer. The radiolabeling mixture was isolated by RP-HPLC combined with SPE to produce the corresponding pure radiolabeled compound [^{11}C]**3** in 30-40% decay-corrected radiochemical yield. This is a 1-pot-2-step radiosynthesis.[22] The best base for the radiolabeling reaction was freshly ground K_2CO_3 powder.



Scheme 5. Synthesis of [^{11}C]GSK1482160 via precursor BOC-protected desmethyl-GSK1482160. Reagents, conditions and yields: (i) [^{11}C]CH_3OTf, DMSO, K_2CO_3, 100 °C, 5 min; (ii) 1 M HCl, 100 °C, 3 min; 30-40%.

Our approach employed more reactive [^{11}C]CH_3OTf, instead of commonly used [^{11}C]methyl iodide ([^{11}C]CH_3I),[32] in [^{11}C]methylation of the amide, and thus the radiochemical yield of [^{11}C]**3** was relatively high. It is important to note that the strong base NaH would help the *N*-[^{11}C]methylation of unprotected amide precursor desmethyl-GSK1482160, and significantly increase the radiochemical yield of [^{11}C]**3**. Therefore, the radiochemical yields for [^{11}C]**3** reported here are much higher than that the *N*-[^{11}C]methylation of amide precursor with other base (KOH) reported previously.[25] For the Boc-protected desmethyl-GSK1482160 precursor, it was better to conduct the *N*-[^{11}C]methylation of amide under mildly basic conditions (K_2CO_3), since the Boc protecting group is not very stable under strongly basic conditions with high reaction temperature. Only a relatively small amount of the precursor (0.3-0.5 mg) was used for radiolabeling, instead of our more commonly used amount of the precursor (1.0-1.5 mg), which improved the chemical purity of the final tracer solution. Furthermore, addition of $NaHCO_3$ solution to quench the radiolabeling reaction and to dilute the radiolabeling mixture prior to the injection onto the semi-preparative HPLC column for purification, gave better separation of [^{11}C]**3** from its unprotected or protected amide precursor (**4** or **5**).[29-31,33]

The radiosynthesis was performed in a home-built automated multi-purpose [^{11}C]-radiosynthesis module.[34-36] This radiosynthesis module facilitated the overall design of the reaction, purification and reformulation capabilities in a fashion suitable for adaptation to preparation of human doses. In addition, the module is designed to allow in-process measurement of [^{11}C]-tracer specific activity (SA, GBq/μmol at EOB) using a radiation detector at the outlet of the HPLC-

portion of the system.[26] For the reported syntheses, product SA was in a range of 370-1110 GBq/μmol at EOB.

The factors that affect the EOB SA significantly to lead to such a wide range from 370 to 1110 GBq/μmol are mainly from two parts: (1) carrier from the [^{11}C]-target, and (2) carrier from the [^{11}C]-radiosynthesis unit.[26] We have optimized the [^{11}C] gas irradiation target system and the [^{11}C]-radiosynthesis unit to eliminate ^{12}C carrier-added as much as possible and to reach the high end of the SA. To help produce high SA [^{11}C]CO_2, we usually do 2-3 10-minute target pre-burns, with the same beam current, prior to the actual production run. These pre-burn warm up the cyclotron target and eliminate significant amount of ^{12}C carrier in the cyclotron's [^{11}C] gas target. We use an Eckert & Ziegler Modular Lab C-11 Methyl Iodide/Triflate module to produce [^{11}C]CH_3OTf, convenient gas phase bromination of [^{11}C]methane, and production of [^{11}C]CH_3OTf. This 'dry' method, using Br_2 to generate a [^{11}C]CH_3Br intermediate, differs from other 'dry' methods using I_2 and 'wet' methods using $LiAlH_4$ and HI, and seems to help minimize introduction of additional ^{12}C carrier after [^{11}C]CO_2 production.[28]

The 1-pot-1-step radiosynthesis via desmethyl-GSK1482160 is a non-specific synthesis route, and 1-pot-2-step radiosynthesis via Boc-protected desmethyl-GSK1482160 is a specific synthesis route. The non-specific route required lower reaction temperature, shorter reaction time, and shorter overall synthesis time. Consequently it gave higher radiochemical yield. In addition, it is easier to synthesize the precursor without Boc group than with Boc group. This route is somewhat simpler to implement as it avoids adding a deprotection step. Fortunately, this route proceeds with high selectivity (~10:1) for [^{11}C]methylation at the *N* of the desired cyclic amide over the acyclic amide, and the desired product has been cleanly isolated after semi-preparative HPLC purification of the radiolabeling reaction mixture. In comparison with these two radiosynthesis approaches, the non-specific route is identified as a more appropriate route for future applications.

