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Synthesis of carbon-11-labeled 4-(phenylamino)-pyrrolo [2,1-*f*][1,2,4]triazine derivatives as new potential PET tracers for imaging of p38α mitogen-activated protein kinase

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ABSTRACT

The reference standards methyl 4-(2-methyl-5-(methoxycarbamoyl)phenylamino)-5-methylpyrrolo [2,1-*f*][1,2,4]triazine-6-carboxylate (**10a**), methyl 4-(2-methyl-5-(ethoxycarbamoyl)phenylamino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carboxylate (**10b**) and corresponding precursors 4-(2-methyl-5-(methoxycarbamoyl)phenylamino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carboxylic acid (**11a**), methyl 4-(2-methyl-5-(ethoxycarbamoyl)phenylamino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carboxylic acid (**11b**) were synthesized from methyl crotonate and 3-amino-4-methylbenzoic acid in multiple steps with moderate to excellent yields. The target tracer [^{11}C]methyl 4-(2-methyl-5-(methoxycarbamoyl)phenylamino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carboxylate ([^{11}C]**10a**) and [^{11}C]methyl 4-(2-methyl-5-(ethoxycarbamoyl)phenylamino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carboxylate ([^{11}C]**10b**) were prepared from their corresponding precursors with [^{11}C]CH_3OTf under basic condition through O-[^{11}C]methylation and isolated by a simplified solid-phase extraction (SPE) method in 50–60% radiochemical yields at end of bombardment (EOB) with 185–555 GBq/μmol specific activity at end of synthesis (EOS).

Mitogen-activated protein kinases (MAPKs) act as an integration point of multiple biochemical signals and are involved in controlling numerous cellular processes, such as differentiation, inflammation, mitogenesis, oncogenesis, and apoptosis.[1–3] The p38 subfamily of the MAPK superfamily, a serine threonine kinase, comprises four isoforms (α, β, γ and δ).[4] The p38α MAPK plays a critical role in regulating the biosynthesis of many inflammatory cytokines, including tumor necrosis factor alpha (TNF-α) and interleukin-1β (IL-1β).[5,6] p38α MAPK is associated with a wide variety of diseases like inflammatory diseases (e.g. rheumatoid arthritis and inflammatory bowel disease), cancer, heart disease, neurodegenerative disease such as Alzheimer's disease, sepsis and asthma.[7–10] Recent studies indicated p38α MAPK has been implicated in diabetes as well.[11] p38α MAPK has emerged as an attractive target for chemotherapeutic intervention for the treatment of the diseases, and many p38α MAPK inhibitors have been discovered.[12,13] p38α MAPK has also become a promising target for molecular imaging of p38α MAPK-related diseases and image-guided therapy using the biomedical imaging technique positron emission tomography (PET). However, radionuclides including carbon-11 and fluorine-18 labeled p38α MAPK inhibitors are still not reported. To address local investigators needs for PET imaging of diabetes, in our previous work, we have redeveloped [^{11}C]acetate-PET and [^{11}C]palmitate-PET to study cardiometabolic response and free fatty acid levels in diabetes.[14–16] In this ongoing study, we first target p38α MAPK and develop radiolabeled p38α MAPK inhibitors. Here we report the design, synthesis and labeling of two carbon-11-labeled 4-(phenylamino)-pyrrolo[2,1-*f*][1,2,4]triazine derivatives as new potential PET agents for imaging of p38α MAPK in diabetes.

The efforts in developing therapeutic agents for p38α MAPK enzyme have been accompanied by a growing interest in translating therapeutic agents to diagnostic agents. Two title compounds methyl 4-(2-methyl-5-(methoxycarbamoyl)phenylamino)-5-methylpyrrolo[2,1-*f*][1,2,4]triazine-6-carboxylate (**10a**), methyl 4-(2-methyl-5-(ethoxycarbamoyl)phenylamino)-5-methylpyrrolo [2,1-*f*] [1,2,4]triazine-6-carboxylate (**10b**) were served as reference standards and selected for radiolabeling. Compounds **10a** and **10b** have been reported previously.[6] They are potent p38α MAPK inhibitors, and the IC_{50} (nM) for **10a** and **10b** are 1.1 and 4.0, respectively.[6] Reference standards **10a** and **10b** and their corresponding precursor 4-(2-methyl-5-(methoxycarbamoyl)phenylamino)-5-methylpyrrolo

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[2,1-*f*][1,2,4]triazine-6-carboxylic acid (**11a**), methyl 4-(2-methyl-5-(ethoxycarbamoyl)phenylamino)-5-methylpyrrolo[2,1-*f*][1,2,4] triazine-6-carboxylic acid (**11b**) were synthesized according to the reported procedures with modifications.[6,17–20]

