Synthesis of carbon-11-labeled imidazopyridine- and purine-thioacetamide derivatives as new potential PET tracers for imaging of nucleotide pyrophosphatase/phosphodiesterase 1 (NPP1)

Mingzhang Gao, Min Wang, Qi-Huang Zheng*

Department of Radiology and Imaging Sciences, Indiana University School of Medicine, 1345 West 16th Street, Room 202, Indianapolis, IN 46202, USA

*Corresponding author. Tel.: +1 317-278-4671. Fax: +1 317-278-9711. E-mail address: qzheng@iupui.edu.

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Abstract—The target tracer carbon-11-labeled imidazopyridine- and purine-thioacetamide derivatives, *N*-(3-[¹¹C]methoxy-4-methoxyphenyl)-2-((5-methoxy-3*H*-imidazo[4,5-*b*]pyridin-2-yl)thio)acetamide (3-[¹¹C]**4a**) and *N*-(4-[¹¹C]methoxy-3-methoxyphenyl)-2-((5-methoxy-3*H*-imidazo[4,5-*b*]pyridin-2-yl)thio)acetamide (4-[¹¹C]**4a**); 2-((6-amino-9*H*-purin-8-yl)thio)-*N*-(3-[¹¹C]methoxy-4-methoxyphenyl)acetamide (3-[¹¹C]**8a**) and 2-((6-amino-9*H*-purin-8-yl)thio)-*N*-(4-[¹¹C]methoxy-3-methoxyphenyl)acetamide (4-[¹¹C]**8a**), were prepared by *O*-[¹¹C]methylation of their corresponding precursors with [¹¹C]CH₃OTf under basic condition (2 N NaOH) and isolated by a simplified solid-phase extraction (SPE) method in 50-60% radiochemical yields based on [¹¹C]CO₂ and decay corrected to end of bombardment (EOB). The overall synthesis time from EOB was 23 min, the radiochemical purity was >99%, and the specific activity at end of synthesis (EOS) was 185-555 GBq/μmol.

Keywords: Carbon-11-labeled imidazopyridine- and purine-thioacetamide derivatives; Nucleotide pyrophosphatase/phosphodiesterase 1 (NPP1); Positron emission tomography (PET); Type 2 diabetes; Brain cancers.

The enzyme family of human nucleotide pyrophosphatases/phosphodiesterases (NPPs), existing both as membrane proteins with an extracellular active site and as soluble proteins in body fluids, is a class of dimeric enzymes that catalyze the hydrolysis of phosphate diester bonds, and it consists of seven subtypes (NPP1-7).¹ NPP1 is a membrane glycoprotein involved in the hydrolysis of extracellular nucleotides, and NPP1 is a vital regulator that influences a wide range of tissues through various signaling pathways and when disrupted can lead to significant pathology.² Overexpression of NPP1 is associated with a wide variety of diseases like type 2 diabetes, calcium pyrophosphate dehydrate deposition disease leading to inflammatory arthritis and brain cancers, and thus NPP1 has been proposed as a novel therapeutic protein target, and many NPP1 inhibitors have been developed for this

purpose.¹⁻⁵ Recently a new class of imidazopyridine- and purine-thioacetamide derivatives has been developed as potent and selective NPP1 inhibitors.¹ NPP1 has also become a promising target for molecular imaging of NPP1-related diseases and image-guided therapy using positron emission tomography (PET) modality. However, radionuclides including carbon-11 and fluorine-18 labeled NPP1 inhibitors are still not reported. To meet investigator needs within our institution for PET imaging of diabetes, we are interested in tracer development and production. In our previous work, we have redeveloped [¹¹C]acetate-PET and [¹¹C]palmitate-PET to study cardiometabolic response and free fatty acid levels; and carbon-11-labeled 4-(phenylamino)-pyrrolo[2,1-*f*][1,2,4]triazine derivatives for imaging of p38α mitogen-activated protein kinase (MAPK) in diabetes, as indicated in

Figure 1.⁶⁻⁹ In this ongoing study, we first target NPP1 and develop radiolabeled NPP1 inhibitors. Here we report the design, synthesis and labeling of four carbon-11-labeled imidazopyridine- and purine-thioacetamide derivatives, *N*-(3-[¹¹C]methoxy-4-methoxyphenyl)-2-((5-methoxy-3*H*-imidazo[4,5-*b*]pyridin-2-yl)thio)acetamide (3-[¹¹C]**4a**) and *N*-(4-[¹¹C]methoxy-3-methoxyphenyl)-2-((5-methoxy-3*H*-imidazo[4,5-*b*]pyridin-2-yl)thio)acetamide (4-[¹¹C]**4a**); 2-((6-amino-9*H*-purin-8-yl)thio)-*N*-(3-[¹¹C]methoxy-4-methoxyphenyl)acetamide (3-[¹¹C]**8a**) and 2-((6-amino-9*H*-purin-8-yl)thio)-*N*-(4-[¹¹C]methoxy-3-methoxyphenyl)acetamide (4-[¹¹C]**8a**) as new potential PET agents for imaging of NPP1 in diabetes.

