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RESEARCH ARTICLE

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Potent and selective CC chemokine receptor 1 antagonists labeled with carbon-13, carbon-14, and tritium

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1-(4-Fluorophenyl)-1*H*-pyrazolo[3,4-c]pyridine-4-carboxylic acid (2-methanesulfonyl-pyridin-4-ylmethyl)-amide (**1**) and its analogs (**2**) and (**3**) are potent CCR1 antagonists intended for the treatment of rheumatoid arthritis. The detailed syntheses of these 3 compounds labeled with carbon-13 as well as the preparation of (**1**) and (**2**) labeled with carbon-14, and (**1**) labeled with tritium, are described.

KEYWORDS
carbon-13, carbon-14, radiosynthesis, tritium, CCR1 antagonists

1 | INTRODUCTION

CCR1 is a G-protein coupled receptor that is involved in the migration and activation of leukocytes to sites of inflammation.[1,2] Therefore, it is a therapeutic target for the treatment of inflammatory diseases.[3-12] By restricting immune cell trafficking to inflammation sites, scientists hoped to reduce disease severity and pain by using small molecule antagonists of CCR1.[13] In this manuscript, we describe the synthesis of potent and selective antagonists of CCR1 labeled with carbon-13, carbon-14, and tritium.

2 | RESULTS AND DISCUSSION

These 3 CCR1 antagonists are structurally similar and have a common aryl acid moiety, (1-(4-fluorophenyl)-1*H*-pyrazolo[3,4-c]pyridine-4-carboxylic acid (**10**) coupled via an amide bond to (2-methylsulfonyl-4-pyridyl)-methaneamine (**11**), 1-(2-(methylsulfonyl)pyridin-4-yl) cyclopropan-1-amine (**12**),[14] and (*S*)-1-(2-(methylsulfonyl) pyridin-4-yl)propan-1-amine (**13**),[15] respectively (Figure 1). Our efforts were concentrated on the synthesis of this acid as a common intermediate to access these compounds labeled with carbon-13 and carbon-14.

Thus, for carbon-13 synthesis, fluorobenzene-$^{13}C_6$ ([$^{13}C_6$]-(**4**)) was converted to 4-nitrofluorobenzene-$^{13}C_6$ ([$^{13}C_6$]-(**5**)) by using nitric acid in TFAA in 82% yield after separation of about 10% of the undesired 2-nitrofluorobenzene.[16] Reduction of the nitro group was accomplished in quantitative yield by using tin chloride dissolved in ethanol and ethyl acetate at 80°C to furnish [$^{13}C_6$]-(**6**) in quantitative yield. Conversion to the hydrazine [$^{13}C_6$]-(**7**) was completed in 82% yield by using sodium nitrate, tin chloride, and 6 M aqueous HCl.[17] Finally, condensation of [$^{13}C_6$]-(**7**) with 3,-5-dibromopyridine-4-carbaldehyde (**8**) gave the bromide [$^{13}C_6$]-(**9**) in 82% yield. This key bromide intermediate was then transformed to the acid [$^{13}C_6$]-(**10**) by using isopropyl magnesium chloride and lithium chloride solution in tetrahydrofuran (THF; turbo-Grignard) and carbon dioxide in 81% yield (Scheme 1).

With this intermediate in hand, the 3 drug candidates labeled with carbon-13 were then obtained via amide bond formation with the amines **11**, **12**, and **13** respectively using known reaction conditions like 1,1′-carbonyldiimidazole (CDI) activation of the acid or using propylphosphonic anhydride (T3P) and *N*-methyl morpholine (NMM) in *N*-methyl-2-pyrrolidone (NMP) as shown in Scheme 2.

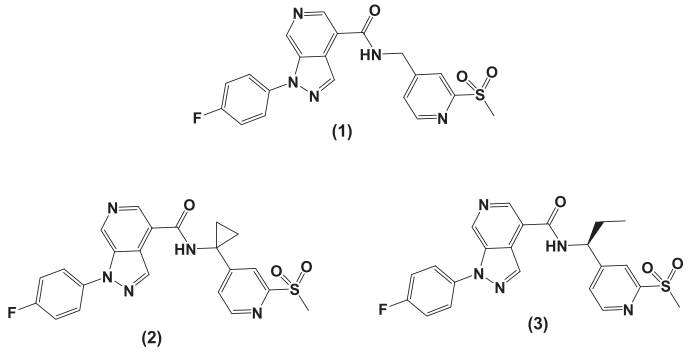
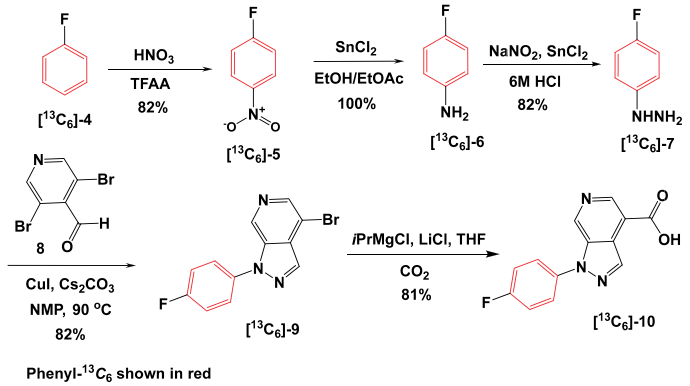
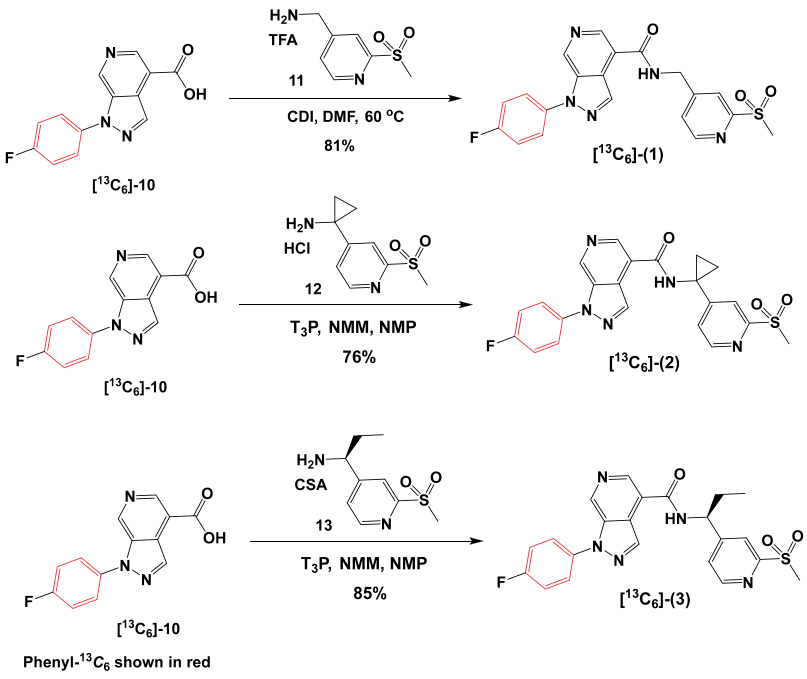


