Synthesis of [^{11}C]HG-10-102-01 as a new potential PET agent for imaging of LRRK2 enzyme in Parkinson's disease

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Abstract—The reference standard (4-((5-chloro-4-(methylamino)pyrimidin-2-yl)amino)-3-methoxyphenyl)(morpholino)methanone (HG-10-102-01) and its precursor (4-((5-chloro-4-(methylamino)pyrimidin-2-yl)amino)-3-hydroxyphenyl)(morpholino)methanone (desmethyl-HG-10-102-01) were synthesized from 2,4,5-trichloropyrimide and 3-methoxy-4-nitrobenzoic acid with overall chemical yield 49% in four steps and 14% in five steps, respectively. The target tracer (4-((5-chloro-4-(methylamino)pyrimidin-2-yl)amino)-3-[^{11}C]methoxyphenyl)(morpholino)methanone ([^{11}C]HG-10-102-01) was prepared from the precursor desmethyl-HG-10-102-01 with [^{11}C]CH_3OTf through *O*-[^{11}C]methylation and isolated by HPLC combined with SPE in 45-55% radiochemical yield, based on [^{11}C]CO_2 and decay corrected to end of bombardment (EOB). The radiochemical purity was >99%, and the specific activity (SA) at EOB was 370-1110 GBq/μmol with a total synthesis time of ~40-minutes from EOB.

Keywords: [^{11}C]HG-10-102-01; Leucine-rich repeat kinase 2 (LRRK2); Radiosynthesis; Positron emission tomography (PET); Parkinson's disease (PD).

Parkinson's disease (PD) is the second most common neurodegenerative disease of the central nervous system (CNS) that mainly affects the motor system in elderly people.[1-3] The exact cause is still unknown, and thus there is presently no cure. Current standards of care have serious limitations and largely address only symptoms, and the major treatment options are medication and surgery to manage PD's symptoms.[4-6] To discover more effective treatments, a reliable diagnostic tool is really needed.[7] Neuroimaging of PD is one of the most active as well as most challenging areas in neuroscience.[8,9] Advanced biomedical imaging technique positron emission tomography (PET) is a promising modality for PD, and significant advances have accomplished in this field of molecular imaging.[10] The representative PET tracers used in PD imaging such as [^{18}F]FDOPA for dopamine synthesis, [^{11}C]β-CFT for dopamine transporter, [^{11}C]DTBZ for vesicular monoamine transporter type 2, [^{11}C]raclopride for D_2 receptor, [^{11}C]WAY100635 for serotonin receptor, [^{11}C]PBR28 for translocator protein, [^{11}C]PIB for aggregated β-amyloid plaques, and [^{18}F]T807 ([^{18}F]AV-1451) for tau protein are listed in Figure 1.[11-16]

The development of new PET imaging probes for *in vivo* detection of PD is critical for early and accurate diagnosis and for the successful discovery of disease-modifying or neuro-protective therapies, as currently only symptomatic treatment is available.[17-19] Recent genetic studies provide new opportunity to discover molecularly targeted therapeutics for neurodegeneration.[20,21] Among the genes associated with PD, leucine-rich repeat kinase 2 (LRRK2) is an unique enzyme in humans linked to an increased risk of

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PD, the most common known cause of parkinsonism.[22] LRRK2 has become an attractive therapeutic target for PD, and many highly potent, selective, and brain-penetrable LRRK2 small molecule inhibitors have been developed, and the lead compounds HG-10-102-01 ((4-((5-chloro-4-(methylamino)pyrimidin-2-yl)amino)-3-methoxyphenyl)(morpholino)methanone), G1023 and G7915 exhibited excellent biochemical IC_{50} values of 3.2 nM against LRRK2[G2019S], 9 nM against pLRRK2, and 9 nM against pLRRK2, respectively.[4,5,23-27] LRRK2 has also become a promising imaging target for PD, since the efforts in developing therapeutic agents for LRRK2 enzyme have been accompanied by a growing interest in translating therapeutic agents to diagnostic agents. We are interested in the development of new PET agents for PD imaging. In our previous works, we have synthesized various PET PD imaging agents like [^{11}C]β-CFT, [^{11}C]β-CIT, [^{11}C]raclopride, [^{18}F]fallypride, [^{11}C]PBR28, [^{11}C]PIB and [^{18}F]T807.[28-34] In this ongoing effort, we target LRRK2 and develop radiolabeled LRRK2 inhibitors as new potential PET agents for imaging of LRRK2 enzyme in PD.