Carbon-11-labeled 4-(phenylamino)-pyrrolo[2,1-*f*][1,2,3]triazine derivatives, R=Me; Et

Figure 1. PET tracers for diabetes.

The efforts in developing therapeutic agents for NPP1 enzyme have been accompanied by a growing interest in translating therapeutic agents to diagnostic agents. Two title compounds imidazopyridine-thioacetamide derivative *N*-(3,4-dimethoxyphenyl)-2-((5-methoxy-3*H*-imidazo[4,5-*b*]pyridin-2-yl)thio)acetamide (**4a**), and purine-thioacetamide derivative 2-((6-amino-9*H*-purin-8-yl)thio)-*N*-(3,4-dimethoxyphenyl)acetamide (**8a**) were served as reference standards and selected for radiolabeling. Compounds **4a** and **8a** have been reported previously.¹ They are potent NPP1 inhibitors with high inhibitory potency, and the *K_i* values for **4a** and **8a** are 29.6 nM and 5.00 nM, respectively.¹ Reference standards **4a** and **8a** and their corresponding precursors *N*-(3-hydroxy-4-methoxyphenyl)-2-((5-methoxy-3*H*-imidazo[4,5-*b*]pyridin-2-yl)thio)acetamide (**4b**) and *N*-(4-hydroxy-3-methoxyphenyl)-2-((5-methoxy-3*H*-imidazo[4,5-*b*]pyridin-2-yl)thio)acetamide (**4c**), 2-((6-amino-9*H*-purin-8-yl)thio)-*N*-(3-hydroxy-4-methoxyphenyl)acetamide (**8b**) and 2-((6-amino-9*H*-purin-8-yl)thio)-*N*-(4-hydroxy-3-methoxyphenyl)acetamide (**8c**) were synthesized according to the reported procedures¹ with modifications.

The imidazopyridine-thioacetamide derivative standard and precursors **4a-c** were synthesized as showed in

Scheme 1. The appropriate amino-phenol or dimethoxyaniline reacted with 2-chloroacetyl chloride to obtain compounds **1a-c** in 60-80% yields. Reduction of 6-methoxy-3-nitropyridin-2-amine was achieved catalytically (using Pd/C as catalyst under hydrogen gas) to give the compound 6-methoxypyridine-2,3-diamine (**2**). This diamino analogue is instable, compound **2** was immediately used in the next step reaction without further purification. Subsequent imidazole ring compound **3** was formed from compound **2** by treating with CS₂ and KOH in 63% yield. Then substituent alkylation of compound **3** with alkyl chloride **1** using NaOH as base and EtOH as solvent led to the target compounds **4a-c** in 50-65% yields.

Scheme 1. Synthesis of imidazopyridine-thioacetamide derivative standard and precursors **4a-c**. Reagents, conditions and yields: (i) 2-chloroacetyl chloride, Et₃N, CH₂Cl₂, RT; 60-80%. (ii) H₂, Pd/C, MeOH, RT. (iii) CS₂, KOH, EtOH, H₂O, reflux; 63%. (iv) NaOH, EtOH, H₂O, RT; 50-65%.

Scheme 2. Synthesis of purine-thioacetamide derivative standard and precursors **8a-c**. Reagent, conditions and yield: (i) NaNO₂, HCl, 0 °C; 78%. (ii) Na₂S₂O₄, H₂O, H₂SO₄; NaOH; 41%. (iii) CS₂, KOH, EtOH, H₂O, reflux; 66%. (iv) NaOH, EtOH, H₂O, 60 °C; 45-60%.

The purine-thioacetamide derivative standard and precursors **8a-c** were synthesized as depicted in Scheme 2. Commercially available 4,6-pyrimidinediamine was first converted to 5-nitrosopyrimidine-4,6-diamine (**5**) by NaNO₂ and HCl in 78% yield. Reduction of

compound **5** by sodium dithionite ($\text{Na}_2\text{S}_2\text{O}_4$) provided pyrimidine-4,5,6-triamine (**6**) in 41% yield. The purine ring formation of compound **6** by treating with CS_2 and KOH in mixed solvents EtOH and H_2O yielded compound **7** in 66% yield. Substituent alkylation of the thio moiety of compound **7** with alkyl chloride **1** using NaOH as base at 60°C gave target compound **8a-c** in 45-60% yields.