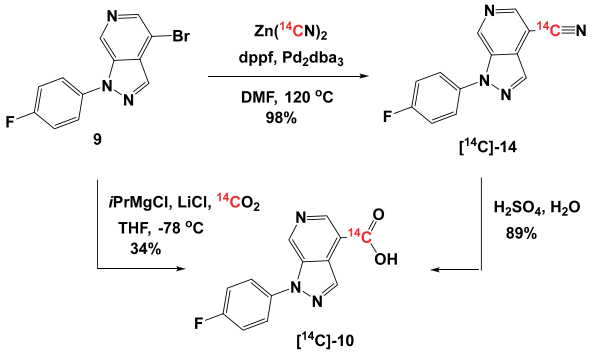
FIGURE 1 CCR1 antagonists



SCHEME 1 Synthesis of aryl carboxylic acid [$^{13}C_6$]-**10**



SCHEME 2 Synthesis of carbon-13 labeled CCR1 antagonists



SCHEME 3 Synthesis of aryl acid intermediate [^{14}C]-(**10**)

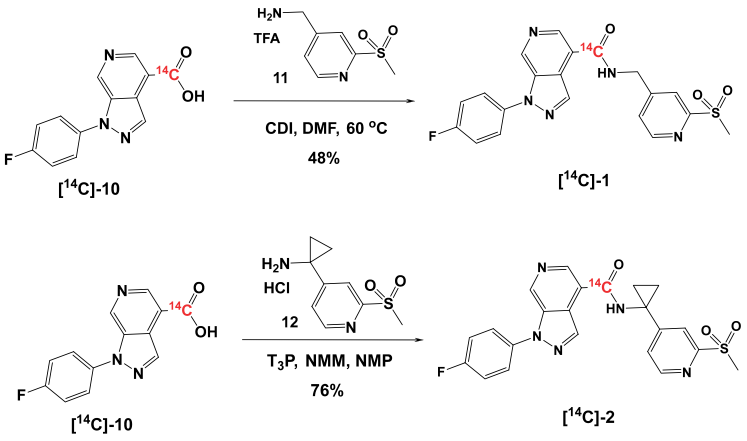
For carbon-14 synthesis, the bromide (**9**)[18] was either subjected to cyanation reaction by using zinc cyanide-^{14}C, followed by nitrile hydrolysis to [^{14}C]-(**10**) in 87% overall yield, or subjected to turbo-Grignard conditions described before and carbon-14 carbon dioxide albeit in poor yield (Scheme 3).

Amide bond formation with amines (**11**) and (**12**) as described before gave the desired carbon-14 labeled analogs (**1**) and (**2**) with specific activities of 59.5 and 58.3 mCi/mmol and radiochemical purities of 99.9% and 99.5%, respectively (Scheme 4).

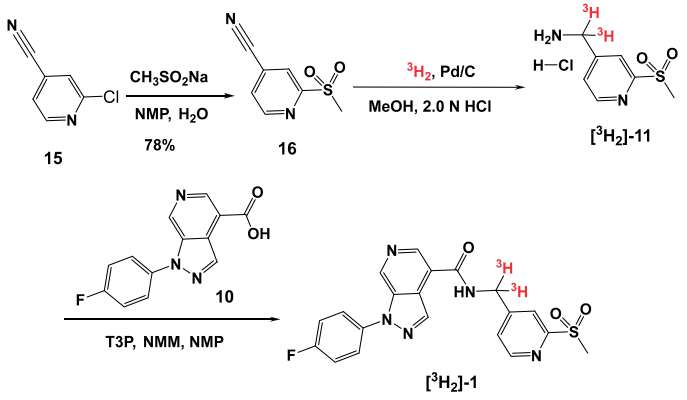
To prepare tritium labeled (**1**), 6-chloronicotinonitrile (**15**) was converted to 6-(methylsulfonyl)nicotinonitrile (**16**) in 78% yield, and then reduced under carrier-free tritium gas and palladium on carbon in methanol in the presence of 2 N aqueous HCl to the amine [$^{3}H_2$]-(**11**). Coupling to the acid, (**10**) gave tritium labeled (**1**) with a specific activity of 42.1 Ci/mmol and radiochemical purity of more than 98% (Scheme 5).