As shown in Scheme 1, 4-chloro-pyrrolo[2,1-*f*][1,2,4]triazine **6** was prepared starting from commercially available β-substituted acrylate, it underwent cycloaddition with tosylmethyl isocyanide (TosMIC) in the presence of NaH in a mixture of DMSO and Et_2O to give pyrrole **1** in 78% yield.[21,22] Acylation at the C-2 position of the pyrrole **1** was achieved in one-pot with two steps. Treatment **1** with trichloroacetyl chloride in the presence of $AlCl_3$ in dichloroethane (DCE) to afford trichloroacetyl substituted pyrrole **2** in 94% yield, which was treated with sodium methoxide in MeOH to provide the tri-substituted pyrrole **3** in 91% yield. A safer and more economical N-amination of pyrrole **4** was accomplished with monochloroamine (NH_2Cl) instead of *O*-(2,4-diphenyl-phosphoryl)hydroxylamine ($DnpONH_2$),[23] in 80% yield. N-aminated pyrrole **4** with formamide underwent cyclization to afford 1,2,4-triazine **5** in 82% yield. Chlorination of **5** with phosphorous oxychloride ($POCl_3$) provided **6** in 88% yield.

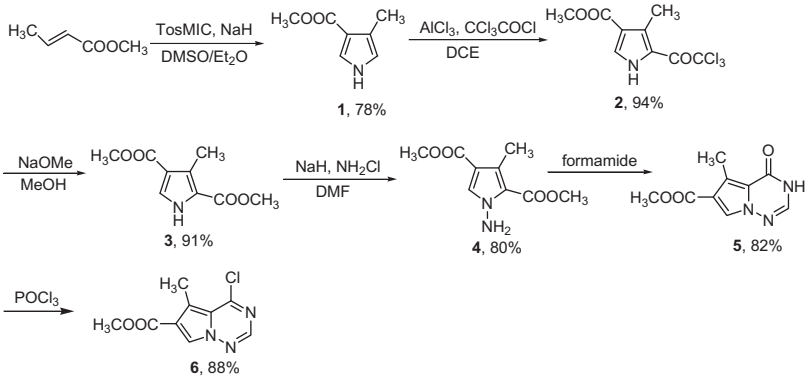
As depicted in Scheme 2, *tert*-butoxycarbonylation of 3-amino-4-methylbenzoic acid with Boc_2O in THF afforded Boc-protected aniline **7** in 88% yield. Benzoic acid **7** was activated with *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide (EDC) and catalytic 1-hydroxy benzotriazole (HOBt), followed by coupling with methoxyamine or ethoxyamine in the presence of *N*,*N*-diisopropylethylamine (DIPEA) in DMF to give amides **8a**–**b** in excellent yields (98% and 92%). The protecting Boc groups in **8a**–**b** were removed with 4 N HCl to afford **9a**–**b** in 74% and 79% yield, respectively.

As indicated in Scheme 3, **9a**–**b** were coupled with **6** in DMF to obtain standards **10a**–**b** in 43% and 48% yield, respectively. The ester groups at C-6 of **10a**–**b** were hydrolyzed with 1 N NaOH in a mixture of MeOH and THF to afford the respective acids **11a**–**b** as precursors in 69% and 80% yield, respectively.

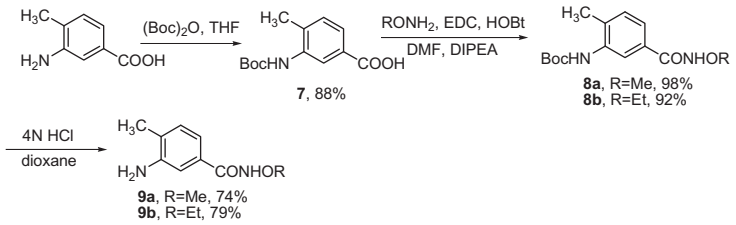
Synthesis of carbon-11-labeled 4-(phenylamino)-pyrrolo [2,1-*f*][1,2,4]triazine derivatives, [^{11}C]methyl 4-(2-methyl-5-(methoxycarbamoyl)phenylamino)-5-methylpyrrolo[2,1-*f*][1,2,4] triazine-6-carboxylate ([^{11}C]**10a**) and [^{11}C]methyl 4-(2-methyl-5-(ethoxycarbamoyl)phenylamino)-5-methylpyrrolo[2,1-*f*][1,2,4] triazine-6-carboxylate ([^{11}C]**10b**), is indicated in Scheme 4. The desmethylated precursors **11a** and **11b** were labeled by a reactive [^{11}C]methylating agent, [^{11}C]methyl triflate ([^{11}C]CH_3OTf)[24,25] prepared from [^{11}C]CO_2, under basic conditions (2 N NaOH) in acetonitrile through the O-[^{11}C]methylation and isolated by a simplified solid-phase extraction (SPE) method[26–29] to provide target tracers [^{11}C]**10a** and [^{11}C]**10b** in 50–60% radiochemical yields, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2.