Chemical purity and radiochemical purity were determined by analytical HPLC.[37] The chemical purity of the precursors and reference standards was >93%. The radiochemical purity of the target tracers was >99% determined by radio-HPLC through γ-ray (PIN diode) flow detector, and the chemical purity of the target tracers was >90% determined by reversed-phase HPLC through UV flow detector.

The experimental details and characterization data for compounds **1-9** and for the tracer [^{11}C]**3** are given.[38]

In summary, synthetic routes with moderate to excellent yields have been developed to produce the precursors desmethyl-GSK1482160 and Boc-protected desmethyl-GSK1482160; the reference standards GSK1482160 and GSK1482160 isomer; and the target PET radiotracer [^{11}C]GSK1482160. The radiosynthesis employed [^{11}C]CH_3OTf for *N*-[^{11}C]methylation at the nitrogen position of the amide precursors, followed by product purification and isolation by a semi-preparative RP HPLC combined with solid-phase extraction. The desired [^{11}C]GSK1482160 was obtained in high radiochemical yield, radiochemical purity and chemical purity, with a reasonably short overall synthesis time, and high specific activity. This will facilitate studies to evaluate [^{11}C]GSK1482160 as a new PET radiopharmaceutical for targeting the $P2X_7$ receptor in animals and humans.

Acknowledgments

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38. *(a) General:* All commercial reagents and solvents were purchased from Sigma-Aldrich and Fisher Scientific, and used without further purification. [^{11}C]CH_3OTf was prepared according to a literature procedure.[28] Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, on a Bruker Avance II 500 MHz NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shift data for the proton resonances were reported in parts per million (ppm, δ scale) relative to internal standard TMS (δ 0.0), and coupling constants (*J*) were reported in hertz (Hz). Liquid chromatography-mass spectra (LC-MS) analysis was performed on an Agilent system, consisting of an 1100 series HPLC connected to a diode array detector and a 1946D mass spectrometer configured for positive-ion/negative-ion electrospray ionization. The high resolution mass spectra (HRMS) were obtained using a Waters/Micromass LCT Classic spectrometer. Chromatographic solvent proportions are indicated as volume:volume ratio. Thin-layer chromatography (TLC) was run using Analtech silica gel GF uniplates (5×10 cm^2). Plates were visualized under UV light. Normal phase flash column chromatography was carried out on EM Science silica gel 60 (230-400 mesh) with a forced flow of the indicated solvent system in the proportions described below. All moisture- and air-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source. Analytical HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6×250 mm; mobile phase 40% CH_3CN/60% 20 mM H_3PO_4; flow rate 1.0 mL/min; and UV (254 nm) and γ-ray (PIN diode) flow detectors. Semi-preparative HPLC was performed

using a Prodigy (Phenomenex) 5 μm C-18 column, 12 nm, 10 × 250 mm; mobile phase 40% CH_3CN/60% H_2O; 5.0 mL/min flow rate; UV (254 nm) and γ-ray (PIN diode) flow detectors. C18 Plus Sep-Pak cartridges were obtained from Waters Corporation (Milford, MA). Sterile Millex-FG 0.2 μm filter units were obtained from Millipore Corporation (Bedford, MA).

