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Synthesis of 2,6-difluoro-*N*-(3-[^{11}C]methoxy-1*H*-pyrazolo[3,4-*b*]pyridine-5-yl)-3-(propylsulfonamidio)benzamide as a new potential PET agent for imaging of B-Raf^{V600E} in cancers

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Radiosynthesis
Automation
Positron emission tomography (PET)
Cancer imaging

ABSTRACT

The authentic standard 2,6-difluoro-*N*-(3-methoxy-1*H*-pyrazolo[3,4-*b*]pyridine-5-yl)-3-(propylsulfonamidio)benzamide was synthesized from 2,6-difluorobenzoic acid and 3-amino-5-hydroxypyrazole in 9 steps with 1% overall chemical yield. Direct desmethylation of the reference standard with TMSCl/NaI gave the precursor 2,6-difluoro-*N*-(3-hydroxy-1*H*-pyrazolo[3,4-*b*]pyridine-5-yl)-3-(propylsulfonamidio)benzamide for radiolabeling in 70% yield. The target tracer 2,6-difluoro-*N*-(3-[^{11}C]methoxy-1*H*-pyrazolo[3,4-*b*]pyridine-5-yl)-3-(propylsulfonamidio)benzamide was prepared from the precursor with [^{11}C]CH_3OTf through *O*-[^{11}C]methylation and isolated by HPLC combined with SPE in 40–50% decay corrected radiochemical yields with 370–740 GBq/μmol specific activity at end of bombardment (EOB).

The mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) signaling pathway transduces signals from cell surface receptors to the nucleus leading to cell proliferation, differentiation, and survival.[1–4] The serine/threonine-protein kinase (Raf) family contains A-Raf, B-Raf and C-Raf (Raf-1), and their biochemical potencies to phosphorylate and activate MAP/ERK kinase (MEK) are B-Raf > C-Raf >> A-Raf.[5] The V600E mutation due to the substitution of valine with glutamic acid at amino acid (aa) residue 600 of B-Raf kinase (B-Raf^{V600E}) results in constitutive activation of the Ras (a G-protein)/B-Raf/MEK/ERK (MAPK) signaling pathway and is found in approximately 7% of all cancers including melanoma, papillary thryroid cancer, colorectal cancer, cholangiocarcinoma, and ovarine cancer.[1–5] B-Raf^{V600E} correlates with increased malignancy and decreased response to chemotherapy, and thus has emerged as an important biomarker for diagnosis, prognosis and therapeutic guidance for human cancers.[2] B-Raf^{V600E} is a highly attractive target for cancer therapy, and several small-molecule protein kinase inhibitors selective for B-Raf^{V600E} including RAF265 (Chiron/Novartis), XL281/BMS-908662 (Exelixis/BMS), GSK2118436 (GlaxoSmithKline), and PLX4032 (Plexxikon/Roche) are currently in clinical trials.[1–5] Recently, a novel series of pyrazolopyridine inhibitors of B-Raf^{V600E} has been developed.[5] The representative compound 2,6-difluoro-*N*-(3-methoxy-1*H*-pyrazolo[3,4-*b*]pyridine-5-yl)-3-(propylsulfonamidio) benzamide (**9**, IC_{50} 4.8 nM for B-Raf^{V600E}) is a selective, orally bioavailable, and efficacious inhibitor and has been selected for further preclinical evaluation.[5] B-Raf^{V600E} is an attractive target for molecular imaging of cancer as well. However, so far no specific and selective cancer B-Raf^{V600E} imaging agent to guide chemotherapy is reported, and the therapeutic efficacy of PLX4032 for the treatment of malignant melanoma patients was observed through the reductions in 2-[^{18}F]fluoro-2-deoxyglucose ([^{18}F]FDG) uptake on biomedical imaging technique positron emission tomography (PET) scanning, in which [^{18}F]FDG is the only PET cancer imaging agent for glucose metabolism used clinically at this point in time.[6] We are interested in the development of new PET imaging agents for cancer detection, diagnosis and image-guided therapy, and here we present the design and synthesis of 2,6-difluoro-*N*-(3-[^{11}C]methoxy-1*H*-pyrazolo[3,4-*b*]pyridine-5-yl)-3-(propylsulfonamidio)benzamide ([^{11}C]**9**) as a new specific and selective potential imaging agent for PET to image B-Raf^{V600E} in cancers, for the first time.

