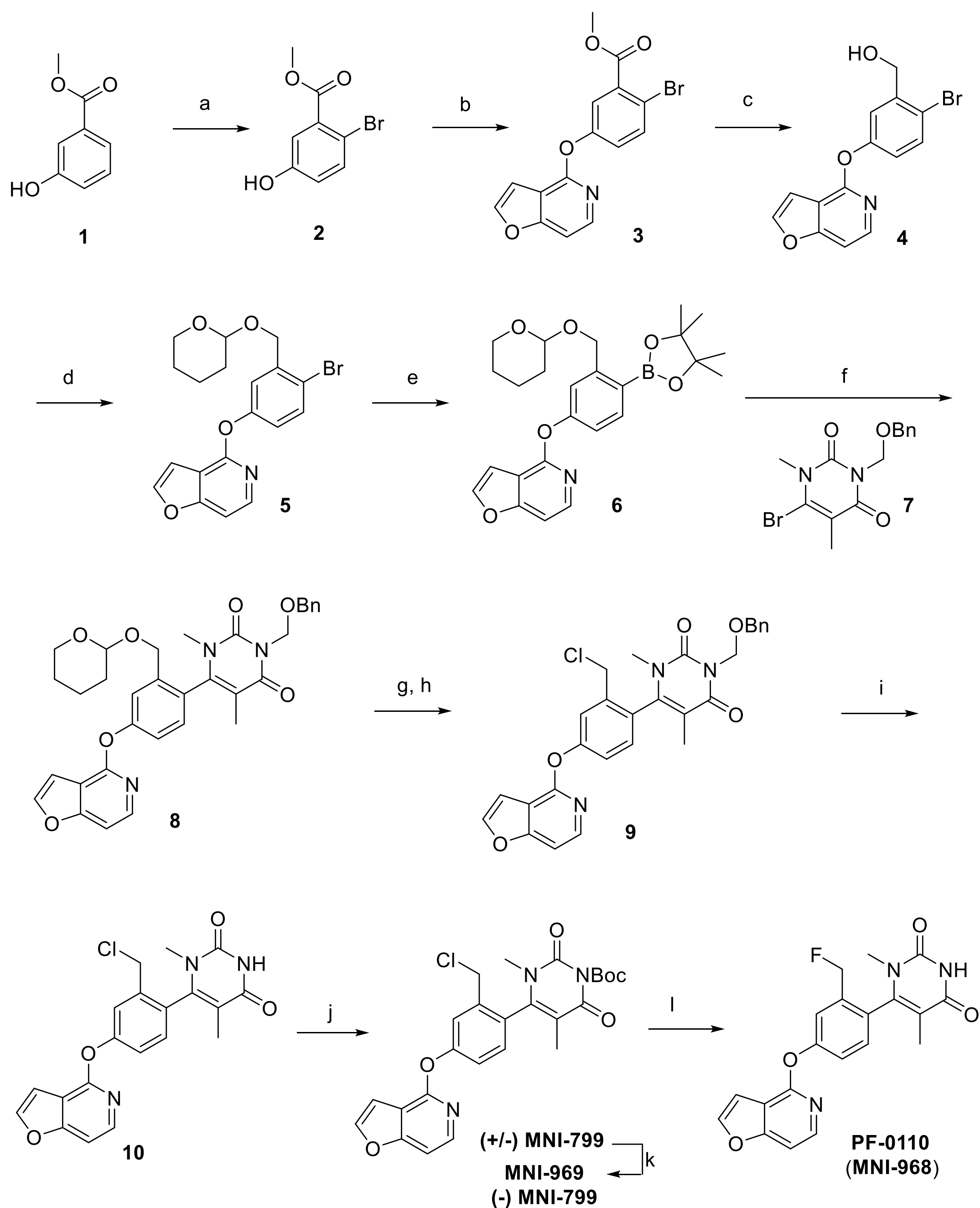
Synthesis of MNI-968 (PF-0110) and MNI-800 (PF-8477) and their precursor MNI-969 and MNI-799, respectively