Synthesis of carbon-11-labeled imidazopyridine- and purine-thioacetamide derivatives 3- ^{11}C **4a** and 4- ^{11}C **4a**, 3- ^{11}C **8a** and 4- ^{11}C **8a** is indicated in Scheme 3. The desmethylated precursors **4b** and **4c**, **8b** and **8c** were labeled by a reactive ^{11}C methylating agent, ^{11}C methyl triflate (^{11}C CH_3OTf)^{10,11} prepared from ^{11}C CO_2 , through the *O*- ^{11}C methylation. The labeling reaction was under basic conditions (2 N NaOH) in acetonitrile, and the labeling mixture was isolated by a simplified solid-phase extraction (SPE) method¹²⁻¹⁵ to provide target tracers 3- ^{11}C **4a** and 4- ^{11}C **4a**, 3- ^{11}C **8a** and 4- ^{11}C **8a**. The radiochemical yields were 50-60%, decay corrected to end of bombardment (EOB), based on ^{11}C CO_2 .

Scheme 3. Synthesis of target tracers 3- ^{11}C **4a** and 4- ^{11}C **4a**, 3- ^{11}C **8a** and 4- ^{11}C **8a**. Reagent, conditions and yield: (i) ^{11}C CH_3OTf , CH_3CN , 2 N NaOH , 80°C , 3 min; SPE; 50-60%.

^{11}C CH_3OTf is more reactive than commonly used ^{11}C methyl iodide (^{11}C CH_3I),¹⁶ and thus, the radiochemical yield of 3- ^{11}C **4a** and 4- ^{11}C **4a**, 3- ^{11}C **8a** and 4- ^{11}C **8a** was relatively high. The large polarity difference between the sodium salt of the phenolic hydroxyl precursor and the corresponding labeled *O*-methylated ether 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 quenching reagent 0.1 M NaHCO_3

combined with an excess of base 2 N NaOH used in the reaction effectively deprotonated a phenolic hydroxyl precursor. Any non-reacted 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 radiolabeled by-product ^{11}C CH_3OH , sodium salt of phenolic hydroxyl precursor and reaction solvent, and total 6 mL (2×3 mL) volume of water was enough to wash off all impurities. 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,¹⁷⁻¹⁹ it is difficult to directly elute the labeled product from a C-18 Sep-Pak to a vial using ethanol (1×1 mL or 2×0.5 mL), due to the back pressure in the C-18 Sep-Pak and dead volume in the transfer tubing. The high back pressure is resulted from the eluent (water, ethanol and saline) change. 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 radiotracer produced for animal study, we used ethanol (2×2 mL) for elution, and rotary evaporation was required before reformulation. For the radiotracer produced for human study, we used ethanol (2×1 mL), no evaporation required, and a C-18 Sep-Pak was used for direct reformulation.²⁰⁻²³ 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. The reason is that our home-built fully automated module used many PTFE (polytetrafluoroethylene)/Silicone liners (septa) and Teflon tubing, and these materials cannot afford too high pressure gas (N_2) push. The pressure of N_2 gas introduced in our module is set at 207 kPa (30 Psi). C-18 Light Sep-Pak cartridge works well in manual or semi-automated radiosynthesis in our lab, because we can easily introduce high pressure gas push during the purification and reformulation process. 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 ether tracer purification using the phenolic hydroxyl precursor for radiolabeling.^{13,24,25}

The radiosynthesis was performed in an automated self-designed multi-purpose ^{11}C -radiosynthesis module, allowing measurement of specific activity at EOB during synthesis.¹⁷⁻¹⁹ On line determination of specific activity at EOB is accurate when reverse-phase (RP) high performance liquid chromatography (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 imidazopyridine- and purine-thioacetamide 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.²⁶ The actual measurement of specific activity at EOS was performed by analytical HPLC^{27,28} and calculated. The exact values of the specific activity for the tracers 3- ^{11}C **4a** and 4- ^{11}C **4a**, 3- ^{11}C **8a** and 4- ^{11}C **8a** were 185-555 GBq/ μmol at EOS, which are in agreement with the estimated values and the “on line” determined values. 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.^{29,30} Furthermore, specific activity for the ^{11}C -tracers synthesized by ^{11}C -methylation with ^{11}C CH₃OTf 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 CH₃OTf 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 CO₂ 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. We use an Eckert & Ziegler Modular Lab C-11 Methyl Iodide/Triflate module to produce ^{11}C CH₃OTf, convenient gas phase bromination of ^{11}C methane, and production of ^{11}C CH₃OTf. This ‘dry’ method, using Br₂ to generate a ^{11}C CH₃Br intermediate, differs from other ‘dry’ methods using I₂ and ‘wet’ methods using LiAlH₄ and HI, and seems to help minimize introduction of additional ^{12}C carrier after ^{11}C CO₂ production.¹¹ To further help produce high specific activity ^{11}C CH₃OTf, we usually do 2 ‘test loop’ procedures when we set up the module for the actual ^{11}C CH₃OTf