The reference standard **9** was synthesized via amide coupling of 2,6-difluoro-3-(propylsulfonamido)benzoic acid (**5**) with 3-methoxy-1*H*-pyrazolo[3,4-*b*]pyridine-5-amine (**8**) using the modifications of the literature method.[5,7]

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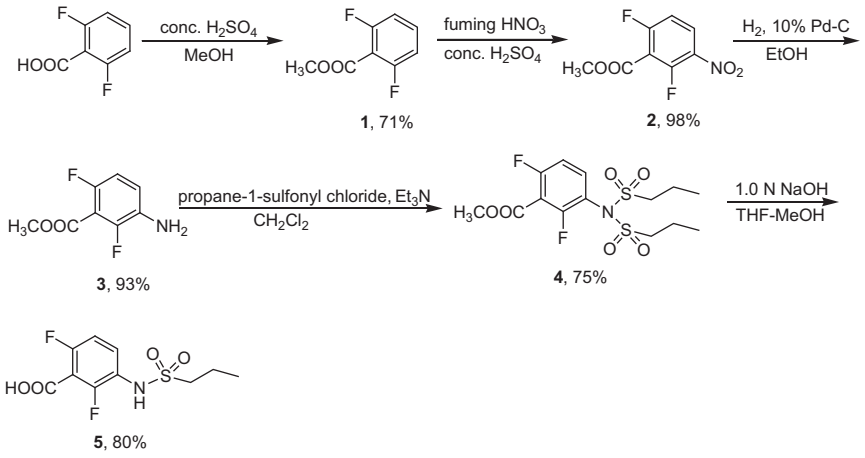
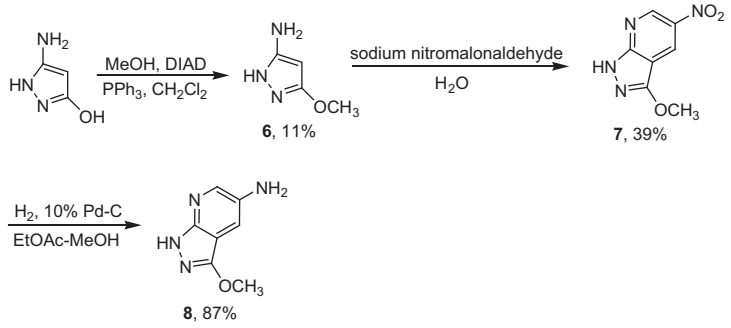


Scheme 1. Synthesis of the key intermediate **5**.



Scheme 2. Synthesis of the key intermediate **8**.

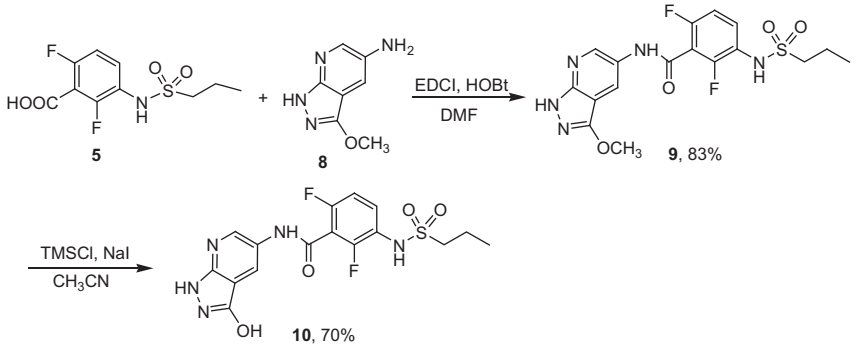
As indicated in Scheme 1, the key intermediate **5** was synthesized starting from low cost and commercially available 2,6-difluorobenzoic acid. Benzoic acid was esterified to methyl benzoate **1** by treatment with MeOH in the presence of concentrated H_2SO_4 as catalyst, in 71% yield. Nitration of methyl benzoate **1** with fuming HNO_3 in concentrated H_2SO_4 afforded nitrobenzoate **2** in 98% yield.[8] Reduction of the nitro group was performed by catalytic hydrogenation of **2** with 10% Pd/C in EtOH to give amino-benzoate **3** in 93% yield. Bis-sulfonamide **4** was obtained by treatment aniline **3** with propane-1-sulfonyl chloride in the presence of Et_3N in CH_2Cl_2, in 75% yield. Concurrent hydrolysis of the ester and one of sulfonamides was achieved with aqueous NaOH in a mixture of THF and MeOH to provide **5** in 80% yield.