3 | CONCLUSION

1-(4-Fluorophenyl)-1*H*-pyrazolo[3,4-c]pyridine-4-carboxylic acid (2-methanesulfonyl-pyridin-4-ylmethyl)-amide (**1**) and its analogs (**2**) and (**3**) are potent CCR1 antagonists intended for the treatment of rheumatoid arthritis. These compounds labeled with carbon-13 were prepared starting from fluorobenzene-$^{13}C_6$ ([$^{13}C_6$]-(**4**)), which was converted in 3 steps to 4-fluorobenzene hydrazine ([$^{13}C_6$]-(**7**)) in 80% overall yield and then condensed with 3,5-dibromo-4-pyridinecarboxaldehyde (**8**) to



SCHEME 4 Synthesis of [^{14}C]-(**1**) and [^{14}C]-(**2**)



SCHEME 5 Synthesis of [$^{3}H_2$]-(**1**)

afford 4-bromo-1-(4-fluorophenyl-$^{13}C_6$)-1-*H*-pyrazolo [3,4-c]pyridine ([$^{13}C_6$]-(**9**)) in 80% yield. Treatment with turbo-Grignard (isopropyl magnesium chloride and lithium chloride solution) followed by carbonylation with carbondioxide gave the acid ([$^{13}C_6$]-(**10**)) in 81% yield. Amide bond formation with amines (**11**), (**12**), and (**13**) gave [$^{13}C_6$]-(**1**), [$^{13}C_6$]-(**2**), and [$^{13}C_6$]-(**3**) in good yields and in 99% isotopic enrichment. In carbon-14 synthesis, bromide (**9**) was converted to acid [^{14}C]-(**10**) by using either turbo-Grignard and [^{14}C]-CO_2 in 34% yield or via a 2-step synthesis utilizing Zn(^{14}CN)$_2$ followed by hydrolysis in 87% overall yield. Amide bond formation afforded [^{14}C]-(**1**) and [^{14}C]-(**2**) in 48% to 76% yield respectively with specific activities higher than 58 mCi/mmol and radiochemical purities higher than 99%. Finally, the amine [$^{3}H_2$]-(**11**) was obtained from the reduction of 4-cyano-2-methanesulfonylpyridine (**16**) under tritium gas in the presence of Pd/C and 2 N HCl in methanol and then coupled to the acid (**10**) to give [$^{3}H_2$]-(**1**) with a specific activity of 42 Ci/mmol and 98% radiochemical purity.

4 | MATERIALS AND METHODS

Fluorobenzene-$^{13}C_6$ ([$^{13}C_6$]-(**4**)) with isotopic enrichment of 99% and chemical purity of 99.7% was purchased from Cambridge Isotope Laboratories, Inc (Tewksbury, MA, USA). Zinc cyanide-^{14}C with a specific activity of 115.5 mCi/mmol was obtained from Moravek Biochemicals (Brea, CA, USA). Carbonylation using carbon-14 carbon dioxide and coupling to the amine (**11**) were performed at Moravek Biochemicals (Brea, CA, USA). Tritium reduction of a nitrile derivative (**16**) was performed by American Radiolabeled Chemicals (Saint Louis, MO, USA). 3,-5-Dibromopyridine-4-carbaldehyde (**8**) and 6-chloronicotinonitrile (**15**) were purchased from Sigma-Aldrich (WI, USA). Ultra performance liquid chromatography-mass spectrometry (UPLCMS) was acquired by using a medium polar method: run time 2.0 minutes, gradient 95% water (0.1% TFA), and 5% MeCN (0.1%TFA) to 5% water in 1.7 minutes, hold to 2 minutes at 5% water, flow 2.5 mL/min; column: Agilent Zorbax C18 SB (4.6 mm × 30 mm, 3.5 μm). The data were acquired on Waters Acquity™ Ultra Performance LC (Milford, MA, USA). Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker 400 and 500-MHz spectrometers by using deuterated NMR solvents (Sigma-Aldrich, USA). High-performance liquid chromatography (HPLC) was performed by using an HPLC system composed of an Agilent 1200, IN/US β-RAM-4, IN-FLOW™ counting solution (LabLogic systems, Inc. Brandon, FL, USA) and using *Laura Lite* software for data evaluation for radiolabeled compounds. High-performance liquid chromatography conditions, unless indicated otherwise: mobile phase: gradient A: MeCN (0.1% TFA), B: H_2O (0.1% TFA) 20% A to 100% A in 22 minutes, flow rate: 1 mL/min, column: Eclipse XDB C18 (4.5 mm × 150 mm, 2.7 μm). Liquid scintillation counting was carried out by using a Beckman LS6500 and Ultima Gold™ cocktail (Beckman, Fullerton, CA, USA).