*(b) Methyl (S)-1-methyl-5-oxopyrrolidine-2-carboxylate (**1**):* Method A (from *L*-pyroglutamic acid). To a stirred suspension of sodium hydride (60% dispersion in mineral oil, 2.1 g, 52 mmol) in DMF (60 mL) at 0 °C under nitrogen, *L*-pyroglutamic acid (2.58 g, 20 mmol) was added during 15 min. The mixture was stirred at room temperature (RT) for 1 h. While ice cooling and stirring, iodomethane (7.76 g, 55 mmol) was added and the reaction mixture was allowed to warm to RT overnight. The solvent was evaporated under reduced pressure, and water was added. After pH was adjust to 5 with adding acetic acid, the aqueous layer was extracted with CH_2Cl_2 (100 mL × 3). The organic layer was concentrated, and the residue was purified by column chromatography on silica gel with eluent (30:70 EtOAc/hexanes) to give colorless oily product **1** (1.35 g, 43%). R_f = 0.68 (1:19 MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 2.07-2.09 (m, 1H, CH), 2.33-2.40 (m, 2H, CH_2), 2.47-2.49 (m, 1H, CH), 2.85 (s, 3H, NCH_3), 3.78 (s, 3H, OCH_3), 4.12 (dd, J = 3.0, 8.0 Hz, 1H, CH). MS (ESI): 158 ($[M+H]^+$, 100%). Method B (from methyl *L*-pyroglutamate). Methyl *L*-pyroglutamate (1.43 g, 10 mmol was dissolved in DMF (25 mL) and cooled to 0 °C. Sodium hydride (60% suspension in mineral oil, 0.6 g, 15 mmol) was added to the reaction mixture and stirred for 1 h. Then methyl iodide (2.11 g, 15 mmol) was added slowly, the mixture was allowed to warm to RT and stirred for another 1 h. The solvent was evaporated in vacuo, and water was added, subsequently acetic acid was added to adjust pH to 5, the aqueous layer was then extracted with CH_2Cl_2 (60 mL × 3). The organic layer was concentrated, and the residue was purified by column chromatography on silica gel with eluent (30:70 EtOAc/hexanes) to yield colorless oily product **1** (0.64 g, 41%). Analytical data was identical with Method A.

*(c) (S)-1-Methyl-5-oxopyrrolidine-2-carboxylic acid (**2**):* Compound **1** (0.47 g, 3.0 mmol) was dissolved in methanol (20 mL), and to this solution was added 2 M NaOH (3 mL, 6.0 mmol). The mixture was refluxed for 3 h, then cooled and evaporated to leave a minimal amount of water. This mixture was acidified to pH 2 using 1 M aqueous HCl. The aqueous was extracted with CH_2Cl_2 (100 mL × 3), and organic layer was washed with water, dried over $MgSO_4$. The solvent was evaporated to afford white solid compound **2** (0.35 g, 82%). R_f = 0.11 (1:5 MeOH/CH_2Cl_2), mp 148–150 °C. ^{1}H NMR (DMSO-d_6): δ 1.92-1.94 (m, 1H, CH), 2.19-2.30 (m, 3H, CH_2 and CH), 2.69 (s, 3H, NCH_3), 4.11 (dd, J = 3.8, 8.8 Hz, 1H, CH), 12.97 (s, 1H, COOH). MS (ESI): 309 ($[2M+Na]^+$, 20%), 287 ($[2M+H]^+$, 100%), 144 ($[M+H]^+$, 60%).

*(d) (S)-N-(2-Chloro-3-(trifluoromethyl)benzyl)-1-methyl-5-oxopyrrolidine-2-carboxamide (GSK1482160, **3**):* Compound **2** (286 mg, 2.0 mmol) was suspended in CH_2Cl_2 (60 mL), and EEDQ (543 mg, 2.2 mmol) was added. The mixture was stirred at RT for 10 min. A solution of 2-chloro-3-(trifluoromethyl)benzylamine (440 mg, 2.1 mmol) in CH_2Cl_2 (20 mL) was then added dropwise to the mixture. The mixture was then stirred at RT overnight. The mixture was washed with saturated aqueous $NaHCO_3$ (40 mL), 2 N aqueous HCl (40 mL × 2), and water. The organic solution was dried over Na_2SO_4, filtered and concentrated in vacuo. The resulting crude product was then purified by column chromatography on silica gel with eluent (2:98 MeOH/CH_2Cl_2) to obtain white solid product **3** (534 mg, 80%). R_f = 0.53 (1:12 MeOH/CH_2Cl_2), mp 166-168 °C. ^{1}H NMR (acetone-d_6): δ 1.96-2.03 (m, 1H, CH), 2.18-2.21 (m, 1H, CH), 2.28-2.36 (m, 1H, CH_2), 2.77 (s, 3H, NCH_3), 4.17 (dd, J = 3.5, 8.5 Hz, 1H, CH), 4.63 (d, J = 6.0 Hz, 2H, CH_2), 7.55 (t, J = 8.0 Hz, 1H, Ph-H), 7.75 (t, J = 8.0 Hz, 2H, Ph-H), 8.05 (br s, 1H, NH). MS (ESI): 335 ($[M+H]^+$, 100%); MS (ESI): 333 ($[M-H]^-$, 50%).