As depicted in Scheme 2, the preparation of another key intermediate **8** began with commercial readily available 3-amino-5-hydroxypyrazole. Methoxyl-substituted aminopyrazole **6** was obtained in one-step without protecting and deprotecting by treatment hydroxyaminopyrazole with MeOH in the presence of triphenylphosphine and diidopropylazodicarboxylate (DIAD) in 11% yield.[9,10] Condensation of amniopyrazole **6** with sodium nitromalonaldehyde monohydrate in water produced the fused 2-ring heterocyclic compound **7** in 39% yield. Reduction of the nitro group was performed by catalytic hydrogenation of **7** with 10% Pd/C in EtOH to give **8** in 87% yield.

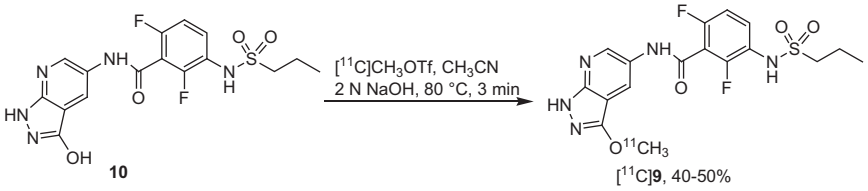
As shown in Scheme 3, amide coupling of **5** and **8** in the presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDCI) and hydroxybenzotriazole (HOBt) in DMF provided the standard **9** in 83% yield. In an effort to obtain the precursor for ^{11}C-labeling, we focused on desmethylation of **9**, because one-step direct desmethylation to prepare the precursor would be better than using a multiple-step short synthesis route to synthesize the precursor. The different commonly used desmethylating agents including HBr, BBr_3, $AlCl_3$/EtSH, LiBr and chlorotrimethylsilane (TMSCl)/NaI were tested for the desmethylation reaction, and we finally selected TMSCl/NaI for this reaction, which provided the best result. The desmethylation of **9** with TMSCl/NaI in acetonitrile afforded the precursor **10** in 70% yield.[11,12]

The overall chemical yield for the standard **9** in 9 steps was 1%. The extremely low yield was because of one-step synthesis of compound **6** in only 11% yield. The way to improve the yield would be using a 3-step synthetic approach including protecting, *O*-methylation and deprotecting reactions to synthesize **6**. However, it would require more materials and labors compared to a low-yield one-step direct and selective *O*-methylation.

Radiosynthesis of the target tracer [^{11}C]**9** is indicated in Scheme 4. The hydroxyl precursor **10** was labeled by [^{11}C]methyl triflate ([^{11}C]CH_3OTf)[13,14] through *O*-[^{11}C]methylation[15–17] in acetonitrile at 80 °C under basic condition (2 N NaOH) and isolated by a semi-preparative high performance liquid chromatography (HPLC) with a Prodigy C-18 column from Phenomenex and a solid-phase extraction (SPE) with a disposable C-18 Plus Sep-Pak cartridge from Waters (a second purification or isolation process)[18–20] to produce the corresponding pure radiolabeled compound [^{11}C]**9** in 40–50% radiochemical yield, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2. Addition of $NaHCO_3$ 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]**9** from its hydroxyl precursor **10**.[18–21] The radiosynthesis was performed in a home-built automated multi-purpose ^{11}C-radiosynthesis module, allowing



Scheme 3. Synthesis of the reference standard **9** and precursor **10**.