5 | EXPERIMENTAL PROCEDURES

5.1 | Synthesis of carbon-13 labeled analogs

5.1.1 | 1-Fluoro-4-nitrobenzene-$^{13}C_6$, [$^{13}C_6$]-(5)

A solution of trifluoroacetic anhydride (TFAA, 13.2 mL, 94 mmol) and 40% nitric acid (5.9 g, 37.5 mmol) was prepared by adding TFAA to HNO_3 at low temperature (ice bath). This solution was then added at room temperature to flurorobenzene-$^{13}C_6$ ([$^{13}C_6$]-(**4**)) (2 g, 19.4 mmol) and stirred at room temperature for 2 hours. The reaction was poured into water (200 mL) and extracted with CH_2Cl_2 (200 mL × 2), dried over $MgSO_4$, filtered, and concentrated in vacuo to give 3.1 g of viscous oil. TLC: in 10% EtOAc:hexanes: R_f of desired product: 0.4, R_f of the 2-nitrofluorobenzene byproduct = 0.22. The crude material was purified by Combi-Flash by using 150-g disposable silica gel column and up to 10% EtOAc:hexanes. The tubes containing the desired product were combined and concentrated to give 2.35 g of material, which solidified at room temperature, in 82% yield. The byproduct 1-fluoro-2-nitrobenzene-$^{13}C_6$ eluted later and gave 0.4-g of a semisolid product in 11% yield. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 8.31 (dm, J = 166.95 Hz, 2H), 7.22 (dm, J = 164.52 Hz, 2H). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 165.5 (tdd, J = 9.71, 71.1, 257 Hz), 144.35 (dt, J = 7.95, 68.83 Hz), 126.27 (dt, J = 9.8, 71.64 Hz), 116.42 (m). ^{19}F-NMR ($CDCl_3$, 376 MHz) δ: −101.5 (d).

5.1.2 | 4-Fluoroaniline-$^{13}C_6$, [$^{13}C_6$]-(6)

A mixture of [$^{13}C_6$]-(**5**) (2.6 g, 17.7 mmol) and tin chloride dihydrate (16 g, 71 mmol) in ethyl acetate (40 mL) and ethanol (40 mL) was heated to reflux at 85°C and stirred for 4 hours. The reaction was then cooled to room temperature and poured into 200 mL of 1 N aqueous NaOH solution and extracted with ethyl acetate (200 mL × 3). The combined extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo to give 2.1 g (100% yield) of a colorless oil, turned amber after standing in the air.

TLC: 10% EtOAc:hexanes, base lane. This material was used as it is in the next step. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 6.95 (dm, J = 150.7 Hz, 2H), 6.71 (dm, J = 154.1 Hz, 2H), 3.61 (brs, 2H). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 158.3 (m), 155.2 (m),142.5 (m), 116.4 (m). ^{19}F-NMR ($CDCl_3$, 376 MHz) δ: −126.2 (d).

5.1.3 | (4-Fluorophenyl-$^{13}C_6$)hydrazine, [$^{13}C_6$]-(7)

A mixture of [$^{13}C_6$]-(**6**) (2.1 g, 18 mmol) and 20 mL of 6 N aq. HCl was stirred in an ice bath, while a solution of sodium nitrite (1.3 g, 18 mmol) in water (10 mL) was added dropwise in a 25-minute period. The resulting yellow solution was stirred for 30 minutes at 0°C before a solution of tin chloride hydrate (10.2 g, 44.3 mmol) in 20 mL of 6 N aqueous HCl solution was added dropwise in a 30-minute period. The resulting mixture was stirred for 14 hours. The solid was filtered and washed with concentrated HCl (10 mL) and dried under reduced pressure to give 2.95 of a solid material. The free base was isolated by treating the solid in water with 40% KOH until pH is about 8, extracted with EtOAc, and concentrated to give 2.1 g of material in 82% yield. TLC in 10% MeOH/CH_2Cl_2: R_f = 0.8 for product and R_f = 0.7 for starting aniline. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 9.35 (s, 1H), 7.18 (dm, J = 136.1 Hz, 2H), 6.92 (dm, J = 156.1 Hz, 2H), 3.33 (s, 2H). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 158.35 (m), 155.4 (m), 142.98 (m), 115.6 (m).

5.1.4 | 4-Bromo-1-(4-fluorophenyl-$^{13}C_6$)-1*H*-pyrazolo[3,4-c]pyridine, [$^{13}C_6$]-(9)

[$^{13}C_6$]-(**7**) (2.1 g, 16 mmol) was added to a solution of 3,5-dibromopyridine-4-carbaldehyde (**8**) (4.24 g, 16 mmol) and NMP (20 mL) as a slurry in NMP (8 mL) with stirring at room temperature. After stirring for 2 hours, HPLC showed no starting aldehyde and a new product at 12.9 minutes. Solid Cs_2CO_3 (14.8 g, 43 mmol) was added portion wise, followed by copper iodide (164 mg, 0.9 mmol), and the mixture was heated to 90°C and stirred for 4 hours. The reaction mixture turned dark. Water (55 mL) was added at 90°C, and the mixture was cooled slowly to room temperature (3-h period). The mixture was filtered, washed with water (20 mL), and dried under suction for 12 hours to give 5.1 g of a yellow fine powder. This material was heated to 80°C with 25 mL of toluene, then heptane (50 mL) was added slowly, keeping the temperature at around 80°C. After the addition was completed, the mixture was cooled to room temperature slowly and then stirred for 14 hours. Filteration of the solid and washing with heptane (40 mL), then drying at 61°C for 3 hours gave 4.2 g of a yellow-brown solid. TLC in 20% EtOAc:CH_2Cl_2: R_f = 0.62. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 9.02 (s, 1H), 8.52 (s, 1H), 8.28 (s, 1H), 7.72 (dm, J = 163.2 Hz, 2H), 7.34 (dm, J = 167.3 Hz, 2H). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 163.1 (ddt, J = 10.3, 71.6, 143.1 Hz), 138.5, 137.5, 136.2, 135.4, 135.1 (dt, J = 9.1, 65.1 Hz), 126.2, 125.2 (dt, J = 8.16, 65.1 Hz), 121.3, 116.6 (m).