[^{11}C]CH_3OTf is a proven methylation reagent with greater reactivity than commonly used [^{11}C]methyl iodide ([^{11}C]CH_3I),[30] and thus, the radiochemical yield of [^{11}C]**10a**–**b** was relatively high. The large polarity difference between the acid precursor and the corresponding labeled O-methylated ester product permitted the use of SPE technique for purification of the labeled product from the radiolabeling reaction mixture. A C-18 Plus Sep-Pak cartridge was used in SPE purification technique. The crude reaction mixture was treated with aqueous $NaHCO_3$ and loaded onto the cartridge by gas pressure. The pH of freshly prepared 0.1 M $NaHCO_3$ might be too low to effectively deprotonate an acid. However, an excess of 2 N NaOH used in the reaction provided a final pH after addition of the 0.1 M $NaHCO_3$ high enough to deprotonate all the acid. Any non-reacted acid precursor was actually converted to the corresponding sodium salt, and any non-reacted [^{11}C]CH_3OTf was actually hydrolyzed to [^{11}C]CH_3OH, which would not be trapped to the C-18 Sep-Pak. The cartridge was washed with water to remove non-reacted [^{11}C]CH_3OTf, remaining acid precursor and reaction solvent, and total 6 mL (2×3 mL) volume of water was enough to wash off all acid. The final labeled product was eluted with ethanol (2×2 mL), concentrated by rotary evaporation and reformulated in saline (10 mL). In our fully automated radiosynthesis module,[31–33] it is difficult to directly elute the labeled product from a C-18 Sep-Pak to a vial using either 1×1 mL ethanol or 2×0.5 mL ethanol, due to the back pressure in the C-18 Sep-Pak and dead volume in the transfer tubing. In order to elute most of the labeled product from the C-18 Sep-Pak, we need to increase the volume of the eluent ethanol. For the radiotracers produced for animal study, we used 2×2 mL ethanol for elution, and rotary evaporation was required before reformulation. For the radiotracer produced for human study, we used 2×1 mL ethanol, no evaporation required, and a C-18 Sep-Pak was used for direct reformulation.[34–37] We have tried to use a C-18 Light Sep-Pak cartridge instead of a C-18 Plus Sep-Pak cartridge to allow smaller volume (1 mL) of ethanol and to avoid laborious rotary evaporation before formulation. However, there is more serious back pressure in the Light Sep-Pak than in the Plus Sep-Pak, in addition, dead volume in the transfer tubing also affects the elution, and thus it is more difficult to efficiently elute the labeled product from a Light Sep-Pak, which resulted in the low radiochemical yield. Overall synthesis time was 23 min from EOB, including approximately 11 min for [^{11}C]CH_3OTf production, 5 min for O-[^{11}C]methylation reaction, and 7 min for SPE purification, evaporation and reformulation. SPE technique is fast, efficient and convenient and works very well for the O-methylated ester tracer purification using the acid precursor for radiolabeling.[26,28]

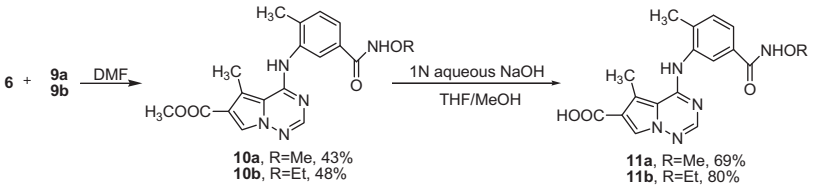
The radiosynthesis was performed in an automated self-designed multi-purpose ^{11}C-radiosynthesis module, allowing measurement of specific activity at EOB during synthesis.[31–33] On line determination of specific activity at EOB is accurate when reverse-phase (RP) high performance liquid chromatography



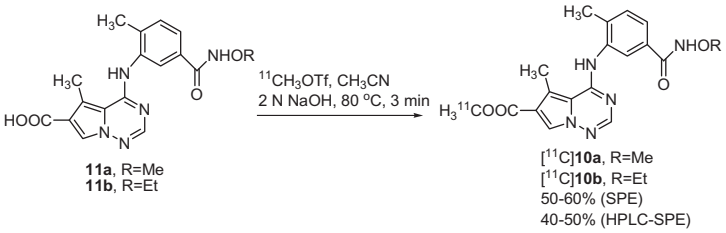
Scheme 1. Synthesis of an intermediate **6**.



Scheme 2. Synthesis of intermediates **9a**–**b**.