The synthetic route used to prepare MNI-968 (PF-0110) and its corresponding labeling precursor MNI-969 is illustrated in Scheme 1. The synthesis of the precursor MNI-969 involves ten steps starting from commercially available methyl 3-hydroxylbenzoate (**1**). Bromination using bromine at room temperature in dichloromethane provided compound **2** in 99% yield. The subsequent SnAr reaction with 4-chlorofurano[3,2-c]pyridine yielded ester **3**, which upon reduction using lithium borohydride provided the corresponding benzyl alcohol **4**. THP protection was successfully achieved in high yield (99%) by treating compound **4** with 3,4-dihydro-2H-pyran in the presence of p-toluenesulfonic acid monohydrate. The resulted compound **5** was then converted to boronic ester **6**, which was subsequently subjected to coupling reaction with benzyloxy methyl (BOM) protected bromouracil **7** to give the desired product **8**. Selective removal of the THP protecting group by 4N HCl in 1,4-dioxane and the resulted benzyl alcohol was converted to the corresponding benzyl chloride **9** in nearly quantitative yield. The BOM protecting group was then removed using a two-step procedure involving treatment with BCl_3 in dichloromethane from -78 ^oC to room temperature, followed by 1N HCl in 1,4-dioxane at 80 ^oC. The resulted uracil **10** was re-protected by the Boc group to give MNI-799, the racemic precursor of MNI-800 (PF-8477). Chiral separation of the two atropisomers was achieved using the condition detailed in Supplemental Scheme 1 to yield the desired atropisomer MNI-969 as a white solid with an optical rotation of -6.6 degrees and chiral purity of 98.15%. Finally, MNI-969 was converted to MNI-968 in one-step upon treatment with potassium fluoride in DMSO at 120 ^oC for 10 min, confirming the viability of late-stage fluorination as a radiolabeling strategy.

Supplemental SCHEME 1: Synthesis of **MNI-968** and its radiolabeling precursor **MNI-969**



Reagents and conditions: a) Br_2, DCM, rt, 99%; b) 4-Chlorofurano[3,2-c]pyridine, Cs_2CO_3, DMSO, 80 ºC, then addition of MeI, rt, 53%; c) $LiBH_4$, THF, 65 ºC, 81%; d) 3,4-DHP, *p*-TsOH, DCM, rt, 99%; e) bis(pinacolato)diboron, Pd(dppf)Cl_2, KOAc, 1,4-dioxane, 100 ºC, 85%; f) compound 7, Pd(dppf)Cl_2, 3M K_2CO_3 aqueous solution, 1,4-dioxane, 71%; g) 4N HCl in 1,4-dioxane, DCM, rt, 63%; h) $SOCl_2$, DCM, rt, 99%; i) BCl_3, DCM, -78 ºC to rt, then 1N HCl, 1,4-dioxane, 80 ºC, 99%; j) Boc_2O, Et_3N, DMAP, THF, rt; k) chiral separation using the following condition. Column: Chiral Tech IC 250mm x 21.2 mm 5 micron; Isocratic Conditions: mobile phase A, 80% CO_2; mobile phase B, 20% 75:25:0.2% MeOH: EtOAc: ammonia; Flow rate: 80.0 mL/min; l) KF, kryptofix, DMSO, 120 ºC, 43%.

Radiochemistry of ^{18}F-MNI-800 (^{18}F -PF-8477) and ^{18}F -MNI-968 (^{18}F -PF-0110)