5.1.5 | 1-(4-Fluorophenyl-$^{13}C_6$)-1*H*-pyrazolo[3,4-c]pyridine-4-carboxylic acid, [$^{13}C_6$]-(10)

[$^{13}C_6$]-(**9**) was placed in a dry 25-mL round bottom flask equipped with magnetic stirrer (0.51 g, 1.68 mmol). Tetrahydrofuran (3.5 mL) was added to the flask under N_2, and the resulting slurry was cooled to −20°C (internal probe). A solution of *i*PrMgCl and LiCl (2.0 M in THF, 1.26 mL) was added with such a rate to keep the internal T < −10°C. In about 1.5 hour, the slurry became brown solution. High-performance liquid chromatography sample was taken after 2.5 hours. Several drops were quenched with MeOH (1 mL). High-performance liquid chromatography indicated about 6% of starting material left. The reaction was stirred at −10°C for an additional 1 hour. Carbon dioxide was bubbled through the reaction solution until there was no rise in the temperature. Initially, the temperature rose from −10°C to 15°C then start dropping. Carbon dioxide was bubbled for 5 additional minutes after the temperature started dropping. The flask was taken out of the cooling bath, and the resulting violet solution was stirred for 14 hours at 20 to 25°C. One drop of the reaction mixture was added to 1 mL of MeOH to prepare an HPLC sample. High-performance liquid chromatography indicated 93% product and 6% of des-bromo compound. Isopropyl acetate (IPAC, 3.2 mL) was added in 1 portion at 25°C forming fine off-white slurry. A solution of 2.6 M aqueous HCl (0.1 mL) was added to the reaction mixture and heated to 55°C (internal probe). More aqueous HCl (2.4 mL) was added dropwise. The color of the reaction turned to yellow, then a slurry started to form and became thick. The reaction was removed from the heating bath and stirred at 25°C for 1 hour. The solid was filtered and washed with IPAC (10 mL) and water (10 mL), providing a yellow solid, which was further dried in a vacuum oven overnight at 45°C to give 350 mg of product in 80% yield. Ultra performance liquid chromatography-mass spectrometry: t_R = 0.69 minutes, MH^+ = 264.1 (100%). High-performance liquid chromatography: t_R = 4.58 minutes (99.8%). TLC: R_f = 0.12 in 10% MeOH/DCM. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 13.69 (s, 1H, exchangeable), 9.46 (s, 1H), 8.90 (s, 1H), 8.72 (s, 1H), 7.91 (dm, J = 175.1 Hz, 2H), 7.51 (dm,

J = 170.4 Hz, 2H). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 166.2, 162.2 (ddt, J = 10.2, 71.1, 244.8 Hz), 154.5, 151.2, 137.2, 138.9, 135.1 (dt, J = 9.12, 65.8 Hz), 134.7, 126.3, 125.1 (dt, J = 6.86, 63.5 Hz), 116.7 (m). HRMS, calculated 264.08746, found 264.08740.

5.1.6 | 1-(4-Fluorophenyl-$^{13}C_6$)-*N*-(1-(2-(methylsulfonyl)pyridin-4-yl)methyl)-1*H*-pyrazolo[3,4-*c*]pyridine-4-carboxamide, [$^{13}C_6$]-(1)

1,1′-Carbonyldiimidazole (75 mg, 0.45 mmol) was added to a mixture of [$^{13}C_6$]-(**10**) (94 mg, 0.36 mmol) in DMF (5 mL) to give a solution. After stirring at room temperature for 10 minutes, the reaction was heated to 60°C. A sample was taken with the tip of a pipet after 45 minutes and treated with 1 mL of MeOH and 3 drops of triethylamine and checked by HPLC; no starting acid was observed and UPLCMS 1 single peak, MH$^+$ = 278.57 corresponding to the methyl ester. The amine TFA-salt (**11**) (118 mg, 0.4 mmol) was added in 1 portion at 60°C and stirred for 1 hour. High-performance liquid chromatography showed a new product and UPLC-MS: MH$^+$ = 432.65 corresponding to the product. Water (10 mL) was added dropwise at 60°C, and the temperature was then ramped to room temperature slowly. The precipitate was filtered, washed with water (10 mL) and heptane (10 mL), and left to dry under vacuum overnight to give 125 mg as an off-white solid in 81% yield. Ultra performance liquid chromatography-mass spectrometry: t_R = 1.33, MH$^+$ = 432.65 (100%). High-performance liquid chromatography: t_R = 6.32 minutes (99%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 9.65 (s, 1H), 9.12 (s, 1H), 8.75 (d, 2H), 8.63 (d, 1H), 8.10 (s, 1H), 7.70 (dm, 2H), 7.71 (t, 1H), 7.28 (dm, 2H), 4.84 (d, 2H), 3.32 (s, 3H). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 162.35, 161.5 (dt), 160.1, 158.03, 150.45, 148.2, 137.47 (d), 135.03, 130.60, 130.07, 129.43 (t), 125.51, 125.12 (d), 121.4, 118.1, 116.27 (m), 42.63, 40.15. HRMS: (MH$^+$), calculated 432.12281, found 432.12303.