Scheme 3. Synthesis of reference standards **10a**–**b** and corresponding precursors **11a**–**b**.



Scheme 4. Synthesis of target tracers [^{11}C]**10a**–**b**.

(HPLC) is used as purification method. However, the on-the-fly technique to determine specific activity at EOB is not applied when SPE is used as purification method. The specific activity for the ^{11}C-tracers produced in our PET chemistry facility usually ranges from 370 to 1110 GBq/μmol at EOB according to our previous works. The specific activity of carbon-11-labeled 4-(phenylamino)-pyrrolo[2,1-*f*][1,2,4]triazine derivatives was estimated in a range of 185–555 GBq/μmol at the end of synthesis (EOS) based on other compounds produced in our facility using the same targetry conditions which have been measured by the on-the-fly technique or the same SPE purification method.[38] The actual measurement of specific activity at EOS was performed by analytical HPLC[39,40] and calculated. The exact values of the specific activity for the tracers [^{11}C]**10a**–**b** were 185–555 GBq/μmol at EOS, which are in agreement with the estimated values and the "on line" determined values. To prove the consistency of specific activity determination between by the on-the-fly technique (semi-preparative HPLC) and by analytical HPLC technique, we also employed HPLC combined with SPE method[34–37] in the radiosynthesis of [^{11}C]**10a**–**b** for comparison, the specific activity determined on line was in a range of 370–1110 GBq/μmol at EOB. Specific activity is defined as the radioactivity per unit mass of a radionuclide or a labeled compound. Specific activity (MBq/mg) = $3.13 \times 10^9/A \times t_{1/2}$, where A is the mass number of the radionuclide, and $t_{1/2}$ is the half-life in hours of the radionuclide. For carbon-11, carrier-free ^{11}C, maximum (theoretical) ^{11}C specific activity = 340,918 GBq/μmol.[41] Actual specific activity of the ^{11}C-tracers in the PET chemistry facility are depended on two parts: (1) carrier from the ^{11}C-target, and (2) carrier from the ^{11}C-radiosynthesis unit.[42] Furthermore, actual specific activity for the ^{11}C-tracers synthesized by ^{11}C-methylation with [^{11}C]CH_3OTf in our PET chemistry facility is depended on two parts: (1) carrier from the cyclotron consisted of the ^{11}C gas irradiation target system, and (2) carrier from the [^{11}C]CH_3OTf system, ^{11}C radiolabeled precursor or called ^{11}C radiolabeled methylating reagent. If we can eliminate ^{12}C carrier-added as much as possible, then we will be able to achieve the highest specific activity. The ^{11}C gas target we used is the Siemens RDS-111 Eclipse cyclotron ^{11}C gas target. The technical trick to produce high specific activity [^{11}C]CO_2 is we will usually do 2–3 times pre-burn with the same beam current and short time like 10 min before production run. This pre-burn will warm up the cyclotron and eliminate significant amount of ^{12}C carrier-added in the cyclotron ^{11}C gas target. The [^{11}C]CH_3OTf production system we used is an Eckert & Ziegler Modular Lab C-11 Methyl Iodide/Triflate module, convenient gas phase bromination of [^{11}C]methane and production of [^{11}C]CH_3OTf, a 'dry' method using Br_2 different with other 'dry' method using I_2 and 'wet' method using $LiAlH_4$ and HI. Our system will have much less ^{12}C carrier-added in comparison with other 'dry' method and 'wet' method.[25] HPLC–SPE method took longer overall synthesis time, and thus radiochemical yields were a little low, 40–50%. On the other hand, HPLC–SPE method gave higher chemical purity of the tracers due to no rotary evaporation.[34–37] Chemical purity and radiochemical purity were determined by analytical HPLC.[40] The chemical purity of the precursors and reference standards was >95%. The radiochemical purity of the target tracers was >98% 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 quality control (QC) of final product was performed to guarantee the final doses are suitable for animal and human injection. Both HPLC–SPE and SPE methods can produce enough activity, although the radiochemical yield was lower for HPLC–SPE method than for SPE method. Both methods had similar radiochemical purity, specific activity and residual solvent analysis results analyzed

by gas chromatography (GC) equipped with a capillary column and flame ionization detector (FID). The only difference is that HPLC–SPE method gave higher chemical purity of the product than SPE method. In our PET chemistry facility, we employ the same QC release criteria of final doses for both human use and animal use.