Scheme 4. Synthesis of the target tracer [^{11}C]**9**.

measurement of specific radioactivity during synthesis.[22–24] This ^{11}C-radiosynthesis module includes the overall design of the reaction, purification and reformulation capabilities of the prototype system. In addition, ^{11}C-tracer specific activity (GBq/μmol at EOB) can be automatically determined prior to product delivery for compounds purified by the HPLC-portion of the system. Briefly, analysis of the chromatographic data utilized PeakSimple software (SRI Instruments, Las Vegas, NV). Immediately following elution of the product peak, the chromatographic data are exported to PeakSimple readable files, and the area of the radioactivity peak is converted to GBq at EOB by comparison to a reference calibration curve previously constructed using the same detector, loop and flow rate. The mass peak from the UV chromatogram (without decay correction) is similarly compared to a standard curve made at the same UV wavelength, mobile phase and flow rate. Simple division of the total EOB radioactivity peak (in GBq) by the total mass peak (in nmoles) gives specific activity at EOB in GBq/μmol. The overall synthesis, purification and reformulation time was 30–40 min from EOB. The specific radioactivity was in a range of 370–740 GBq/μmol at EOB. The specific activity can also be measured by analytical HPLC, which is consistent with the on-the-fly technique to determine the specific activity by semi-preparative HPLC. Chemical purity and radiochemical purity were determined by analytical HPLC.[25] The chemical purity of the precursor **10** and reference standard **9** was >97%. The radiochemical purity of the target tracer [^{11}C]**9** was >99% determined by radio-HPLC through γ-ray (PIN diode) flow detector, and the chemical purity of [^{11}C]**9** was >93% determined by reverse-phase HPLC through UV flow detector. A C-18 Plus Sep-Pak cartridge was used to significantly improve the chemical purity of the tracer solution.[18–20,25] The chemical purity of the [^{11}C]**9** tracer solution with Sep-Pak purification was usually increased higher 10–20% than that without Sep-Pak purification.[18–20]

The experimental details and characterization data for compounds **1**–**10** and for the target tracer [^{11}C]**9** are given.[26]

In summary, an efficient and convenient synthetic route to the pyrazolopyridine standard **9**, normethyl precursor **10** and target tracer [^{11}C]**9** has been developed. An automated self-designed multi-purpose [^{11}C]-radiosynthesis module for the synthesis of [^{11}C]**9** has been built, featuring the measurement of specific activity by the on-the-fly technique. The radiosynthesis employed *O*-[^{11}C]methylation radiolabeling on oxygen position of the hydroxyl precursor. Radiolabeling procedures incorporated efficiently with the most commonly used [^{11}C]methylating agent, [^{11}C]CH_3OTf, which was produced by gas-phase production of [^{11}C]methyl bromide ([^{11}C]CH_3Br) from our laboratory.[14] The target tracer was isolated and purified by a semi-preparative HPLC combined with SPE procedure in high radiochemical yields, short overall synthesis time, and high specific activity. These chemistry results combined with the reported in vitro and in vivo biological data of pyrazolopyridine inhibitors[5] encourage further in vivo biological evaluation, preclinical and clinical PET studies of new radiolabeled pyrazolopyridine inhibitors as cancer B-Raf^{V600E} imaging agents in animals and humans.

Acknowledgments

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26. (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.[14] Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^1H 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). 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 1:1 CH_3CN/H_2O; flow rate 1.5 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 1:1 CH_3CN/H_2O; flow rate 5.0 mL/min; 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 2,6-difluorobenzoate (**1**)*. To a solution of 2,6-difluorobenzoic acid (30.0 g, 190.0 mmol) in MeOH (100 mL) was added concentrated sulfuric acid (5 mL) dropwise at room temperature. The reaction mixture was heated under reflux overnight. The solvent was removed in vacuo. The residue was dissolved in EtOAc and washed with saturated $NaHCO_3$ and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo to give **1** (23.2 g, 71%) as a yellow oil. ^1H NMR ($CDCl_3$): δ 7.45–7.39 (m, 1H), 6.98–6.93 (m, 2H), 3.96 (s, 3H).