production run, and 1 actual ^{11}C CH₃OTf production run before we do ^{11}C methylation labeling reaction. These procedures avoid any leak in the module to introduce additional ^{12}C carrier and eliminate significant amount of original ^{12}C carrier accumulated in the ^{11}C CH₃OTf production system. Therefore, the specific activity of our ^{11}C -tracers is significantly improved.

Chemical purity and radiochemical purity were determined by analytical HPLC.²⁸ The chemical purity of the precursors and reference standards was >93%. 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 experimental details and characterization data for compounds **1-8** and for the tracers 3- ^{11}C **4a** and 4- ^{11}C **4a**, 3- ^{11}C **8a** and 4- ^{11}C **8a** are given.³¹

In summary, a simple and moderate-to-high-yield synthetic route to synthesize phenolic hydroxyl precursors **4b-c** and **8b-c**, ether standards **4a** and **8a**, and carbon-11-labeled imidazopyridine- and purine-thioacetamide derivatives 3- ^{11}C **4a** and 4- ^{11}C **4a**, 3- ^{11}C **8a** and 4- ^{11}C **8a** has been developed. An automated self-designed multi-purpose ^{11}C -radiosynthesis module for the synthesis of 3- ^{11}C **4a** and 4- ^{11}C **4a**, 3- ^{11}C **8a** and 4- ^{11}C **8a** 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 CH₃OTf, 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 specific activity 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 data¹ encourage further *in vivo* biological evaluation of carbon-11-labeled imidazopyridine- and purine-thioacetamide derivatives as new candidate PET agents for imaging of NPP1 in diabetes and other heart, cancer and brain diseases.

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- Wang, M.; Gao, M.; Xu, Z.; Zheng, Q.-H. *Bioorg. Med. Chem. Lett.* **2015**, *25*, 4587.
- (a) *General*: All commercial reagents and solvents were purchased from Sigma-Aldrich and Fisher Scientific, and used without further purification. [¹¹C]CH₃OTf was prepared according to a literature procedure.¹¹ Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ¹H NMR and ¹³C 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 cm²). 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 RP HPLC was performed using a Prodigy (Phenomenex) 5 μ m C-18 column, 4.6 $\times$ 250 mm; mobile phase 40% CH₃CN/60% H₂O; flow rate 1.5 mL/min; and UV (254 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 μ m filter units were obtained from Millipore Corporation (Bedford, MA).

(b) *2-Chloro-N-(3,4-dimethoxyphenyl)acetamide (1a)*: To a solution of 3,4-dimethoxyaniline (3.0 g, 19.58 mmol) in CH₂Cl₂ (50 mL) was added Et₃N (2.97 g, 29.37 mmol), the mixture was cooled to 0 °C on an ice bath, and 2-chloroacetyl chloride (2.76 g, 24.48 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature (RT) and stirred overnight. Then the mixture was evaporated under reduced pressure, and the crude product was washed with ice-cold water. The precipitate was filtered off, washed with water and dried, affording a gray solid **1a** (4.5 g, 80%). R_f = 0.82 (1:19 MeOH/CH₂Cl₂), mp 99–101 °C. ¹H NMR (CDCl₃): δ 3.87 (s, 3H, OCH₃), 3.89 (s, 3H, OCH₃), 4.18 (s, 2H, CH₂), 6.82 (d, J = 8.5 Hz, 1H, Ph-H), 6.94 (dd, J = 2.0, 8.5 Hz, 1H, Ph-H), 7.27 (d, J = 2.0 Hz, 1H, Ph-H), 8.15 (s, 1H, NH). MS (ESI): 230 ([M+H]⁺, 100%).