Materials: Components and reagents used for the radiolabeling were obtained from the following sources: Acetonitrile anhydrous (Sigma-Aldrich, part # 271004), Acetonitrile HPLC (Sigma-Aldrich, part # 34998), Sep-Pak QMA (ABX, part # K-925), Potassium carbonate (Sigma-Aldrich, part # 367877), Kryptofix 222 (ABX, part # 8000.1000), Dimethylsulfoxide anhydrous (Sigma-Aldrich, part # 276855), Hydrochloric acid (Sigma-Aldrich, part # 1.09057), Ethanol 200 Proof (Pharco-Aaper, part # 111000200), Water HPLC (Sigma-Aldrich, part # 270733), triethylamine (Sigma-Aldrich, part # 471283), Ammonium acetate (Sigma-Aldrich, part # 17836), Sodium ascorbate (Mylan, part # NDC 67457-118-50), Sep-Pak tC18 (Waters, part # WAT036805), Sep-Pak Oasis HLB light (Waters, part # 186005125), Methanol HPLC (Sigma-Aldrich, part # 34860), Saline 0.9% (Hospira, part # NDC0409488806), Water for injection (Hospira, part # NDC04094887-24), Filter 0.2-0.22µm (Millipore, part # SLLG013SL), Sterile vial (Hospira, part # 5816-31).

^{18}F-MNI-800 (3): In a typical procedure, ^{18}F-fluoride in a shipping vial (target water obtained from a cyclotron facility) is transferred onto and trapped on a Sep-Pak QMA light ion exchange cartridge. It is then eluted with an aqueous acetonitrile solution (1 mL) of potassium carbonate (1.25 mg) and Kryptofix 222 (10 mg) into the reaction vessel of the TRACERlab® module. The solution is first evaporated by heating at 95°C for 4 min under vacuum and helium flow. Acetonitrile (1 mL) is added to the reactor and the evaporation is continued under the same conditions for 2 min under vacuum. After a second addition of acetonitrile (1 mL), final evaporation is carried out at 95°C for 2 min under vacuum and helium flow. The reactor is then cooled to 50°C. A solution of the precursor MNI-799 (**1**, 1.0 mg) in anhydrous dimethylsulfoxide

(1.0 mL) is added to the reaction vessel and the reaction mixture is heated at 80°C for 5 min. After 5 min, the reactor is cooled to 70°C, hydrochloric acid (1M, 1.0 mL) is added and heated at 70°C for 5 min before being cooled to 40°C. The mixture is transferred to the HPLC loading vial. The reactor is rinsed with HPLC mobile phase (2.0 mL) and transferred to the HPLC loading vial, pre-filled with NaOH (1M, 1.1 mL). The entire contents of vial are transferred into the HPLC injector loop for purification. Purification is performed by column with a Phenomenex Luna C18 (2) (10 µm, 250 x10 mm) and eluted with a mixture of ethanol/water/triethyl amine solution (30/70/0.1 v/v/v) at a flow rate of 4 mL/min. The product fraction is collected into the collection flask, containing 25 mL of ascorbic acid (10 mg/mL) in WFI. The diluted product mixture is passed through a tC18 solid-phase extraction cartridge and the cartridge is rinsed with 10 mL of ascorbic acid (10 mg/mL) in WFI. The radiolabeled product is eluted from the SPE cartridge with 1 mL of 200-proof USP grade ethanol into the formulation flask, pre-loaded with 10 mL of formulation base (ascorbic acid (0.7 mg/mL) in 0.9% saline). The cartridge is rinsed with 4 mL of formulation base and the rinse is mixed with the contents of the formulation flask. The resulting solution is passed through a sterilizing 0.2 µm membrane filter into a sterile, filter-vented vial (final product vial, FPV), pre-filled with 15 mL of saline.

^{18}F-MNI-968 (4): In a typical procedure, ^{18}F-fluoride in a shipping vial (target water obtained from a cyclotron facility) is transferred onto and trapped on a Sep-Pak QMA light ion exchange cartridge. It is then eluted with an aqueous acetonitrile solution (1 mL) of potassium carbonate (1.25 mg) and Kryptofix 222 (10 mg) into the reaction vessel of the TRACERlab® module. The solution is first evaporated by heating at 95°C for 4 min under vacuum and helium flow. Acetonitrile (1 mL) is added to the reactor and the evaporation is continued under the same