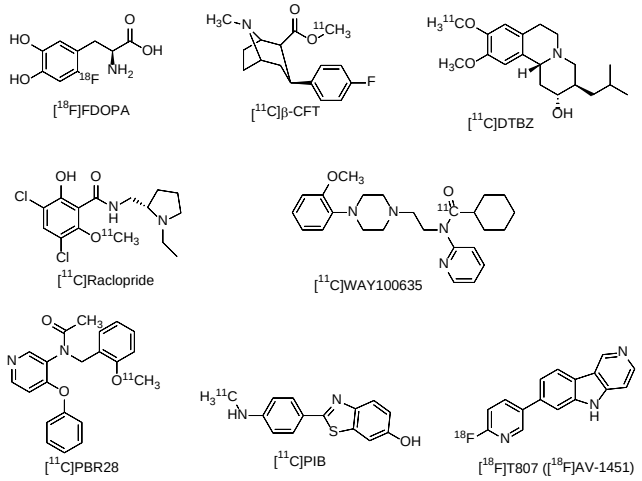


Figure 1. PET tracers for imaging of PD.

The PubMed search showed no records on radiolabeled LRRK2 inhibitors. The Google search indicated that Genentech has filed a patent entitled fluorine-18 and carbon-11 labeled radioligands for positron emission tomography (PET) imaging for LRRK2, and several radiolabeled LRRK2 inhibitors such as ^{11}C-G1023, ^{18}FE-G1023, ^{18}FM-G1023, ^{11}C-G7915, ^{18}FE-G7915, and ^{18}FM-G7915 have been patented (Figure 2).[35] However, the patented synthesis of radiolabeled LRRK2 inhibitors is complicated or lacks synthetic details, and certain key steps gave poor yield or were difficult to reproduce in our hands. On the other hand, HG-10-102-01 was reported earlier than Genentech compounds G1023 and G7915 with simpler chemistry and better binding affinity,[4,5,23-27] although they have similar chemical structures. Here we report the synthesis of a new radiolabeled LRRK2 inhibitor [^{11}C]HG-10-102-01 ((4-((5-chloro-4-(methylamino)pyrimidin-2-yl)amino)-3-[^{11}C]methoxyphenyl)(morpholino)methanone).

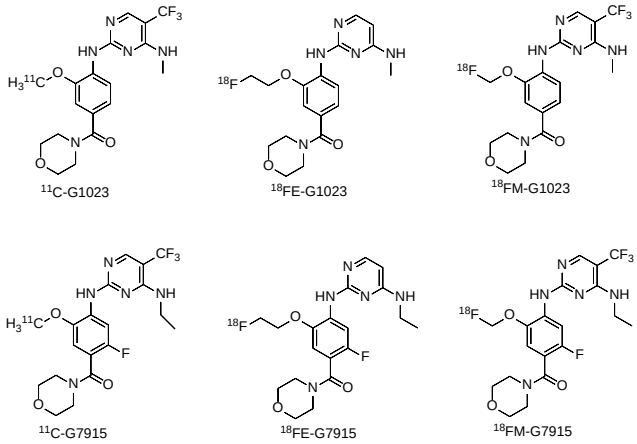
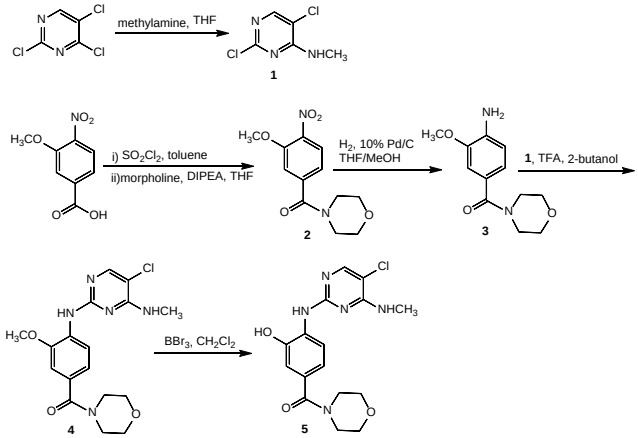


Figure 2. Fluorine-18 and carbon-11 labeled radioligands for LRRK2.