(c) *2-Chloro-N-(3-hydroxy-4-dimethoxyphenyl)acetamide (1b)*: To a solution of 5-amino-2-methoxyphenol (2.78 g, 20 mmol) in CH₂Cl₂ (50 mL) was added Et₃N (3.03 g, 30 mmol), the mixture was cooled to 0 °C, and 2-chloroacetyl chloride (2.37 g, 21 mmol) was added dropwise. The reaction mixture was allowed to warm to RT and stirred overnight. After the reaction completed, the solvents were removed, and the residue was purified by column chromatography on silica gel with eluent (0:100 to 1:99 MeOH/CH₂Cl₂) to give a black solid product **1b** (2.58 g, 60%). R_f = 0.69 (1:19 MeOH/CH₂Cl₂), mp 122–124 °C. ¹H NMR (DMSO-*d*₆): δ 3.72 (s, 3H, OCH₃), 4.18 (s, 2H, CH₂), 6.83 (d, J = 8.5 Hz, 1H, Ph-H), 6.93 (dd, J = 2.0, 8.5 Hz, 1H, Ph-H), 7.15 (d, J = 2.0 Hz, 1H, Ph-H), 9.08 (s, 1H, NH), 10.02 (s, 1H, OH). MS (ESI): 216 ([M+H]⁺, 100%); MS (ESI): 214 ([M-H][−], 20%).

(d) *2-Chloro-N-(4-hydroxy-3-methoxyphenyl)acetamide (1c)*: Compound **1c** was prepared from 4-amino-2-methoxyphenol and 2-chloroacetyl chloride according to the procedure for the synthesis of **1b**, as a brown solid, yield 62%. R_f = 0.65 (1:19 MeOH/CH₂Cl₂), mp 126–128 °C. ¹H NMR (DMSO-*d*₆): δ 3.71 (s, 3H, OCH₃), 4.19 (s, 2H, CH₂), 6.70 (d, J = 8.5 Hz, 1H, Ph-H), 6.94 (dd, J = 2.5, 8.5 Hz, 1H, Ph-H), 7.24 (d, J = 2.0 Hz, 1H, Ph-H), 8.79 (s, 1H, NH), 10.06 (s, 1H, OH). MS (ESI): 216 ([M+H]⁺, 70%); MS (ESI): 214 ([M-H][−], 100%).

(e) *6-Methoxypyridine-2,3-diamine (2)*: To a solution of 6-methoxy-3-nitropyridin-2-amine (25 g, 0.148

mol) in methanol was added palladium on carbon (10% Pd/C, 0.70 g). The reaction mixture was stirred at RT for 15 h under an atmosphere of hydrogen gas (55 psi). The mixture was filtered over Celite, and the filtrate was collected. The solvent was evaporated to afford a black solid crude product **2** (20.4 g). Because of the instability of the diamino analogue, the product was immediately used in next step reaction without further purification. R_f = 0.40 (1:19 MeOH/CH₂Cl₂). MS (ESI): 140 ([M+H]⁺, 100%).

(f) *5-Methoxy-1,3-dihydro-2H-imidazo[4,5-b]pyridine-2-thione (3)*: To a solution of compound **2** (20.56 g, 148 mmol) in a mixture of EtOH (200 mL) and water (40 mL) was added KOH (9.75 g, 148 mmol) and CS₂ (22.5 g, 296 mmol). The reaction mixture was stirred at reflux for 12 h. The solvents were evaporated, and the residue was purified by column chromatography on silica gel with eluent (2:98 to 10:90 MeOH/CH₂Cl₂), affording a gray solid product **3** (16.9 g, 63%). R_f = 0.50 (1:19 MeOH/CH₂Cl₂), mp 246–248 °C. ¹H NMR (DMSO-*d*₆): δ 3.83 (s, 3H, OCH₃), 6.56 (d, J = 8.5 Hz, 1H, Ar-H), 7.44 (d, J = 8.5 Hz, 1H, Ar-H), 12.73 (s, 2H, NH). MS (ESI): 182 ([M+H]⁺, 100%); MS (ESI): 180 ([M-H][−], 23%).

(g) *General procedure to prepare compounds 4a-c*: To a solution of compound **3** (360 mg, 2.0 mmol) in a mixture of EtOH (60 mL) and 1 N aq. NaOH (2.2 mL, 2.2 mmol), compound **1** (2.1 mmol) was added. The resulting reaction mixture was stirred overnight at RT. Then solvents were removed under reduced pressure, and the crude product was purified by column chromatography on silica gel with eluent (0:100 to 3:97 MeOH/CH₂Cl₂) to give a white solid product **4** (50–65% yields).