5.1.7 | 1-(4-Fluorophenyl-$^{13}C_6$)-*N*-(1-(2-(methylsulfonyl)pyridin-4-yl)cyclopropyl)-1*H*-pyrazolo[3,4-*c*]pyridine-4-carboxamide, [$^{13}C_6$]-(2)

The acid [$^{13}C_6$]-(**10**) (170 mg, 0.63 mmol) and amine HCl-salt (**12**) (241 mg, 0.95 mmol) were placed in a 15-mL round-bottom flask, and the flask was flushed with N_2. *N*-Methyl-2-pyrrolidone (0.6 mL) was added via syringe under N_2 forming a suspension. *N*-Methyl morpholine (522 mg, 5.07 mmol) was added to the suspension at room temperature and stirred for 30 minutes, forming a light-brown thick reaction mixture. Propylphosphonic anhydride (604 mg, 0.95 mmol, 50% in EtOAc) was added dropwise with cooling (water bath). The reaction was heated at 70°C for 2.5 hours. High-performance liquid chromatography showed complete conversion. Water (1.6 mL) was added to the reaction mixture at 70°C and allowed to stirr at room temperature for 1 hour. The formation of an off-white residue was observed. The reaction vessel was cooled for 1 hour by using an ice bath to complete the crystallization. The residue was filtered by using a paper filter, washed with water (10 mL), then IPAC (5 mL). The solid was air-dried on the filter for 3 hours and then in a vacuum oven for 14 hours at 60°C to give 220 mg of material in 76% yield. Ultra performance liquid chromatography-mass spectrometry: 0.73 minute (100%), MH$^+$ = 458.11. High-performance liquid chromatography: t_R = 2.48 minutes (99.8%). ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 9.75 (s, 1H, NH), 9.73 (s, 1H), 9.42 (s, 1H), 8.94 (s, 1H), 8.68 (d, 1H), 8.66 (d, 1H), 7.92 (dm, 2H), 7.80 (d, 1H), 7.53 (d, 1H), 6.98 (dm, 2H), 3.28 (s, 3H), 1.61 (m, 4H). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 166.2, 161.8, 160.2 (dt), 158.2, 156.3, 150.3, 138.6, 137.7, 135.3 (m), 135.1, 126.7, 124.9 (t), 124.1, 122.9, 121.4, 116.7 (m), 115.1, 39.8, 34.5, 20.4. HRMS: [$C_{16}{}^{13}C_6H_{19}N_5O_3SF$]$^+$, calculated 458.13884, found 458.13852.

5.1.8 | (*S*)-1-(4-Fluorophenyl-$^{13}C_6$)-*N*-1-(2-(methylsulfonyl)pyridin-4-yl)propyl)-1*H*-pyrazolo[3,4-*c*]pyridine-4-carboxamide, [$^{13}C_6$]-(3)

N-Methyl morpholine (5 mL) was added to a mixture of [$^{13}C_6$]-(**10**) (346 mg, 1.3 mmol) and the amine camphorsulfonic acid-salt (**13**) (0.59 g, 1.32 mmol) in NMP (5 mL), and the solution was stirred for 15 minutes. Propylphosphonic anhydride (50% solution in EtOAc, 1.1 mL, 1.85 mmol) was added dropwise at room temperature. The solution was then heated to 60°C and stirred for 90 minutes. High-performance liquid chromatography indicated about 30% of remaining starting material. One more equivalent of the amine and 1.4 equivalent of T3P were added successively and stirred for 14 hours. Water was added slowly, and the mixture was cooled to room temperature overnight. The aqueous was extracted with EtOAc (150 mL × 2), and the combined extracts were washed with water (100 mL × 3), dried over $MgSO_4$, filtered, and concentrated in vacuo to give 887.5 mg of a yellow residue. The crude product was crystallized from 2-butanone (3 mL) and heptane (3 mL) by dissolving first the solid in 2-butanone at 75°C, then adding heptane slowly and cooling to room temperature. The

product was filtered and dried in vacuo to give 513 mg of product more than 99% pure in 85% yield. Ultra performance liquid chromatography-mass spectrometry: t_R = 0.82 minute (100%), MH^+ = 458.38. High-performance liquid chromatography: Eclipsee, XDB-C18 (4.6 × 150 mm, 1.8 μm). A = 0.2% H_3PO_4 and 60-mM NH_4PF_6 in water, B MeCN, flow rate 1.5 mL/min, run time 12 minutes, column temperature 65°C, DAD 210 nm, R_t = 4.81 minutes, 99.5%. Chiral HPLC: column Chiralcel AD-H (0.46 × 250 mm), mobile phase: A heptane with 0.1% acetic acid; B = EtOH isocratic A/B: 50/50. Flow 1 mL/min, run time 16 minutes, column temperature 20°C 5-μL injection, DAD 220 nm. The sample was prepared in methanol. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 9.41 (s, 1H), 9.37 (d, 1H), 8.95 (s, 1H), 8.75 (d, 1H), 8.64 (s, 1H), 8.17 (s, 1H), 7.91 (dm, 2H), 7.82 (dd, 1H), 7.51 (dm, 2H), 5.18 (m, 1H), 3.30 (s, 3H), 1.93 (m, 2H), 1.01 (t, 3H). ^{13}C-NMR (DMSO-d_6, 100 MHz) δ: 164.7, 161.1 (dt), 158.1, 155.8, 153.4, 150.2, 138.6, 137.5, 135.3 (dt), 135.1, 126.7, 125.9, 124.2 (dt), 121.6, 118.1, 116.6 (m), 39.7, 35.6, 28.3, 11.1. HRMS: calculated 460.15449, found 460.15429.