*(e) (S)-N-(2-Chloro-3-(trifluoromethyl)benzyl)-5-oxopyrrolidine-2-carboxamide (desmethyl-GSK1482160, **4**):* *L*-Pyroglutamic acid (516 mg, 4.0 mmol) and 2-chloro-3-(trifluoromethyl)benzylamine (838 mg, 4.0 mmol) were mixed with EDAC·HCl (959 mg, 5.0 mmol) and HOBt (676 mg, 5.0 mmol) in dry CH_2Cl_2 (100 mL). The reaction mixture was stirred at RT for overnight. Then the resultant mixture was washed with 2 N HCl (50 mL × 3) and saturated aqueous $NaHCO_3$ (40 mL). The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel with eluent (2:98 MeOH/CH_2Cl_2) to afford white solid product **4** (975 mg, 76%). R_f = 0.47 (1:12 MeOH/CH_2Cl_2), mp 173-175 °C. ^{1}H NMR (acetone-d_6): δ 2.10-2.19 (m, 2H, CH_2), 2.23-2.28 (m, 1H, CH), 2.44-2.49 (m, 1H, CH), 4.23-4.25 (m, 1H, CH), 4.55-4.65 (m, 2H, CH_2), 7.07 (br s, 1H, NH), 7.51 (t, J = 8.0 Hz, 1H, Ph-H), 7.73 (t, J = 7.5 Hz, 2H, Ph-H), 8.00 (br s, 1H, NH). ^{13}C NMR (acetone-d_6): δ 26.65, 29.84, 41.36, 57.34, 123.07 (q, $J_{C\text{-}F}$ = 270.8 Hz, CF_3), 127.28 (q, $J_{C\text{-}F}$ = 5.5 Hz), 128.08, 128.79 (q, $J_{C\text{-}F}$ = 30.6 Hz), 131.34, 133.84, 140.21, 173.80, 178.43. MS (ESI): 321 ($[M+H]^+$, 100%). HRMS (ESI): calcd for $C_{13}H_{12}N_2O_2ClF_3Na$ ($[M+Na]^+$) 343.0437, found 343.0432.

*(f) tert-Butyl (S)-(2-chloro-3-(trifluoromethyl)benzyl)(5-oxopyrrolidine-2-carbonyl)carbamate (Boc-protected desmethyl-GSK1482160, **5**):* Compound **4** (300 mg, 0.93 mmol) and $(Boc)_2O$ (430 mg, 1.97 mmol) were mixed with DMAP (115 mg, 0.93 mmol) and Et_3N (142 mg, 1.4 mmol) in acetonitrile (50 mL). The reaction mixture was stirred at RT for 24 h. The resulting mixture was

concentrated, and the residue was purified by column chromatography on silica gel with eluent (2:98 MeOH/CH_2Cl_2) to give white solid product **5** (173 mg, 44%). R_f = 0.46 (1:19 MeOH/CH_2Cl_2), mp 145-147 °C. ^{1}H NMR ($CDCl_3$): δ 1.39 (s, 9H, 3 × CH_3), 2.14-2.20 (m, 1H, CH), 2.22-2.29 (m, 1H, CH), 2.43-2.49 (m, 1H, CH), 2.70-2.78 (m, 1H, CH), 4.53 (dd, J = 2.8, 7.8 Hz, 1H, CH), 4.57-4.67 (m, 2H, NCH_2), 6.88 (s, 1H, Ph-H), 7.34 (t, J = 7.5 Hz, 1H, Ph-H), 7.63 (d, J = 7.5 Hz, 2H, Ph-H). ^{13}C NMR ($CDCl_3$): δ 22.08, 27.82, 31.66, 41.55, 60.17, 83.99, 121.74 (q, J_{C-F} = 271.6 Hz, CF_3), 126.83, 126.90 (q, J_{C-F} = 5.3 Hz), 128.93 (q, J_{C-F} = 30.9 Hz), 131.51, 133.53, 137.90, 150.03, 171.01, 173.89. MS (ESI): 419 ([M-H]$^-$, 1%). HRMS (ESI): calcd for $C_{18}H_{20}N_2O_4ClF_3Na$ ([M+Na]$^+$) 443.0961, found 443.0972.