The experimental details and characterization data for compounds **1**–**11a**–**b** and for the tracer [^{11}C]**10a**–**b** are given.[43]

In summary, a simple and high-yield synthetic route to acid-precursors **11a**–**b**, ester-standards **10a**–**b** and carbon-11-labeled 4-(phenylamino)-pyrrolo[2,1-*f*][1,2,4]triazine derivatives [^{11}C]**10a**–**b** has been developed. An automated self-designed multi-purpose [^{11}C]-radiosynthesis module for the synthesis of [^{11}C]**10a**–**b** has been built. The target tracers were easily prepared by O-[^{11}C]methylation of their corresponding precursors using a reactive [^{11}C]methylating agent, [^{11}C]CH_3OTf, and isolated by a simplified SPE purification procedure in high radiochemical yields, short overall synthesis time, and high specific radioactivities. These methods are efficient and convenient. It is anticipated that the approaches for the design, synthesis and automation of new tracer, authentic standard and radiolabeling precursor, and improvements to increase radiochemical yield and chemical purity of the tracer described here can be applied with advantages to the synthesis of other ^{11}C-radiotracers for PET imaging. These chemistry results combined with the reported in vitro biological data[6] encourage further in vivo biological evaluation of carbon-11-labeled 4-(phenylamino)-pyrrolo[2,1-*f*][1,2,4]triazine derivatives as new candidate PET agents for imaging of p38α MAPK in diabetes and other heart, cancer and brain diseases.

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43. (a) *General:* All commercial reagents and solvents were purchased from Sigma–Aldrich and Fisher Scientific, and used without further purification. ^{1}H NMR spectra were recorded 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. 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 UV 254 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; mobile phase 30% CH_3CN/70% H_2O/0.1% TFA; flow rate 1.5 mL/min; and UV (220 nm) and γ-ray (PIN diode) flow detectors. Semi-preparative RP HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 12 nm, 10×250 mm; mobile phase 30% CH_3CN/70% H_2O/0.1% TFA; 7.0 mL/min flow rate; UV (220 nm) and γ-ray (PIN diode) flow detectors. C18 Plus Sep-Pak cartridges were obtained from Waters Corporation (Milford, MA). Sterile Millex-GS 0.22 μm and Millex-FG 0.2 μm filter units were obtained from Millipore Corporation (Bedford, MA).
(b) *Methyl 4-methyl-1H-pyrrole-3-carboxylate* (***1***): To a stirred suspension of NaH (60% dispersion in mineral oil, 13.2 g, 330 mmol) in anhydrous Et_2O (200 mL) was added a solution of methyl crotonate (15.0 g, 150 mmol) and tosylmethyl isocyanide (32.1 g, 164 mmol) in anhydrous mixture of DMSO (180 mL) and Et_2O (360 mL) dropwise under nitrogen atmosphere. After stirring at room temperature (rt) for 1 h, the resulting mixture was poured into ice-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, and the crude product was purified by column chromatography (100:1 CH_2Cl_2/MeOH) to afford **1** (16.2 g, 78%) as a yellow

solid. ^{1}H NMR ($CDCl_3$): δ 8.43 (br s, 1H), 7.38–7.37 (m, 1H), 6.54–6.53 (m, 1H), 3.80 (s, 3H), 2.29 (d, J = 1.0 Hz, 2H).

(c) *Methyl 4-methyl-5-(2,2,2-trichloroacetyl)-1H-pyrrole-3-carboxylate (**2**)*: To a suspension of aluminum chloride (51.16 g, 384 mmol) in DCE (200 mL) was added trichloroacetyl chloride (43 mL, 384 mmol) dropwise at −40 °C under nitrogen atmosphere, followed by a solution of compound **1** (10.6 g, 76.7 mmol) in DCE (50 mL). The reaction mixture was gradually warmed to rt and stirred for 2 days. The mixture was poured into ice-water carefully and extracted with CH_2Cl_2. The combined organic layer was washed with 3 N HCl, brine, dried over anhydrous Na_2SO_4, and filtered. The solvent was evaporated in vacuo to afford **2** (20.3 g, 94%) as a dark oil which was used for next step without further purification.

(d) *Dimethyl 3-methyl-1H-pyrrole-2,4-dicarboxylate (**3**)*: To a cooled and stirred solution of compound **2** (20.0 g, 70.7 mmol) in MeOH (80 mL) was added a solution of sodium methoxide in MeOH (25% wt, 70 mL, 306 mmol) at 0 °C under nitrogen atmosphere. After finishing the addition, the reaction mixture was allowed to warm to rt and stirred for 1 h. The mixture was concentrated in vacuo, diluted with ice-water and then adjusted pH to 6 with 2 N HCl. 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, and the crude product was purified by column chromatography (7:3 hexanes/EtOAc) to afford **3** (12.6 g, 91%) as a pale yellow solid. ^{1}H NMR ($CDCl_3$): δ 9.33 (br s, 1H), 7.48 (d, J = 3.5 Hz, 1H), 3.88 (s, 3H), 3.82 (s, 3H), 2.60 (s, 3H).