5.2 | Carbon-14 synthesis

5.2.1 | 1-(4-Fluoro-phenyl)-1*H*-pyrazolo[3,4-c]pyridine-4-carboxylic acid-^{14}C, [^{14}C]-(10)

4-Bromo-1-(4-fluoro-phenyl)-1*H*-pyrazolo[3,4-c]pyridine (**9**) (1.1 g, 3.62 mmol) in THF (10 mL) was cooled to 0°C. A solution of *i*-PrMgCl.LiCl (1.3 M THF, 2.89 mL, 3.75 mmol) was added slowly. The resulting solution was stirred at 0°C for 1 hour. Carbonation was performed at −20°C for 2 hours. Then, a solution of aqueous 3 N HCl (12 mL) was added. The aqueous was extracted with CH_2Cl_2, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography by using 3:1 CH_2Cl_2:MeOH to give 81 mCi of product in 38% yield. The product coeluted on TLC and HPLC with the unlabeled (**10**).

5.2.2 | 1-(4-Fluoro-phenyl)-1*H*-pyrazolo[3,4-c]pyridine-4-carboxylic-^{14}C-acid-(2-methanesulfonyl-pyridin-4-ylmethyl)-amide, [^{14}C]-(1)

The above acid (81 mCi, 1.4 mmol) and CDI (291 mg, 1.79 mmol) were stirred in DMF (6 mL) for 20 minutes at room temperature. The temperature of the mixture was slowly raised to about 60°C. After 2 hours at this temperature, the amine-TFA salt (**11**) (441 mg, 1.46 mmol) in DMF (1.5 mL) is added to the above solution at 55 to 60°C. Stirring was continued for 1.5 hour. Water (10 mL) was added at a rate to maintain the batch at 55 to 60°C and held for 10 minutes at about 60°C. The mixture was then cooled down to room temperature over 2 hours and held for at least 1 hour at this temperature. The solid was filtered and washed with water (2 mL × 3) and heptane (2 mL) and then dried under vacuum to give 280 mg (38.6 mCi) after flash chromatography purification. The product was obtained as a light yellow solid, with a radiopurity of 99.9% by using the following HPLC conditions: Supelco Discovery C18 (4.6 × 250 mm), flow rate; 1 mL/min, mobile phase: A: water, 0.1% TFA; B: MeCN, 0.1% TFA, 10 to 30 minutes, 20-100%B held to 40 minutes. MS: MH^+ = 426.24 (100%), 427.27 (20%). Specific activity = 59.5 mCi/mmol. ^{1}H-NMR (DMSO-d_6, 500 MHz) δ: 9.65 (t, 1H, NH), 9.45 (s, 1H), 8.93 (s, 1H), 8.74 (s, 2H), 8.08 (s, 1H), 7.94 (m, 2H), 7.77 (d, 1H), 7.47 (m, 2H), 4.75 (d, 2H), 3.30 (s, 3H).

5.2.3 | 1-(4-Fluorophenyl)-1*H*-pyrazolo[3,4-c]pyridine-4-carbonitrile-^{14}C, [^{14}C]-14

A mixture of (**9**) (0.5 g, 1.73 mmol) and zinc cyanide-^{14}C (100 mCi, SA = 115.5 mCi/mmol) in anhydrous DMF (8.0 mL) in a screw cap 24 mL vial was degassed 3 times, and Argon was introduced in every cycle. Dppf (261 mg, 0.5 mmol) and $Pd_2(dba)_3$ (916 mg, 0.21 mmol) were introduced to the reaction vial and degassed 3 more times. The mixture was heated to 120°C and stirred for 14 hours. A solution of 2 N NH_4OH (50 mL) was added, and the mixture was extracted with EtOAc. The combined extracts were filtered through a short pad of silica gel and rinsed with EtOAc. The solution was concentrated to give a dark residue (0.45 g), which was used as is in the next step.

5.2.4 | 1-(4-Fluorophenyl)-1*H*-pyrazolo[3,4-c]pyridine-4-carboxylic-^{14}C acid, [^{14}C]-(10)

Sulfuric acid (20 mL) and water (30 mL) were added to the above crude nitrile (0.42 g, 1.74 mmol). The mixture was heated to 124°C and stirred for 4 hours. The resulting mixture was filtered off and washed with water. The aqueous solution was cooled in an ice bath and treated with aqueous 4 N NaOH (100 mL) until a precipitate appeared. The mixture was extracted with EtOAc (150 mL × 3), and the combined extracts were dried over $MgSO_4$, filtered, and concentrated in vacuo to give 400 mg (90 mCi) of a yellowish powder in 89% yield. High-performance liquid chromatography coeluted with unlabeled (**10**).