N-(3,4-Dimethoxyphenyl)-2-((5-methoxy-3H-imidazo[4,5-b]pyridin-2-yl)thio)acetamide (4a): R_f = 0.61 (1:19 MeOH/CH₂Cl₂), mp 175–177 °C. ¹H NMR (DMSO-*d*₆): δ 3.66 (s, 6H, 2 $\times$ OCH₃), 3.84 (s, 3H, OCH₃), 4.20 (s, 2H, CH₂), 6.59 (d, J = 8.5 Hz, 1H, Ar-H), 6.88 (d, J = 8.5 Hz, 1H, Ar-H), 7.07 (d, J = 8.0 Hz, 1H, Ar-H), 7.28 (d, J = 2.5 Hz, 1H, Ar-H), 7.80 (d, J = 8.5 Hz, 1H, Ar-H), 10.30 (br s, 1H, NH), 12.76 and 13.10 (2s, 1H, NH). MS (ESI): 375 ([M+H]⁺, 100%); MS (ESI): 373 ([M-H][−], 20%).

N-(3-Hydroxy-4-methoxyphenyl)-2-((5-methoxy-3H-imidazo[4,5-b]pyridin-2-yl)thio)acetamide (4b): R_f = 0.35 (1:19 MeOH/CH₂Cl₂), mp 216–218 °C. ¹H NMR (DMSO-*d*₆): δ 3.71 (s, 3H, OCH₃), 3.85 (s, 3H, OCH₃), 4.19 (s, 2H, CH₂), 6.59 (d, J = 8.5 Hz, 1H, Ar-H), 6.82 (d, J = 8.5 Hz, 1H, Ar-H), 6.92 (dd, J = 2.0, 8.5 Hz, 1H, Ar-H), 7.14 (d, J = 2.5 Hz, 1H, Ar-H), 7.73 and 7.80 (2s, 1H, Ar-H), 9.06 (s, 1H, OH), 10.17 (br s, 1H, NH), 12.74 and 13.09 (2s, 1H, NH). ¹³C NMR (DMSO-*d*₆): δ 35.99, 53.34, 55.88, 104.56, 107.62, 109.83, 112.54, 121.00, 128.20, 130.10, 132.50, 143.97, 146.48, 148.00, 159.96, 165.37. MS (ESI): 361 ([M+H]⁺, 100%); MS (ESI): 359 ([M-H][−], 25%).

HRMS (ESI): calcd for $C_{16}H_{17}N_4O_4S$ ($[M+H]^+$) 361.0971, found 361.0954.

N-(4-Hydroxy-3-methoxyphenyl)-2-((5-methoxy-3H-imidazo[4,5-*b*]pyridin-2-yl)thio)acetamide (**4c**): R_f = 0.33 (1:19 MeOH/ CH_2Cl_2), mp 119-121 °C. 1H NMR (DMSO- d_6): δ 3.72(s, 3H, OCH_3), 3.84 and 3.85 (2s, 3H, OCH_3), 4.18 and 4.21 (2s, 2H, CH_2), 6.60 (t, J = 8.5 Hz, 1H, Ar-H), 6.68 (d, J = 8.5 Hz, 1H, Ar-H), 6.91 and 6.94 (2d, J = 8.5, 1H, Ar-H), 7.26 (s, 1H, Ar-H), 7.71 and 7.81 (2d, J = 8.5 Hz, 1H, Ar-H), 8.77 (s, 1H, OH), 10.18 and 10.24 (2br s, 1H, NH), 12.74 and 13.10 (2s, 1H, NH). ^{13}C NMR (DMSO- d_6): δ 35.91, 53.37, 55.47, 104.04, 104.69, 115.24, 121.30, 122.05, 128.04, 130.22, 131.00, 142.70, 147.24, 147.92, 159.94, 165.29. MS (ESI): 361($[M+H]^+$, 100%); MS (ESI): 359 ($[M-H]^-$, 30%). HRMS (ESI): calcd for $C_{16}H_{17}N_4O_4S$ ($[M+H]^+$) 361.0971, found 361.0955.

(h) 5-Nitrosopyrimidine-4,6-diamine (**5**): A solution of 4,6-pyrimidinediamine (16.0 g, 110 mmol) in 3 N HCl (367 mL) was cooled in an ice bath, and then $NaNO_2$ (8.73 g, 126 mmol) in H_2O (30 mL) was added dropwise. The mixture was stirred for 30 min at 0 °C, and then 2 h at RT. The violet solution was neutralized with $NaHCO_3$ to pH 8, the resulting precipitate collected, suspended in H_2O and head to 70 °C, filtered, washed with MeOH, and dried to give a blue solid product **5** (14.5 g, 78%). R_f = 0.81 (1:5 MeOH/ CH_2Cl_2), mp > 350 °C. 1H NMR (DMSO- d_6): δ 7.93 (s, 1H, Ar-H), 8.04 (s, 1H, NHH), 8.44 (s, 1H, NHH), 9.11 (s, NHH), 10.05 (s, 1H, NHH). MS (ESI): 140 ($[M+H]^+$, 100%).