(e) *Preparation of anhydrous ethereal monochloroamine (NH_2Cl)*: To a cooled and stirred suspension of NH_4Cl (12.0 g, 224 mmol) in Et_2O (440 mL) was added concentrated ammonium hydroxide (18.8 mL) at −5 °C, followed by commercial bleach (Clorox, 288 mL) slowly. After stirring at same temperature for 15 min, the organic layer was separated and washed with brine. The organic layer was dried over powered $CaCl_2$ in a freezer for 1 h and stored at −40 °C.

(f) *Dimethyl 1-amino-3-methyl-1H-pyrrole-2,4-dicarboxylate (**4**)*: To a stirred solution of compound **3** (8.73 g, 44.3 mmol) in DMF (60 mL) was added NaH (60% dispersion in mineral oil, 2.30 g, 57.5 mmol). After stirring for 1 h, anhydrous ethereal monochloroamine (340 mL) was added under nitrogen atmosphere. The reaction mixture was stirred for 10 min and then quenched with aqueous $Na_2S_2O_3$ (100 g/L, 200 mL). The mixture was diluted with water. The organic layer was separated and washed with brine, dried over anhydrous Na_2SO_4, and filtered. The solvent was evaporated in vacuo, and the crude product was purified by column chromatography (3.5:1 hexanes/EtOAc) to afford **4** (7.55 g, 80%) as a white solid. ^{1}H NMR ($CDCl_3$): δ 7.48 (s, 1H), 5.22 (br s, 2H), 3.88 (s, 3H), 3.79 (s, 3H), 2.56 (s, 3H).

(g) *Methyl 4-oxo-5-methyl-1,4-dihydropyrrolo[2,1-f][1,2,4]triazine-6-carboxylate (**5**)*: A suspension of compound **4** (6.0 g, 28.3 mmol) in formamide (60 mL) was heated and stirred at 160 °C overnight. The resulting mixture was cooled. The solid was collected by filtration and washed with water, cold Et_2O to afford **5** (4.78, 82%) as a white solid. ^{1}H NMR (DMSO-d_6): δ 11.64 (s, 1H), 7.93 (s, 1H), 7.84 (s, 1H), 3.76 (s, 3H), 2.61 (s, 3H).

(h) *Methyl 4-chloro-5-methylpyrrolo[2,1-f][1,2,4]triazine-6-carboxylate (**6**)*: A suspension of compound **5** (4.1 g, 19.8 mmol) in $POCl_3$ (45 mL) was heated and stirred at 115 °C overnight. The resulting mixture was cooled, then diluted with CH_2Cl_2 and poured into ice cold mixture of saturated $NaHCO_3$ (150 mL) and CH_2Cl_2 (50 mL) with rapidly stirring to ensure quenching of the excess $POCl_3$. The organic layer was separated and washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to afford **6** (3.91 g, 88%) as a tan solid which was used without further purification.

(i) *3-(tert-Butoxycarbonylamino)-4-methylbenzoic acid (**7**)*: A suspension of 3-amino-4-methylbenzoic acid (20.0 g, 132 mmol) and *N*-(*tert*-butoxycarbonyl)anhydride (30.0 g, 219 mmol) in THF (200 mL) was heated and stirred at 50 °C overnight. The resulting mixture was cooled to rt. The solvent was evaporated in vacuo, and the crude product was recrystallized from EtOAc to afford **7** (29.1 g, 88%) as a pink solid. ^{1}H NMR (DMSO-d_6): δ 12.8 (s, 1H), 8.66 (s, 1H), 7.97 (s, 1H), 7.59 (dd, J = 2.0, 8.0 Hz, 1H), 7.28 (d, J = 8.0 Hz, 1H), 3.25 (s, 3H), 1.47 (s, 9H).

(j) *tert-Butyl 2-methyl-5-(methylcarbamoyl)phenylcarbamate (**8a**)*: To a stirred solution of compound **7** (5.0 g, 19.9 mmol) in DMF (30 mL) was added EDC (3.9 mL, 21.9 mmol), HOBt (3.35 g, 21.9 mmol). After stirring at rt for 30 min, methoxyamine hydrochloride (1.83 g, 21.9 mmol) was added. The resulting mixture was stirred for another 10 min, and cooled to 0 °C. DIPEA (8.09 mL, 46.4 mmol) was added slowly to maintain the internal reaction temperature below 25 °C. After finishing the addition, the reaction mixture was allowed to warm to rt and stirred at rt overnight. The resulting mixture was poured into water and extracted with EtOAc. The combined organic layer was washed with cold 0.5 N HCl and water. The organic layer was then extracted with cold 0.5 N NaOH, and the combined basic aqueous extract was adjusted pH to 8 by a slow addition of cold 0.5 N HCl. The resulting precipitate was collected by filtration, and washed with cold water. The crude product was recrystallized in EtOH to afford **8a** (5.17 g, 98%) as a white solid. ^{1}H NMR (DMSO-d_6): δ 11.65 (s, 1H), 8.65 (s, 1H), 7.75 (s, 1H), 7.40 (dd, J = 1.5, 8.0 Hz, 1H), 7.25 (d, J = 8.0 Hz, 1H), 3.69 (s, 3H), 2.23 (s, 3H), 1.47 (s, 9H).