(c) *Methyl 2,6-difluoro-3-nitrobenzoate (**2**)*. To a solution of compound **1** (21.0 g, 122.0 mmol) in concentrated sulfuric acid (50 mL) was added fuming nitric acid (8 mL) dropwise at 0 °C. After the reaction mixture was stirred at 0 °C for 1 h, it was poured into ice-water. The precipitate was collected by filtration and rinsed with water to give **2** (26.0 g, 98%) as a white solid, mp 58–60 °C. ^1H NMR ($CDCl_3$): δ 8.25–8.22 (m, 1H), 7.15–7.11 (m, 1H), 4.01 (s, 3H).

(d) *Methyl 3-amino-2,6-difluorobenzoate (**3**)*. A solution of compound **2** (15.0 g, 69.1 mmol) in EtOH (250 mL) was hydrogenation over 10% Pd/C (4.0 g) at 50 psi for 22 h. The catalyst was filtered off through a layer of Celite, and the filtrate was concentrated in vacuo. The crude product was purified by silica gel column chromatography (4:1–3:1 hexanes/EtOAc) to give **3** (12.9 g, 93%) as a pale yellow oil. ^1H NMR (DMSO-d_6): δ 6.93–6.91 (m, 2H), 5.26 (br s, 2H), 3.87 (s, 3H).

(e) *Methyl 2,6-difluoro-3-(N-(propylsulfonyl)propylsulfonamido)benzoate (**4**)*. To a solution of compound **3** (11.5 g, 61.4 mmol) and triethylamine (25.67 mL, 184.2 mmol) in CH_2Cl_2 (55 mL) was added propane-1-sulfonyl chloride (17.25 mL, 153.3 mmol) dropwise at 0 °C. The reaction mixture was stirred at room temperature for 1 h. Water (150 mL) was added, and the organic layer was separated, washed with water, brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by silica gel column chromatography (6:1–4:1 hexanes/EtOAc) to give **4** (18.4 g, 75%) as an off-white solid, mp 76–77 °C. ^1H NMR ($CDCl_3$): δ 7.51–7.47 (m, 1H), 7.07–7.03 (m, 1H), 3.97 (s, 3H), 3.67–3.61 (m, 2H), 3.52–3.46 (m, 2H), 1.99–1.91 (m, 4H), 1.10 (t, J = 7.5 Hz, 6H).

(f) *2,6-Difluoro-3-(propylsulfonamido)benzoic acid (**5**)*. To a solution of compound **4** (15.0 g, 37.6 mmol) in THF/MeOH (4:1, 150 mL) was added 1.0 N NaOH (113 mL, 113.0 mmol). After the reaction mixture was stirred at room temperature overnight, the majority of the organic solvent was removed in vacuo. The mixture was cooled with ice bath, and then neutralized with 1.0 N HCl (115 mL) slowly. The precipitate was collected by filtration and rinsed with water to give **5** (8.42 g, 80%) as a white solid, mp 218–220 °C. ^1H NMR (DMSO-d_6): δ 14.1 (br s, 1H), 9.75 (s, 1H), 7.56–7.52 (m, 1H), 7.22–7.19 (m, 1H), 3.10–3.08 (m, 2H), 1.78–1.70 (m, 2H), 0.98 (t, J = 7.5 Hz, 3H).