conditions for 2 min under vacuum. After a second addition of acetonitrile (1 mL), final evaporation is carried out at 95°C for 2 min under vacuum and helium flow. The reactor is then cooled to 50°C. A solution of the precursor MNI-969 (**2**, 0.7 mg) in anhydrous acetonitrile (0.7 mL) is added to the reaction vessel and the reaction mixture is heated at 80°C for 5 min. After 5 min, the reactor is cooled to 70°C, hydrochloric acid (1M, 0.8 mL) is added and heated at 70°C for 4 min before being cooled to 40°C and diluted with water for injection (WFI) (10 mL). The mixture is transferred from the reactor onto an intermediate solid phase extraction cartridge (SPE, Oasis HLB light). The reactor is rinsed with methanol (2.0 mL) and transferred through SPE to elute the product into HPLC loading vial, pre-filled with WFI (3.0 mL). The entire contents of vial are transferred into the HPLC injector loop for purification. Purification is performed by HPLC using a semi-preparative Chiralcel OJ-H column (5 µm, 250 x 10 mm) connected to a Phenomenex Luna C18 (2) (10 µm, 250 x10 mm) and eluted with a mixture of acetonitrile/ammonium acetate solution (5 mM) (40/60, v/v) at a flow rate of 4 mL/min. The product fraction is collected into the collection flask, containing 25 mL of ascorbic acid (10 mg/mL) in WFI. The diluted product mixture is passed through a tC18 solid-phase extraction cartridge and the cartridge is rinsed with 10 mL of ascorbic acid (10 mg/mL) in WFI. The radiolabeled product is eluted from the SPE cartridge with 1 mL of 200-proof USP grade ethanol into the formulation flask, pre-loaded with 10 mL of formulation base (ascorbic acid (0.7 mg/mL) in 0.9% saline). The cartridge is rinsed with 4 mL of formulation base and the rinse is mixed with the contents of the formulation flask. The resulting solution is passed through a sterilizing 0.2 µm membrane filter into a sterile, filter-vented vial (final product vial, FPV).

Supplemental TABLE 1

^{18}F-MNI-800 kinetic parameters of 2T model in pooled rhesus and cynomolgus macaques (n = 4, mean ± standard deviation (cov)).

Region	K_1	k_2	k_3	k_4	K_1/k_2	k_3/k_4
Striatum	**0.28 ± 0.06 (21%)**	**0.11 ± 0.04 (38%)**	**0.039 ± 0.052 (133%)**	**0.069 ± 0.050 (72%)**	**2.69 ± 0.45 (17%)**	**0.39 ± 0.36 (92%)**
Caudate	**0.25 ± 0.05 (20%)**	**0.10 ± 0.04 (40%)**	**0.041 ± 0.054 (130%)**	**0.062 ± 0.044 (70%)**	**2.52 ± 0.53 (21%)**	**0.46 ± 0.40 (88%)**
Putamen	**0.31 ± 0.08 (24%)**	**0.11 ± 0.04 (37%)**	**0.037 ± 0.051 (137%)**	**0.076 ± 0.055 (73%)**	**2.86 ± 0.41 (14%)**	**0.33 ± 0.32 (96%)**
Nucleus accumbens	**0.24 ± 0.05 (19%)**	**0.11 ± 0.03 (26%)**	**0.020 ± 0.024 (119%)**	**0.050 ± 0.036 (72%)**	**2.19 ± 0.19 (9%)**	**0.33 ± 0.18 (53%)**
Globus pallidus	**0.21 ± 0.06 (27%)**	**0.10 ± 0.05 (45%)**	**0.052 ± 0.066 (127%)**	**0.084 ± 0.072 (86%)**	**2.18 ± 0.45 (20%)**	**0.39 ± 0.38 (97%)**
Thalamus	0.27 ± 0.06 (21%)	0.21 ± 0.15 (70%)	0.037 ± 0.046 (125%)	0.081 ± 0.048 (59%)	1.61 ± 0.62 (39%)	0.35 ± 0.30 (84%)
Frontal cortex	0.21 ± 0.02 (8%)	0.12 ± 0.00 (2%)	0.007 ± 0.002 (27%)	0.025 ± 0.001 (4%)	1.79 ± 0.12 (7%)	0.27 ± 0.08 (30%)
Cerebellum	0.29 ± 0.04 (15%)	0.20 ± 0.04 (19%)	0.025 ± 0.019 (73%)	0.075 ± 0.052 (69%)	1.45 ± 0.07 (5%)	0.34 ± 0.13 (39%)

Supplemental TABLE 2

^{18}F-MNI-968 kinetic parameters of 2T model in pooled rhesus and cynomolgus macaques (n = 3, mean ± standard deviation (cov)).