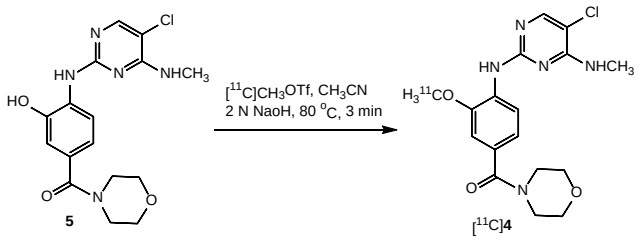


Scheme 1. Synthesis of HG-10-102-01 (**4**) and desmethyl-HG-10-102-01 (**5**).

The reference standard HG-10-102-01 (**4**) and its demethylated precursor (4-((5-chloro-4-(methylamino)pyrimidin-2-yl)amino)-3-hydroxyphenyl)(morpholino)methanone (desmethyl-HG-10-102-01, **5**) were synthesized as depicted in Scheme 1.[4,5] Commercially available 2,4,5-trichloropyrimide was regioselectively aminated with methylamine in THF to obtain 2,5-dichloro-*N*-methylpyrimidin-4-amine (**1**) in 98% yield. Amide derivative **2** was achieved by chlorination of 3-methoxy-4-nitrobenzoic acid with thionly chloride in toluene, followed by amination with morpholine in the presence of *N*,*N*-diisopropylethylamine (DIPEA) in THF in 75% yield. The nitro group of compound **2** was reduced by hydrogenation under H_2 with 10% Pd/C as catalyst in THF and MeOH to afford aniline **3** in 92% yield. Amination of compound **1** by trifluoroacetic acid (TFA) catalyzed S_NAr reaction of aniline **3** in 2-butanol gave reference standard **4** in 73% yield. The methoxyl

group of compound **4** was demethylated with BBr_3 in CH_2Cl_2 to afford the precursor desmethyl-HG-10-102-01 (**5**) in 28% yield.

Synthesis of [^{11}C]HG-10-102-01 ([^{11}C]**4**) is shown in Scheme 2. Desmethyl-HG-10-102-01 underwent *O*-[^{11}C]methylation[36,37] using the reactive [^{11}C]methylating agent [^{11}C]methyl triflate ([^{11}C]CH_3OTf)[38,39] in acetonitrile at 80 °C under basic conditions (2 N NaOH). 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)[40,41] with a disposable C-18 Light Sep-Pak cartridge to produce the corresponding pure radiolabeled compound [^{11}C]**4** in 45-55% radiochemical yield, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2.



Scheme 2. Synthesis of [^{11}C]HG-10-102-01 ([^{11}C]**4**).

The radiosynthesis included three stages: 1) labeling reaction; 2) purification; and 3) formulation. We employed more reactive [^{11}C]CH_3OTf, instead of commonly used [^{11}C]methyl iodide ([^{11}C]CH_3I),[42] in *O*-[^{11}C]methylation to improve radiochemical yield of [^{11}C]**4**. We used an Eckert & Ziegler Modular Lab C-11 Methyl Iodide/Triflate module to produce [^{11}C]methylating agent either [^{11}C]CH_3OTf or [^{11}C]CH_3I ([^{11}C]CH_3Br passed through a NaI column). The direct comparison between [^{11}C]CH_3OTf and [^{11}C]CH_3I confirmed the result. The labeling reaction was conducted using a V-vial method. Addition of aqueous $NaHCO_3$ 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]**4** from its phenyl hydroxyl precursor **5**. We used Sep-Pak trap/release method instead of rotatory evaporation for formulation to improve the chemical purity of radiolabeled product [^{11}C]**4**. In addition, a C18 Light Sep-Pak to replace a C18 Plus Sep-Pak allowed final product formulation with ≤5% ethanol.[43] Overall, it took ~40 min for synthesis, purification and dose formulation.