(g) *3-Methoxy-1H-pyrazol-5-amine (**6**)*. A mixture of 3-amino-5-hydroxypyrazole (25.0 g, 252.3 mmol) and triphenylphosphine (77.8 g, 296.7 mmol) in CH_2Cl_2 (400 mL) was cooled to 0 °C and diisopropyl azodicarboxylate (58.8 mL, 298.6 mmol) was added dropwise at 0 °C to give a dark brown mobile slurry. The reaction mixture was stirred at 0 °C for 1 h. Beige slurry formed after 20 min. MeOH (25 mL) was then added dropwise at 0 °C as the slurry thinned considerably to give lighter yellow slurry. After the reaction mixture was held at 0 °C for 1 h, it was allowed to warm to room temperature and then stirred at room temperature overnight. Solid was filter off, the filtrate was concentrated in vacuo. The crude product was purified by silica gel column chromatography (100:3–100:10 CH_2Cl_2/MeOH) to give **6** (3.17 g, 11%) as a yellow solid, mp 46–48 °C. ^1H NMR (DMSO-d_6): δ 4.73 (s, 1H), 3.65 (s, 3H). ^{13}C NMR (DMSO-d_6): δ 162.8, 149.3, 73.0, 55.1. LC/MS (ESI, m/z): 114 ($[M+H]^+$, 100%).

(h) *3-Methoxy-5-nitro-1H-pyrazolo[3,4-b]pyridine (**7**)*. A mixture of compound **6** (2.0 g, 20.6 mmol) and sodium nitromalonaldehyde monohydrate (3.52 g, 22.4 mmol) in water (70 mL) was heated to 95 °C overnight. The reaction mixture was cooled to room temperature and acidified with acetic acid to pH 5. The mixture was extracted with EtOAc, and the combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by silica gel column chromatography (4:1 hexanes/EtOAc) to give **7** (1.57 g, 39%) as a yellow solid, mp 202–204 °C. ^1H NMR (DMSO-d_6): δ 13.4 (br s, 1H), 9.29 (d, J = 2.5 Hz, 1H), 8.93 (d, J = 2.5 Hz, 1H), 4.07 (s, 3H).

(i) *3-Methoxy-1H-pyrazolo[3,4-b]pyridine-5-amine (**8**)*. A solution of compound **7** (1.4 g, 7.2 mmol) in EtOAc/MeOH (1:1, 40 mL) was hydrogenation over 10% Pd/C (0.8 g) at 60 psi for 7 h. The catalyst was filtered off through a layer of Celite, and the filtrate was concentrated in vacuo. The crude product was purified by silica gel column chromatography (100:4 CH_2Cl_2/MeOH) to give **8** (1.03 g, 87%) as an off-white solid, mp 174–176 °C. ^1H NMR (DMSO-d_6): δ 11.9 (br s, 1H), 8.01 (d, J = 2.5 Hz, 1H), 7.07 (dd, J = 2.5, 0.5 Hz, 1H), 4.97 (s, 2H), 3.94 (s, 3H).

(j) *2,6-Difluoro-N-(3-methoxy-1H-pyrazolo[3,4-b]pyridine-5-yl)-3-(propylsulfonamido)benzamide (**9**)*. A mixture of compound **5** (682 mg, 2.44 mmol), compound **8** (400 mg, 2.44 mmol), HOBt·H_2O (374 mg, 2.44 mmol), EDCI (443 mg, 2.44 mmol) in DMF (10 mL) was stirred at room temperature overnight. The solvent was removed in vacuo to give a dark viscous mixture. A solution of water/saturated aqueous $NaHCO_3$ (10 mL) was added dropwise with rapid stirring at 0 °C. The mixture was stirred at 0 °C for 1 h, and the precipitate was collected by filtration and rinsed with water to give a tan solid. The crude product was purified by silica gel column chromatography (100:4–100:10 CH_2Cl_2/MeOH) to give **9** (861 mg, 83%) as a beige solid, mp 206–207 °C. ^1H NMR (DMSO-d_6): δ 12.6 (br s, 1H), 11.1 (s, 1H), 9.81(s, 1H), 8.59 (d, J = 2.0 Hz, 1H), 8.49 (d, J = 2.0 Hz, 1H), 7.59–7.54 (m, 1H), 7.28 (t, J = 8.5 Hz, 1H), 4.01 (s, 3H), 3.14–3.11 (m, 2H), 1.81–1.73 (m, 2H), 0.99 (t, J = 7.5 Hz, 3H). ^{13}C NMR (DMSO-d_6): δ 158.0, 154.7, 149.7, 143.4, 128.0, 118.4, 102.4, 55.7, 53.8, 16.9, 12.6. LC/MS (ESI, m/z): 426 ($[M+H]^+$, 100%). HRMS (ESI, m/z): calcd for $C_{17}H_{17}N_5O_4SF_2Na$ ($[M+Na]^+$) 448.0867; found 448.0849.