Region	K_1	k_2	k_3	k_4	K_1/k_2	k_3/k_4
Striatum	**0.30 ± 0.07 (23%)**	**0.21 ± 0.22 (104%)**	**0.325 ± 0.535 (165%)**	**0.111 ± 0.095 (86%)**	**2.48 ± 1.43 (58%)**	**1.55 ± 2.24 (145%)**
Caudate	**0.26 ± 0.04 (17%)**	**0.15 ± 0.13 (84%)**	**0.206 ± 0.322 (157%)**	**0.103 ± 0.071 (70%)**	**2.36 ± 1.24 (53%)**	**1.25 ± 1.63 (131%)**
Putamen	**0.34 ± 0.10 (28%)**	**0.26 ± 0.30 (116%)**	**0.444 ± 0.747 (168%)**	**0.118 ± 0.120 (101%)**	**2.59 ± 1.64 (63%)**	**1.84 ± 2.86 (155%)**
Nucleus accumbens	**0.28 ± 0.12 (42%)**	**0.20 ± 0.19 (95%)**	**0.162 ± 0.254 (156%)**	**0.094 ± 0.080 (86%)**	**1.88 ± 0.78 (42%)**	**1.04 ± 1.22 (118%)**
Globus pallidus	**0.22 ± 0.09 (41%)**	**0.13 ± 0.12 (89%)**	**0.309 ± 0.528 (171%)**	**0.159 ± 0.214 (135%)**	**2.12 ± 0.82 (39%)**	**0.83 ± 1.25 (150%)**
Thalamus	0.26 ± 0.05 (21%)	0.18 ± 0.12 (68%)	0.168 ± 0.274 (163%)	0.124 ± 0.120 (97%)	1.77 ± 0.68 (39%)	0.72 ± 0.97 (134%)
Frontal cortex	0.25 ± 0.05 (18%)	0.13 ± 0.02 (17%)	0.013 ± 0.008 (58%)	0.041 ± 0.027 (64%)	1.93 ± 0.07 (4%)	0.33 ± 0.03 (8%)
Cerebellum	0.30 ± 0.08 (25%)	0.20 ± 0.07 (33%)	0.332 ± 0.487 (147%)	0.125 ± 0.083 (66%)	1.53 ± 0.14 (9%)	0.28 ± 0.18 (64%)

Supplemental TABLE 3

^{18}F-MNI-800 (n=2) and ^{18}F-MNI-968 (n=1) V_T and BP_{ND} test-retest variability. ^{18}F-MNI-800 (^{18}F-MNI-968).

	V_T		BP_{ND}			
Region	**2T**	**LGA**	**2T**	**LGA**	**SRTM**	**NI-LGA**
Striatum	**1.4% (7.5%)**	**3.3% (8.5%)**	**1.7% (16.2%)**	**3.6% (16.4%)**	**3.9% (14.8%)**	**3.7% (14.7%)**
Caudate	**1.9% (4.9%)**	**3.4% (6.2%)**	**3.2% (12.5%)**	**3.9% (13.1%)**	**4.3% (11.1%)**	**4.1% (11.1%)**
Putamen	**0.9% (9.7%)**	**3.3% (10.5%)**	**2.5% (18.9%)**	**3.4% (18.7%)**	**3.7% (17.5%)**	**3.5% (17.3%)**
Nucleus accumbens	**6.2% (0.7%)**	**2.2% (1.2%)**	**23.2% (1.9%)**	**11.1% (4.0%)**	**11.0% (0.0%)**	**11.2% (0.3%)**
Globus pallidus	**4.2% (1.4%)**	**3.8% (3.5%)**	**6.4% (7.2%)**	**6.8% (10.2%)**	**7.4% (9.8%)**	**6.9% (9.1%)**
Thalamus	1.5% (9.1%)	2.0% (7.6%)	8.3% (37.9%)	6.9% (37.5%)	7.1% (41.2%)	6.8% (39.1%)
Frontal cortex	8.2% (4.3%)	3.1% (1.3%)	43.0% (11.5%)	24.7% (4.3%)	24.5% (15.1%)	24.9% (11.6%)
Cerebellum	2.1% (1.5%)	3.5% (0.4%)				