(i) Pyrimidine-4,5,6-triamine (**6**): Compound **5** (10.0 g, 72 mmol) was dissolved in water (110 mL) and treated with sodium dithionite (27.6 g, 158 mmol), which was added in portions at RT. The resulting yellow mixture was then treated with 50% aq. H_2SO_4 (141 g, 720 mmol) and heated to 80 °C for 5 min, and then cooled to 10 °C in an ice bath. The resulting precipitate was filtered off and washed with aq. ethanol and dried to afford a yellowish solid intermediate 4,5,6-triaminopyrimidine sulfate (8.81 g). A mixture of 4,5,6-triaminopyrimidine sulfate (8.81 g, 39.5 mmol), NaOH (3.48 g, 87 mmol) and water (80 mL) was heated to 80 °C until all the solid was dissolved. The solution was cooled to 0 °C and pH was adjusted to 7.0 with 1 N HCl, whereupon the free base precipitated as an off-white solid product **6** (3.69 g, 41% from **5**). R_f = 0.26 (1:5 MeOH/ CH_2Cl_2), mp 288-290 °C. 1H NMR (DMSO- d_6): δ 4.33 (br s, 2H, NH_2), 6.45 (br s, 4H, $2 \times NH_2$), 7.68 (s, 1H, Ar-H). MS (ESI): 126 ($[M+H]^+$, 100%).

(j) 6-Amino-7,9-dihydro-8H-purine-8-thione (**7**): To a mixture of compound **6** (4.13 g, 33 mmol) in solvents of EtOH (200 mL) and water (10 mL) was added KOH (2.28 g, 35 mmol) and CS_2 (3.33 g, 43.8 mmol). The reaction mixture was stirred at reflux for overnight. The solvents were evaporated, and the residue were rinsed with water and acetone to obtain a yellow product **7** (3.64 g, 66%). R_f = 0.24 (1:9

MeOH/ CH_2Cl_2), mp > 350 °C. 1H NMR (DMSO- d_6): δ 6.59 (s, 2H, NH_2), 7.98 (s, 1H, Ar-H), 12.20 (br s, 2H, $2 \times NH$). MS (ESI): 168 ($[M+H]^+$, 90%); MS (ESI): 166 ($[M-H]^-$, 100%).

(k) General procedure to prepare compounds **8a-c**: To a solution of compound **7** (334 mg, 2.0 mmol) in a mixture of EtOH (100 mL) and 1 N aq. NaOH (2.6 mL, 2.6 mmol), compound **1** (2.0 mmol) was added. The resulting reaction mixture was stirred overnight at 60 °C. Then solvents were removed under reduced pressure, and the crude product was purified by column chromatography on silica gel with eluent (2:98 to 10:90 MeOH/ CH_2Cl_2) to give a gray solid product **8** (45-60% yields).

2-((6-Amino-9H-purin-8-yl)thio)-*N*-(3,4-dimethoxyphenyl)acetamide (**8a**): R_f = 0.26 (1:9 MeOH/ CH_2Cl_2), mp 270-272 °C. 1H NMR (DMSO- d_6): δ 3.70 (s, 6H, $2 \times OCH_3$), 4.12 (s, 2H, CH_2), 6.87 (d, J = 8.5 Hz, 1H, Ar-H), 7.08-7.10 (m, 3H, Ar-H and NH_2), 7.25 (d, J = 2.5 Hz, 1H, Ar-H), 8.05 (s, 1H, Ar-H), 10.18 (s, 1H, NH), 13.14 (s, 1H, NH). MS (ESI): 361($[M+H]^+$, 100%); MS (ESI): 359 ($[M-H]^-$, 30%).

2-((6-Amino-9H-purin-8-yl)thio)-*N*-(3-hydroxy-4-methoxyphenyl)acetamide (**8b**): R_f = 0.29 (1:7 MeOH/ CH_2Cl_2), mp 261-263 °C. 1H NMR (DMSO- d_6): δ 3.70 (s, 3H, OCH_3), 4.12 (s, 2H, CH_2), 6.81 (d, J = 8.5 Hz, 1H, Ar-H), 6.90 (dd, J = 2.5, 8.5 Hz, 1H, Ar-H), 7.05 (br s, 2H, NH_2), 7.14 (d, J = 2.5 Hz, 1H, Ar-H), 8.05 (s, 1H, Ar-H), 9.05 (s, 1H, OH), 10.57 (s, 1H, NH), 13.09 (s, 1H, NH). MS (ESI): 347($[M+H]^+$, 100%); MS (ESI): 345 ($[M-H]^-$, 70%). HRMS (ESI): calcd for $C_{14}H_{15}N_6O_3S$ ($[M+H]^+$) 347.0926, found 347.0912.