(k) *2,6-Difluoro-N-(3-hydroxy-1H-pyrazolo[3,4-b]pyridine-5-yl)-3-(propylsulfonamido)benzamide (**10**)*. To a suspension of compound **9** (200 mg, 0.47 mmol) and NaI (290 mg, 1.93 mmol) in acetonitrile (4 mL) was added chlorotrimethylsilane (0.24 mL, 1.93 mmol) dropwise under nitrogen atmosphere. The reaction mixture was heated under reflux overnight. The reaction was quenched with MeOH (2 mL). The solvent was removed in vacuo, and the residue was dissolved in MeOH and purified via reversed-phase semi-preparative HPLC using a Shimadzu LC-20AT pump, a SPD-M20A diode array detector (DAD), a Luna C18 column (10×250 mm, 5 μm), a gradient mobile phase composed of 20% CH_3CN (0.1% TFA)—80% H_2O (0.1% TFA) to 60% CH_3CN (0.1% TFA)—40% H_2O (0.1% TFA), flow rate 5.0 mL/min and UV 254 nm. The product fraction was collected at ~10 min to afford **10** (135 mg, 70%) as a pale pink solid, mp 265–267 °C. ^1H NMR (DMSO-d_6): δ 12.6 (br s, 1H), 11.0 (s, 1H), 9.81(s, 1H), 8.58 (d, J = 1.5 Hz, 1H), 8.53 (d, J = 2.0 Hz, 1H), 7.59–7.54 (m, 1H), 7.28 (t, J = 8.5 Hz, 1H), 3.14 (t, J = 7.5 Hz, 1H), 1.82–1.74 (m, 2H), 1.00 (t, J = 7.5 Hz, 3H). ^{13}C NMR (CD_3OD): δ 160.8, 158.3, 152.6, 146.2, 129.5, 123.7, 107.0, 55.2, 18.3, 13.1. LC/MS (ESI, m/z): 412 ($[M+H]^+$, 100%). HRMS (ESI, m/z): calcd for $C_{16}H_{15}N_5O_4SF_2Na$ ($[M+Na]^+$) 434.0711; found 434.0694.

(l) *2,6-Difluoro-N-(3-[^{11}C]methoxy-1H-pyrazolo[3,4-b]pyridine-5-yl)-3-(propylsulfonamidio)benzamide ([^{11}C]**9**)*. $[^{11}C]CO_2$ 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 55 μA beam current and 15 min on target. The production run produced approximately 28.5 GBq of

[^{11}C]CO_2 at EOB. In a small reaction vial (5 mL), the precursor **10** (0.3–0.5 mg) was dissolved in CH_3CN (300 μL). To this solution was added 2 N NaOH (3 μL). No carrier-added (high specific activity) [^{11}C]CH_3OTf that was produced by the gas-phase production method[14] 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 room temperature, 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), and injected onto the semi-preparative 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]**9**. 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 was noted for tracer dose dispensing. Retention times in the semi-preparative HPLC system were: t_R **10** = 5.87 min, t_R **9** = 8.32 min, t_R [^{11}C]**9** = 8.32 min. Retention times in the analytical HPLC system were: t_R **10** = 2.28 min, t_R **9** = 4.17 min, t_R [^{11}C]**9** = 4.17 min. The decay corrected radiochemical yield of [^{11}C]**9** from [^{11}C]CO_2 was 40–50%.