(k) *tert-Butyl 5-(ethylcarbamoyl)-2-methylphenylcarbamate (**8b**)*: Compound **8b** was prepared according to the procedure described in **8a** from *O*-ethylhydroxylamine hydrochloride as a white solid (5.07 g, 92%). ^{1}H NMR (DMSO-d_6): δ 11.53(s, 1H), 8.65 (s, 1H), 7.75 (d, J = 1.0 Hz, 1H), 7.40 (dd, J = 1.5, 8.0 Hz, 1H), 7.24 (d, J = 8.0 Hz, 1H), 3.91 (q, J = 7.0 Hz, 2H), 2.22 (s, 3H), 1.47 (s, 9H), 1.19 (t, J = 7.0 Hz, 3H).

(l) *3-Amino-N-methoxy-4-methylbenzamide hydrochloride (**9a**)*: To a stirred suspension of compound **8a** (5.0 g, 18.9 mmol) in dioxane (25 mL) was added a 4 N solution of HCl in dioxane (30 mL). The reaction mixture was stirred overnight at rt. The solid was collected by filtration and wash with dioxane to afford **9b** (3.02 g, 74%) as a white solid. ^{1}H NMR (DMSO-d_6): δ 11.83 (s, 1H), 7.71 (s, 1H), 7.53 (d, J = 8.0 Hz, 1H), 7.36 (d, J = 8.0 Hz, 1H), 3.70 (s, 3H), 2.36 (s, 3H).

(m) *3-Amino-N-ethoxy-4-methylbenzamide hydrochloride (**9b**)*: Compound **9b** was prepared according to the procedure described in **9a** from compound **8b** as a white solid (2.96 g, 79%). ^{1}H NMR (DMSO-d_6): δ 11.73 (s, 1H), 7.75 (d, J = 1.0 Hz, 1H), 7.58 (d, J = 1.0, 8.0 Hz, 1H), 7.37 (d, J = 8.0 Hz, 1H), 3.92 (q, J = 7.0 Hz, 2H), 2.38 (s, 3H), 1.20 (t, J = 7.0 Hz, 3H).

(n) *Methyl 4-(2-methyl-5-(methoxycarbamoyl)phenylamino)-5-methylpyrrolo[2,1-f][1,2,4]triazine-6-carboxylate (**10a**)*: To a stirred suspension of compound **6** (300 mg, 1.33 mmol) in DMF (5 mL) was added compound **9a** (347 mg, 1.60 mmol). After the reaction mixture was stirred overnight at rt, it was poured into ice-water and neutralized with saturated $NaHCO_3$. The precipitate was collected by filtration and washed with water. The crude product was purified by semi-preparative HPLC to afford **10a** (212 mg, 43%) as a white solid. ^{1}H NMR (DMSO-d_6, rotameric): δ 11.78 (s, 1H), 10.22, 8.86 (s+s, 1H), 8.09–7.20 (m, 5H), 3.80 (s, 3H), 3.70 (s, 3H), 2.83 (s, 3H), 2.23 (s, 3H). LC–MS (ESI, m/z): Calcd for $C_{18}H_{19}N_5O_4$ ([M+H]$^+$) 370.1, found: 370.0.

(o) *Methyl 4-(2-methyl-5-(ethoxycarbamoyl)phenylamino)-5-methylpyrrolo[2,1-f][1,2,4]triazine-6-carboxylate (**10b**)*: Compound **10b** was prepared according to the procedure described in **10a** from methyl compound **6** (300 mg, 1.33 mmol) and compound **9b** (369 mg, 1.60 mmol) as a white solid (243 mg, 48%). ^{1}H NMR (DMSO-d_6, rotameric): δ 11.59 (s, 1H), 10.18, 8.86 (s+s, 1H), 8.02-7.37 (m, 5H), 3.92 (q, J = 7.0 Hz, 2H), 3.41 (s, 3H), 2.80 (s, 3H), 2.20 (s, 3H), 1.20 (t, J = 7.0 Hz, 3H).