2-((6-Amino-9H-purin-8-yl)thio)-*N*-(4-hydroxy-3-methoxyphenyl)acetamide (**8c**): R_f = 0.37 (1:7 MeOH/ CH_2Cl_2), mp 266-268 °C. 1H NMR (DMSO- d_6): δ 3.71 (s, 6H, $2 \times OCH_3$), 4.11 (s, 2H, CH_2), 6.65 (d, J = 8.5 Hz, 1H, Ar-H), 6.93 (dd, J = 2.0, 8.5 Hz, 1H, Ar-H), 7.09 (br s, 2H, NH_2), 7.23 (d, J = 2.0 Hz, 1H, Ar-H), 8.05 (s, 1H, Ar-H), 8.77 (s, 1H, OH), 10.09 (s, 1H, NH), 13.11 (s, 1H, NH). ^{13}C NMR (DMSO- d_6): δ 35.89, 55.48, 103.50, 104.72, 106.40, 108.10, 111.79, 115.19, 119.20, 130.89, 142.73, 142.90, 147.23, 151.57, 165.44. MS (ESI): 347($[M+H]^+$, 80%); MS (ESI): 345 ($[M-H]^-$, 100%). HRMS (ESI): calcd for $C_{14}H_{15}N_6O_3S$ ($[M+H]^+$) 347.0926, found 347.0914.

(l) General procedure to prepare target tracers *N*-(3-[^{11}C]methoxy-4-methoxyphenyl)-2-((5-methoxy-3H-imidazo[4,5-*b*]pyridin-2-yl)thio)acetamide (3-[^{11}C]**4a**) and *N*-(4-[^{11}C]methoxy-3-methoxyphenyl)-2-((5-methoxy-3H-imidazo[4,5-*b*]pyridin-2-yl)thio)acetamide (4-[^{11}C]**4a**); 2-((6-amino-9H-purin-8-yl)thio)-*N*-(3-[^{11}C]methoxy-4-methoxyphenyl)acetamide (3-[^{11}C]**8a**) and 2-((6-amino-9H-purin-8-yl)thio)-*N*-(4-[^{11}C]methoxy-3-methoxyphenyl)acetamide (4-[^{11}C]**8a**): [^{11}C]CO $_2$ was produced by the $^{14}N(p,\alpha)^{11}C$ 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 μ A beam current for 15 min on target. The production run produced approximately 25.9 GBq of [^{11}C]CO₂ at EOB. The phenolic hydroxyl precursor **4b** (**4c**, **8b** or **8c**) (0.1-0.3 mg) was dissolved in CH₃CN (300 μ L). To this solution was added 2 N NaOH (2 μ L). The mixture was transferred to a 5-mL small reaction vial. No-carrier-added (high specific activity) [^{11}C]CH₃OTf (13.9 GBq) that was produced by the gas-phase production method¹¹ within 11 min from [^{11}C]CO₂ (25.9 GBq) through [^{11}C]CH₄ (21.8 GBq) and [^{11}C]CH₃Br (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 NaHCO₃ (0.1 M, 1 mL). 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 H₂O (2 $\times$ 3 mL), and the aqueous washing was discarded. The product was eluted from the cartridge with EtOH (2 $\times$ 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 μ m 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]CO₂, were 50-60%. The same procedure was used to prepare the target tracers 3- ^{11}C **4a** and 4- ^{11}C **4a**, 3- ^{11}C **8a** and 4- ^{11}C **8a** from their corresponding precursors **4b** and **4c**, **8b** and **8c**. Retention times in the analytical HPLC system were: t_{R} **4b** = 5.42 min, t_{R} **4c** = 5.35 min, t_{R} **4a** = 7.74 min, t_{R} 3- ^{11}C **4a** = 7.85 min, t_{R} 4- ^{11}C **4a** = 7.79 min; and t_{R} **8b** = 3.57 min, t_{R} **8c** = 3.46 min, t_{R} **8a** = 5.53 min, t_{R} 3- ^{11}C **8a** = 5.68 min, t_{R} 4- ^{11}C **8a** = 5.61 min.