*(g) tert-Butyl (2-chloro-3-(trifluoromethyl)benzyl)carbamate (**6**):* 2-Chloro-3-(trifluoromethyl)benzylamine (210 mg, 1.0 mmol) was dissolved in THF-water (1:1) and then $(Boc)_2O$ (229 mg, 1.05 mmol) was added to the solution. The reaction mixture was stirred at RT for overnight. Then the mixture was concentrated in vacuo, and cooled down. The resulting precipitate was filtered, washed with water, and dried, affording white solid product **6** (278 mg, 90%). R_f = 0.60 (1:5 EtOAc/hexanes), mp 98-100 °C. ^{1}H NMR ($CDCl_3$): δ 1.45 (s, 9H, 3 × CH_3), 4.45 (d, J = 6.0 Hz, 2H, CH_2), 5.05 (s, 1H, NH), 7.35 (t, J = 7.5 Hz, 1H, Ph-H), 7.59 (d, J = 7.5 Hz, 1H, Ph-H), 7.62 (d, J = 7.5 Hz, 1H, Ph-H). MS (ESI): 308 ([M-H]$^-$, 1%).

*(h) tert-Butyl (2-chloro-3-(trifluoromethyl)benzyl)(methyl)carbamate (**7**):* Sodium hydride (60% dispersion in mineral oil, 152 mg, 3.8 mmol) was added dropwise into a solution of compound **6** (588 mg, 1.9 mmol) in THF (60 mL) at 0 °C. After 30 min methyl iodide (809 mg, 5.7 mmol) was added. The mixture was reacted at RT for 3 h. The reaction mixture was concentrated in vacuo, and thereto was added water in an ice bath. Then it was extracted with ethyl acetate, and washed with brine. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel with eluent (10:90 EtOAc/hexanes) to give colorless oily product **7** (559 mg, 91%). R_f = 0.63 (1:5 EtOAc/hexanes). ^{1}H NMR ($CDCl_3$): δ 1.41 and 1.50 (s, 9H, 3 × CH_3), 2.89 and 2.92 (s, 3H, NCH_3), 4.57 and 4.62 (s, 2H, CH_2), 7.36 (d, J = 7.0 Hz, 2H, Ph-H), 7.62 (s, 1H, Ph-H).

*(i) 1-(2-Chloro-3-(trifluoromethyl)phenyl)-N-methylmethanamine (**8**):* Compound **7** (485 mg, 1.5 mmol) with TFA (1.0 mL) was dissolved in CH_2Cl_2 (10 mL) at RT. After stirring for 3 h, the solvent was removed, and the residue was dissolved in ethyl acetate, which was washed with aqueous Na_2CO_3 followed by brine. The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography on silica gel with eluent (2:98 MeOH/CH_2Cl_2) to afford yellowish oily product **8** (305 mg, 91%). R_f = 0.52 (1:9 MeOH/CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 2.48 (s, 3H, NCH_3), 3.91 (s, 2H, CH_2), 7.35 (t, J = 8.0 Hz, 1H, Ph-H), 7.6 (d, J = 8.0 Hz, 2H, Ph-H). MS (ESI): 224 ([M+H]$^+$, 100%).

*(j) (S)-N-(2-Chloro-3-(trifluoromethyl)benzyl)-N-methyl-5-oxopyrrolidine-2-carboxamide (GSK1482160 isomer, **9**):* This compound was prepared from compound **8** and *L*-pyroglutamic acid using similar procedure for the preparation of compound **3**, affording white solid product **9** in 78% yield. R_f = 0.64 (1:9 MeOH/CH_2Cl_2). ^{1}H NMR (acetone-d_6): δ 2.09-2.28 (m, 3H, CH_2 and CH), 2.24-2.54 (m, 1H, CH), 2.96 and 3.19 (s, 3H, NCH_3), 4.57-4.86 (m, 3H, CH_2 and CH), 7.00 and 7.07 (s 1H, Ph-H), 7.51-7.63 (m, 1H, Ph-H), 7.74-7.82 (m, 1H, Ph-H). MS (ESI): 335 ([M+H]$^+$, 100%); MS (ESI): 333 ([M-H]$^-$, 6%).