(p) *4-(2-Methyl-5-(methoxycarbamoyl)phenylamino)-5-methylpyrrolo[2,1-f][1,2,4]triazine-6-carboxylic acid (**11a**)*: To a stirred suspension of compound **10a** (150 mg, 0.41 mmol) in THF (2 mL) and MeOH (2 mL) was added 1 N NaOH (1.5 mL, 1.5 mmol). The reaction mixture was heated and stirred at 60 °C overnight. The solvent was evaporated in vacuo, the residual was diluted with ice-water and 1 N HCl was added to adjust pH to 5–6. The precipitate was collected by filtration and washed with water, Et_2O/EtOAc (1:1), and dried in vacuo to afford **11a** (100 mg, 69%) as a white solid. ^{1}H NMR (DMSO-d_6, rotameric): δ 12.42 (br s, 1H), 11.71 (s, 1H), 10.12, 8.77 (s+s, 1H), 8.03-7.19 (m, 5H), 3.69 (s, 3H), 2.82 (s, 3H), 2.23 (s, 3H). LC–MS (ESI, m/z): Calcd for $C_{17}H_{17}N_5O_4$ ([M+H]$^+$) 356.1, found: 356.1.

(q) *4-(2-Methyl-5-(ethoxycarbamoyl)phenylamino)-5-methylpyrrolo[2,1-f][1,2,4]triazine-6-carboxylic acid (**11b**)*: Compound **11b** was prepared according to the procedure described in **11a** from compound **10b** (150 mg, 0.39 mmol) as a white solid (115 mg, 80%). ^{1}H NMR (DMSO-d_6, rotameric): δ 12.43 (br s, 1H), 11.60 (s, 1H), 10.11, 8.78 (s+s, 1H), 8.03-7.19 (m, 5H), 3.90 (q, J = 6.5 Hz, 2H), 2.83 (s, 3H), 2.23 (s, 3H), 1.19 (t, J = 6.5 Hz, 3H). LC–MS (ESI, m/z): Calcd for $C_{18}H_{19}N_5O_4$ ([M+H]$^+$) 370.1, found: 370.1.

(r) *General procedure for the preparation of the target tracers [^{11}C]**10a–b***: [^{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 50 μA beam current and 15 min on target. The production run produced approximately 25.9 GBq of [^{11}C]CO_2 at EOB. The acid precursor **11a** or **11b** (0.1–0.3 mg) was dissolved in CH_3CN (300 μL). To this solution was added 2 N NaOH (2 μL). The mixture was transferred to a small reaction vial. No-carrier-added (high specific activity) [^{11}C]CH_3OTf (13.9 GBq) that was produced by the gas-phase production method[25] within 11 min from [^{11}C]CO_2 (25.9 GBq) through [^{11}C]CH_4 (21.8 GBq) and [^{11}C]CH_3Br (13.9 GBq) 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 $NaHCO_3$ (0.1 M, 1 mL). *Method **A**:* The reaction vial was connected to a C-18 Plus Sep-Pak cartridge. The labeled product mixture solution was passed onto the cartridge for SPE purification by gas pressure. The cartridge was washed with H_2O (2 × 3 mL), and the aqueous washing was discarded. The product was eluted from the cartridge with EtOH (2 × 2 mL), and then passed onto a rotatory evaporator. The solvent was removed by evaporation (3 min) under vacuum. The final volume of ethanol after evaporation was ~1 mL. The labeled product was reformulated with saline (10 mL), sterile-filtered through a sterile vented Millex-GS 0.22 μm cellulose acetate membrane and collected into a sterile vial. Total radioactivity (4.7–7.1 GBq) was assayed and the total volume (10–11 mL) was noted for tracer dose dispensing. The overall synthesis time including SPE purification and reformulation was 23 min. The radiochemical yields decay corrected to EOB, from [^{11}C]CO_2, were 50–60%. *Method **B**:* 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 Plus cartridge, and washed with water (5 mL × 4). The cartridge was eluted with EtOH (1 mL × 2) to release the labeled product, followed by saline (10 mL). The eluted product was then sterile-filtered through a Millex-FG 0.2 μm membrane into a sterile vial. Total radioactivity (3.8–5.9 GBq) 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 40–45 min. The same procedure was used to prepare the target tracers

[^{11}C]**10a** and [^{11}C]**10b** from their corresponding precursors **11a** and **11b**. Retention times in the analytical HPLC system were: t_R **11a** = 3.43 min, t_R **10a** = 5.68 min, t_R [^{11}C]**10a** = 5.72 min; and t_R **11b** = 3.60 min, t_R **10b** = 6.64 min, t_R [^{11}C]**10b** = 6.77 min. Retention times in the preparative HPLC system were: t_R **11a** = 3.55 min, t_R **10a** = 9.86 min, t_R [^{11}C]**10a** = 9.93 min; and t_R **11b** = 3.70 min, t_R **10b** = 11.37 min, t_R [^{11}C]**10b** = 11.48 min.