The radiosynthesis was performed in a home-built automated multi-purpose [^{11}C]-radiosynthesis module.[44-46] 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. For the reported syntheses, product SA was in a range of 370-1110 GBq/μmol at EOB. The major factors including [^{11}C]-target and [^{11}C]-radiosynthesis unit that affect the EOB SA significantly to lead to such a wide range from 370 to 1110 GBq/μmol have been discussed in our previous works.[47] The general methods to increase SA have been described as well, and the SA of our [^{11}C]-tracers is significantly improved.[47] The 'wide range' of SA we reported is for the same [^{11}C]-tracer produced in different days, because very different [^{11}C]-target and [^{11}C]-radiosynthesis unit situations would make SA in a wide range. For a [^{11}C]-tracer produced in the same day, the SA of the same tracer in different production runs will be in a small range, because [^{11}C]-target and [^{11}C]-radiosynthesis unit would not be much different in the same day. Likewise, the methods to minimize such wide range of SA from practice perspective have been provided in our previous works.[47] At the end of synthesis (EOS), the SA of [^{11}C]-tracer was determined again by analytical HPLC,[48] calculated, decay corrected to EOB, and based on [^{11}C]CO_2, which was in agreement with the 'on line' determined value. In each our [^{11}C]-tracer production, if semi-preparative HPLC was used for purification, then the SA of [^{11}C]-tracer was assessed by both semi-preparative HPLC (during synthesis) and analytical HPLC (EOS); if SPE was used for purification, then the SA of [^{11}C]-tracer was only measured by analytical HPLC at EOS.[37]

Chemical purity and radiochemical purity were determined by analytical HPLC.[48] The chemical purity of the precursor and reference standard was >90%. The radiochemical purity of the target tracer was >99% determined by radio-HPLC through γ-ray (PIN diode) flow detector, and the chemical purity of the target tracer was >90% determined by reversed-phase HPLC through UV flow detector.

ChemOffice 2015 indicated that the Log P for desmethyl-HG-10-102-01 and HG-10-102-01 is 1.43 and 1.7, respectively. We assume the retention time of HG-10-102-01 should be longer than the retention time of desmethyl-HG-10-102-01 in RP-HPLC. However, the precursor has longer retention time than reference standard. This atypical elution order is difficult to explain, but fortunate from the radiosynthetic standpoint, because the radiochemical product eluted before the precursor. We experienced the same case when we radiosynthesized [^{11}C]β-CIT from Nor-β-CIT

precursor.[29] It is important to note that the Log P for Nor-β-CIT and β-CIT is 3.31 and 3.69, respectively, from ChemOffice 2015.

The experimental details and characterization data for compounds **1-5** and for the tracer [^{11}C]**4** are given.[49]

In summary, synthetic routes with moderate to high yields have been developed to produce HG-10-102-01, desmethyl-HG-10-102-01 and [^{11}C]HG-10-102-01. The radiosynthesis employed [^{11}C]CH_3OTf for *O*-[^{11}C]methylation at the phenyl hydroxyl position of the desmethyl precursor, followed by product purification and isolation using a semi-preparative RP HPLC combined with SPE. [^{11}C]HG-10-102-01 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]HG-10-102-01 as a new potential PET agent for imaging of LRRK2 enzyme in PD.