5.2.5 | 1-(4-Fluorophenyl)-*N*-{1-[2-methylsulfonyl)pyridin-4-yl]cyclopropyl}-1*H*-pyrazolo[3,4-c]pyridine-4-carboxamide-^{14}C, [^{14}C]-(2)

N-Methyl morpholine was added to a mixture of the above acid (0.4 g, 1.54 mmol) and the HCl-salt of amine (**12**) (0.5 g, 2.0 mmol) in NMP (5 mL), and the solution was stirred for 15 minutes. Propylphosphonic anhydride (3.0 mL, 50% solution in EtOAc) was added dropwise at room temperature. The solution was then heated to 65°C and stirred overnight. High-performance liquid chromatography showed that about 4% of the acid was left. Another batch of T3P (0.5 mL) was added, and the dark mixture was stirred for another 3 hours. Water (40 mL) was added, and the mixture was cooled to room temperature. The mixture was extracted with CH_2Cl_2 (50 mL × 3), and the combined extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo to give 5.6 g of an NMP solution of the product. The organic phase was heated to 65°C, and water 7 mL was added slowly. The mixture was stirred at 65°C for 1 hour and then at room temperature for 14 hours. The mixture was filtered, washed with water (20 mL), then with heptane (10 mL). The solid was dried to give 470 mg of an off-white solid in 76% yield, 90% pure. Further purification by silica gel chromatography by using 40-g disposable silica gel column and up to 5% MeOH/CH_2Cl_2 afforded 230 mg of a white solid, 29.5 mCi. Ultraviolet and rad indicated a chemical and radiochemical purity of more than 99% and with a specific activity of 58.3 mCi/mmol.^{1}H-NMR ($DMSO_6$, 500 MHz) δ: 9.74 (s, 1H), 9.42 (s, 1H), 8.94 (s, 1H), 8.69 (s, 1H), 8.66 (d, 1H), 7.92 (dd, 1H), 7.81 (s, 1H), 7.51 (m, 3H), 3.28 (s, 3H), 1.60 (brs, 4H). High-performance liquid chromatography: column Zorbax Eclipse XDB C18 (4.6 × 50 mm, 1.8 μm). Mobile phase: A: water (0.1% H_3PO_4, 0.2% $HClO_4$); B: MeCN: MeOH (19:10), t_R = 6.37 minutes, 99.4%, rad HPLC 99.5%.

5.3 | Tritium synthesis

5.3.1 | 4-Cyano-2-methylsulfonylpyridine (16)

A slurry of 2-chloro-4-cyanopyridine (**15**) (5 g, 36.1 mmol) and sodium methane sulfinate (4.4 g, 43 mmol) in NMP (10 mL) was heated to 125°C and stirred for 14 hours. After cooling to 35°C, water (30 mL) was added slowly. The mixture was cooled to room temperature and stirred for 4 hours. The mixture was filtered, washed with water (50 mL), and dried under suction. The off-white solid was further dried in a vacuum oven at 65°C for 48 hours to give 5.1 g of a solid. ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 9.21 (d, 1H), 8.62 (d, 1H), 7.95 (d,1H), 3.32 (s, 3H). ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 161.3, 152.4, 131.8, 123.7, 122.1, 119.6, 43.7 ppm.

5.3.2 | (2-Methylsulfonyl-4-pyridyl)-ditritio-methanamine hydrochloride, [$^3H_2$]-(11)

Methanol (0.5-1.0 mL) and aqueous HCl (2.0 M, 25 μL) were added to a mixture of (**16**) (9 mg, 39.7 μmol) and Pd/C (10%, 10 mg). The mixture was stirred under 1 atm of tritium gas for 20 hours. High-performance liquid chromatography showed complete conversion to desired product. A batch of 1.75 Ci of crude product was obtained.

5.3.3 | 1-(4-fluorophenyl)-*N*-[(2-methylsulfonyl-4-pyridyl)-ditritio-methyl] pyrazolo[3,4-c]pyridine-4-carboxamide, [$^3H_2$]-(1)

N-Methyl morpholine (50 μL, 0.44 mmol) was added to a mixture of the acid (**10**) (4.24 mg, 16.5 μmol) and [$^3H_2$]-(**11**) (3.74 mg, 16.5 μmol) in NMP (2 mL), and the solution was stirred for 15 minutes. Propylphosphonic anhydride (50% solution in EtOAc, 0.3 mL) was added dropwise at room temperature. The solution was then heated to 65°C and stirred overnight. After cooling to room temperature, the solution was treated with water (10 mL) and extracted with EtOAc (5 mL × 3). The combined extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue with some NMP was diluted in 6 mL of methanol and saved at −20°C. A portion of this material (1 mL, about 300 mCi) was purified by semipreparative HPLC to give 175 mCi of product with a specific activity of 42.1 Ci/mmol. Semipreparative conditions: system Agilent 1100 using Zorbax XDB C18 (4.6 × 150 mm, 5 μm) column, 50-μL injection size, 2-mL/min flow rate, mobile phase A: water (0.1% TFA) and B: MeCN; gradient 0 to 6 minutes 80% A, 6 to 15 minutes 80% A to 5% A. High-performance liquid chromatography, t_R = 6.76 minutes (98.5%). Column: Halo C18 (4.6 × 150 mm, 2.7 μm) gradient 10% to 95% MeCN: water (0.1% TFA) in 15 minutes, co-eluted with unlabeled material. ^{3}H-NMR (DMSO-d_6, 427 MHz) δ: ^{1}H-decoupled, 4.72(s); ^{1}H-coupled, 4.72 (d, J = 8.5 Hz).

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