Supplemental TABLE 4

^{18}F-MNI-800 (n=2) and ^{18}F-MNI-968 (n=1) test-retest variability of kinetic parameters of 2T model. ^{18}F-MNI-800 (^{18}F-MNI-968).

Region	K_1	k_2	k_3	k_4	K_1/k_2	k_3/k_4
Striatum	**7.2% (12.7%)**	**10.0% (9.5%)**	**83.0% (80.1%)**	**75.3% (67.0%)**	**5.2% (3.1%)**	**22.7% (13.4%)**
Caudate	**7.1% (11.4%)**	**11.5% (4.7%)**	**80.1% (46.1%)**	**61.8% (51.8%)**	**5.4% (6.7%)**	**23.0% (6.1%)**
Putamen	**7.1% (13.6%)**	**11.1% (13.4%)**	**87.2% (105.6%)**	**87.5% (78.2%)**	**5.2% (0.1%)**	**25.5% (34.5%)**
Nucleus accumbens	**5.4% (9.8%)**	**9.4% (4.3%)**	**77.9% (9.2%)**	**89.8% (35.7%)**	**4.7% (5.6%)**	**48.2% (26.8%)**
Globus pallidus	**8.3% (14.3%)**	**21.8% (25.9%)**	**126.8% (157.5%)**	**80.1% (122.2%)**	**13.6% (11.6%)**	**66.5% (68.0%)**
Thalamus	11.0% (2.6%)	55.3% (7.2%)	166.0% (38.3%)	128.6% (69.8%)	46.4% (4.7%)	111.4% (101.3%)
Frontal cortex	6.2% (6.0%)	10.1% (10.3%)	15.5% (3.3%)	60.4% (40.2%)	5.0% (4.3%)	46.6% (36.9%)
Cerebellum	11.6% (20.4%)	13.1% (48.0%)	115.6% (154.1%)	104.9% (101.6%)	3.8% (28.3%)	19.4% (86.3%)

Supplemental TABLE 5

^{18}F-MNI-800 radiation absorbed dose estimates from whole body PET studies in 1 male and 1 female adult rhesus monkeys. The whole-body effective doses (ED) were estimated using the tissue weighting factors from ICRP-60.

	Dose (mSv/MBq)	
	Female	Male
Adrenals	1.58E-02	1.37E-02
Brain	8.05E-03	5.64E-03
Breasts	8.36E-03	NA
Gallbladder Wall	**1.17E-01**	**7.88E-02**
LLI Wall	2.21E-02	1.82E-02
Small Intestine	3.95E-02	3.16E-02
Stomach Wall	1.81E-02	1.12E-02
ULI Wall	4.31E-02	3.50E-02
Heart Wall	2.44E-02	1.92E-02
Kidneys	2.43E-02	2.89E-02
Liver	**6.69E-02**	**6.38E-02**
Lungs	1.83E-02	1.64E-02
Muscle	1.05E-02	8.54E-03
Ovaries	1.80E-02	NA
Pancreas	1.64E-02	1.36E-02
Red Marrow	1.22E-02	1.19E-02
Osteogenic Cells	1.61E-02	1.28E-02
Skin	7.31E-03	5.85E-03
Spleen	1.00E-02	1.09E-02
Testes	NA	1.05E-02
Thymus	1.05E-02	8.17E-03
Thyroid	8.00E-03	6.59E-03
Urinary Bladder Wall	**1.46E-01**	**1.26E-01**
Uterus	2.03E-02	NA
Total Body	1.27E-02	1.06E-02
ED (ICRP-60)	2.47E-02	2.11E-02