Acknowledgments

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48. Zheng Q-H, Mock BH. *Biomed Chromatogr*.2005;19:671-676.
49. *(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.[39] Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II 500 MHz NMR Fourier transform spectrometer at 500 and 125 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) relative to an internal standard tetramethylsilane (TMS, δ 0.0) (^{1}H NMR) and to the solvent signal (^{13}C NMR), and coupling constants (J) are 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. Preparative TLC was run using Analtech silica gel UV254 plates (20×20 cm^2). 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 RP HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6×250 mm; a gradient mobile phase 40%CH_3CN/60% H_2O to 70%CH_3CN/30%H_2O within 10 min; flow rate 1.0 mL/min; UV (254 nm) and γ-ray (PIN diode) flow detectors. Semi-preparative RP HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 10×250 mm; mobile phase 43%CH_3CN/57%H_2O; flow rate 7 mL/min; UV (254 nm) and γ-ray (PIN diode) flow detectors. C18 Light 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). 2,5-Dichloro-N-methylpyrimidin-4-amine (**1**)*: To a stirred solution of 2,4,5-trichloropyrimide (3.0 g, 16.4 mmol) in anhydrous THF (45 mL) was added methylamine (2.0 M solution in THF, 25 mL, 50.0 mmol) dropwise at 0 °C. After the reaction mixture was warmed to room temperature (RT) and stirred overnight, it was diluted with water and extracted with EtOAc. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated *in vacuo* to afford **1** as a white solid (2.9 g, 98%), which was used for next step without purification. ^{1}H NMR (DMSO-d_6): δ 8.14 (s, 1H), 7.91 (br s, 1H), 2.87 (d, J = 3.0 Hz, 3H).
*(c). (3-Methoxy-4-nitrophenyl)(morpholino)methanone (**2**):* To a stirred solution of 3-methoxy-4-nitrobenzoic acid (5.0 g, 25.4 mmol) in anhydrous toluene (50 mL) was added thionyl chloride (6 mL, 82.3 mmol). The reaction mixture was heated under reflux for 2 h, and the solvent was evaporated *in vacuo*. To the residual was added anhydrous THF (50 mL), followed by DIPEA (9.1 mL, 50.7 mmol) and morpholine (3.3 mL, 38.0 mmol) at 0 °C. After the reaction mixture was warmed to RT and stirred for 1 h, it was diluted with water and extracted with EtOAc. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated *in vacuo*. The crude product was purified by silica gel column chromatography with CH_2Cl_2/MeOH (100:1 to 100:3) as eluent to afford **2** as a yellow solid (5.04 g, 75%); mp 104-105 °C. ^{1}H NMR (DMSO-d_6): δ 7.93 (d, J = 8.5 Hz, 1H), 7.37 (d, J = 8.5 Hz, 1H), 7.12 (dd, J = 1.5, 8.0 Hz, 1H), 3.95 (s, 3H), 3.67 (m, 2H), 3.63 (m, 2H), 3.55 (m, 2H), 3.30 (m, 2H).
*(d). (4-Amino-3-methoxyphenyl)(morpholino)methanone (**3**)*: A solution of compound **2** (5.0 g, 18.8 mmol) in anhydrous THF (15 mL) and MeOH (50 mL) was hydrogenated with H_2 (60 psi) over 10% Pd-C (520 mg) at RT for 5 h. The catalyst was removed by filtration, and the solvent was evaporated *in vacuo*. The crude product was purified by silica gel column chromatography with CH_2Cl_2/MeOH (100:1 to 100:8) as eluent to afford **2** as a white solid (4.09 g, 92%); mp 146-148 °C. ^{1}H NMR (DMSO-d_6): δ 6.85 (d, J = 1.5 Hz, 1H), 6.80 (dd, J = 2.0, 8.0 Hz, 1H), 6.61 (d, J = 8.0 Hz, 1H), 5.15 (s, 2H), 3.77 (s, 3H), 3.59-3.57 (m, 4H), 3.51-3.49 (m, 4H).

*(e). (4-((5-Chloro-4-(methylamino)pyrimidin-2-yl)amino)-3-methoxyphenyl)(morpholino)methanone (HG-10-102-01, **4**)*: To a stirred suspension of compound **1** (300 mg, 1.68 mmol) in 2-butanol (3 mL) was added TFA (0.3 mL), followed by the addition of compound **3** (380 mg, 1.61 mmol). The reaction mixture was heated under reflux overnight, and the solvent was evaporated *in vacuo*. The residual was diluted with CH_2Cl_2, washed with saturated aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated *in vacuo*. The crude product was purified by silica gel column chromatography with CH_2Cl_2/MeOH (100:5) as eluent to afford **4** as a white solid (350 mg, 73%); mp 167-169 °C. ^{1}H NMR (DMSO-d_6): δ 8.41 (d, J = 8.5 Hz, 1H), 7.96 (s, 1H), 7.68 (s, 1H), 7.33-7.30 (m, 1H), 7.05 (d, J = 1.5 Hz, 1H), 7.01 (dd, J = 1.5, 8.0 Hz, 1H), 3.90 (s, 3H), 3.60 (m, 4H), 3.52 (m, 4H), 2.91 (d, J = 4.5 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ 169.0, 158.1, 157.4, 152.4, 147.2, 130.5, 128.0, 119.8, 117.4, 109.8, 104.9, 66.1, 56.0, 27.7. LC-MS (ESI, *m/z*): Calcd for $C_{17}H_{21}ClN_5O_3$ ($[M+H]^+$) 378.1, found: 378.2. HRMS (ESI, *m/z*): Calcd for $C_{17}H_{21}ClN_5O_3$ ($[M+H]^+$) 378.1333, found: 378.1345.