*(k) (S)-N-(2-Chloro-3-(trifluoromethyl)benzyl)-1-[^{11}C]methyl-5-oxopyrrolidine-2-carboxamide, ([^{11}C]GSK1482160, [^{11}C]**3**):* Method A (from desmethyl-GSK1482160, **4**). [^{11}C]CO_2 was produced by the ^{14}N(p,α)^{11}C nuclear reaction in the small volume (9.5 cm^3) aluminum gas target provided with the Siemens RDS-111 Eclipse cyclotron. The target gas consisted of 1% oxygen in nitrogen purchased as a specialty gas from Praxair, Indianapolis, IN. Typical irradiations used for the development were 55 μA beam current and 30 min on target. The production run produced approximately 45.5 GBq of [^{11}C]CO_2 at EOB. In a small reaction vial (5 mL), the precursor **4** (0.3-0.5 mg) was dissolved in CH_3CN (500 μL). To this solution was added NaH (1 mg). No carrier-added (high specific activity) [^{11}C]CH_3OTf that was produced by the gas-phase production method[28] from [^{11}C]CO_2 through [^{11}C]CH_4 and [^{11}C]CH_3Br with silver triflate (AgOTf) column was passed into the reaction vial at RT, until radioactivity reached a maximum (~2 min), and then the reaction vial was isolated and heated at 80 ^{o}C for 3 min. The contents of the reaction vial were diluted with $NaHCO_3$ solution (0.1 M, 1 mL), and injected onto the semi-preparative RP HPLC column with 3 mL injection loop for purification. The product fraction was collected in a recovery vial containing 30 mL water. The diluted tracer solution was then passed through a C-18 Sep-Pak Plus cartridge, and washed with water (5 mL × 4). The cartridge was eluted with EtOH (1 mL × 2), followed by 10 mL saline, to release [^{11}C]**3**. The eluted product was then sterile-filtered through a sterile vented Millex-FG 0.2 μm filter, and collected into a sterile vial. Total radioactivity (4.6-8.2 GBq) was assayed and total volume (10-11 mL) was noted for tracer dose dispensing. The overall synthesis, purification and formulation time was 30-40 min from EOB. Retention times in the analytical HPLC system were: t_R **4** = 2.84 min, t_R **3** = 4.39 min, , t_R **9** = 3.56 min, t_R [^{11}C]**3** = 4.41 min, and t_R [^{11}C]**9** = 3.60 min. Retention times in the semi-preparative HPLC system

were: t_R **4** = 3.63 min, t_R **3** = 7.13 min, , t_R **9** = 5.68 min, t_R [^{11}C]**3** = 7.21 min, and t_R [^{11}C]**9** = 5.79 min. The radiochemical yield of [^{11}C]**3** was 40-50% decay corrected to EOB, based on [^{11}C]CO_2. Method B (from Boc-protected desmethyl-GSK1482160, **5**). The precursor **5** (0.3-0.5 mg) was dissolved in DMSO (500 μL) in a 5-mL V-vial. To this solution was added dry K_2CO_3 powder (1 mg). [^{11}C]CH_3OTf was passed into the reaction vial at RT until radioactivity reached a maximum (~2 min), and then the reaction vial was isolated and heated at 100 °C for 5 min to form a radiolabeled intermediate Boc-protected [^{11}C]GSK1482160. Then, a solution of 1 N HCl (500 μL) was introduced to the reaction vial. The reaction mixture was sealed and heated at 100 °C for 3 min. The contents of the reaction vial were diluted with $NaHCO_3$ (0.1 M, 1 mL), and injected onto the semi-preparative HPLC column with 3 mL injection loop for purification. The purification and formulation procedures were same with Method A. The overall synthesis, purification and formulation time was ~40 min from EOB. Retention times in the analytical HPLC system were: t_R **5** = 5.27 min, t_R **3** = 4.39 min, and t_R [^{11}C]**3** = 4.41 min. Retention times in the semi-preparative HPLC system were: t_R **5** = 8.92 min, t_R **3** = 7.13 min, and t_R [^{11}C]**3** = 7.21 min. The decay corrected radiochemical yields were 30-40%.