*(f). (4-((5-Chloro-4-(methylamino)pyrimidin-2-yl)amino)-3-hydroxyphenyl)(morpholino)methanone (desmethyl-HG-10-102-01, **5**)*: To a stirred solution of compound **4** (345 mg, 0.91 mmol) in anhydrous CH_2Cl_2 (6 mL) was added BBr_3 (1.0 M solution in CH_2Cl_2, 4 mL, 4.0 mmol) dropwise at -78 °C under N_2 atmosphere. After the reaction mixture was warmed to RT and stirred overnight, it was quenched with ice-water and neutralized with aqueous 1 N $NaHCO_3$ solution. The mixture was extracted with CH_2Cl_2. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4 and filtered. The solvent was evaporated *in vacuo*. The crude product was purified by preparative TLC plates with CH_2Cl_2/MeOH (150:7) as eluent to afford **5** as an off-white solid (94 mg, 28%); mp 213-215 °C. ^{1}H NMR (DMSO-d_6): δ 10.4 (br s, 1H), 8.19 (d, J = 8.4 Hz, 1H), 7.95 (s, 1H), 7.86 (s, 1H), 7.34-7.31 (m, 1H), 6.91 (d, J = 2.0 Hz, 1H), 6.87 (dd, J = 2.0, 8.4 Hz, 1H), 3.59-3.58 (m, 4H), 3.50 (m, 4H), 2.92 (d, J = 4.4 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ 169.6, 158.6, 158.1, 152.6, 146.1, 130.4, 128.9, 119.0, 118.8, 114.6, 105.1, 66.7, 28.2. LC-MS (ESI, *m/z*): Calcd for $C_{16}H_{19}ClN_5O_3$ ($[M+H]^+$) 364.1, found: 364.1. HRMS (ESI, *m/z*): Calcd for $C_{16}H_{19}ClN_5O_3$ ($[M+H]^+$) 364.1176, found: 364.1182.

*(g). (4-((5-Chloro-4-(methylamino)pyrimidin-2-yl)amino)-3-[^{11}C]methoxyphenyl)(morpholino)methanone ([^{11}C]HG-10-102-01, [^{11}C]**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 58 μA beam current and 15 min on target. The production run produced approximately 25.9 GBq of [^{11}C]CO_2 at EOB. Desmethyl-HG-10-102-01 (**5**, 0.1-0.3 mg) was dissolved in CH_3CN (300 μL). To this solution was added aqueous NaOH (2 N, 2 μL). The mixture was transferred to a small reaction vial. No-carrier-added (high specific activity) [^{11}C]CH_3OTf that was produced by the gas-phase production method[39] within 12 min 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 °C for 3 min. The contents of the reaction vial were diluted with aqueous $NaHCO_3$ (0.1 M, 1 mL). The reaction vial was connected to a 3-mL HPLC injection loop. The labeled product mixture solution was injected onto the semi-preparative HPLC column 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 Light cartridge, and washed with water (3 × 10 mL). The cartridge was eluted with EtOH (3 × 0.4 mL) to release the labeled product, followed by saline (10-11 mL). The eluted product was then sterile-filtered through a Millex-FG 0.2 μm membrane into a sterile vial. Total radioactivity was assayed and total volume (10-11 mL) was noted for tracer dose dispensing. The overall synthesis time including HPLC-SPE purification and reformulation was ~40 min from EOB. The decay corrected radiochemical yield was 45-55%. Retention times in the analytical HPLC system were: t_R **5** = 7.87 min, t_R **4** = 6.05 min, t_R [^{11}C]**4** = 6.16 min. Retention times in the preparative HPLC system were: t_R **5** = 17.65 min, t_R **4** = 11.32 min, t_R [^{11}C]**4** = 11.58 min.