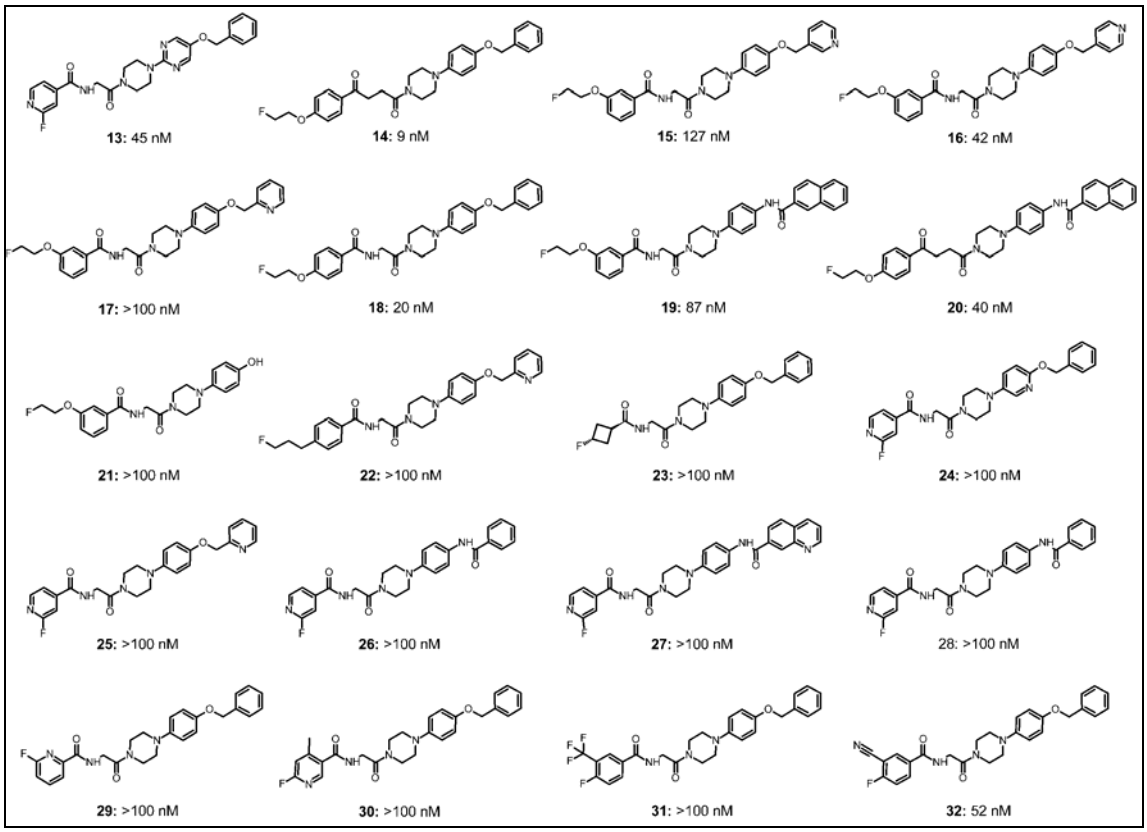
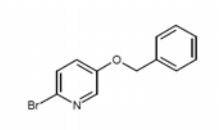
SUPPLEMENTAL FIGURE 1 Structures and IC50 values of compounds **13**–**32**



Synthesis of [^{19}F]1 ([^{19}F]-N-(2-{4-[5-(benzyloxy)pyridin-2-yl]piperazin-1-yl}-2-oxoethyl)-2-fluoropyridine-4-carboxamide) and precursor for radiolabeling

The synthesis of compound [^{19}F]**1** and the iodo-radiolabeling precursor is given below.

a) 5-Benzyloxy-2-bromo-pyridine



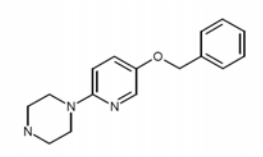
To a solution of 10.0 g (57.47 mmol) of 2-bromo-5-hydroxypyridine in 400 mL DMF was added 14.75 g (86.21 mmol) of benzyl bromide and 23.82 g (172.4 mmol) of potassium carbonate. The mixture was stirred for 6 h at 60°C and overnight at room temperature. The suspension was filtered off and after evaporation of the solvent the residue was chromatographed on silica gel using a dichloromethane/methanol gradient.

Yield: 14.82 g (96.7 %).

MS (ESIpos): m/z = 264, 266 $[M+H]^+$

^{1}H-NMR (300MHz, CHLOROFORM-d): δ [ppm]= 5.10 (s, 2H), 7.16 (dd, 1H), 7.32 - 7.47 (m, 6H), 8.14 (d, 1H).

b) 1-(5-Benzyloxy-pyridin-2-yl)-piperazine



All glassware was dried at 100°C. To a solution of 5.27 g (61.22 mmol) of piperazine in 180 mL toluene was added 561 mg (0.61 mmol) of tris(dibenzylidene acetone) dipalladium(0) and 520 mg (0.83 mmol) of BINAP (2,2'-bis(diphenylphosphino)-1,1'-binaphthyl). Then, a solution of 14.7 g (55.66 mmol) of 5-benzyloxy-2-bromo-pyridine in THF was added followed by a suspension of 8.02 g (83.48 mmol) of sodium t-butylate in THF. The reaction mixture was

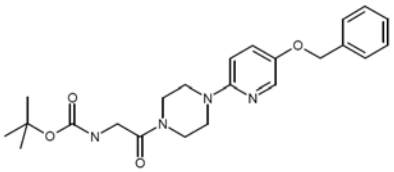
refluxed for 6 h and stirred at room temperature overnight. After evaporation of the solvents the residue was chromatographed on silica gel using a dichloromethane/methanol gradient.

Yield: 7.12 g (47.0 %).

MS (ESIpos): m/z = 270 $[M+H]^+$

^{1}H-NMR (300MHz, CHLOROFORM-d): δ [ppm]= 2.97 - 3.07 (m, 4H), 3.36 - 3.46 (m, 4H), 5.04 (s, 2H), 6.63 (d, 1H), 7.21 (dd, 1H), 7.29 - 7.48 (m, 5H), 8.00 (d, 1H).

c) tert-butyl (2-{4-[5-(benzyloxy)pyridin-2-yl]piperazin-1-yl}-2-oxoethyl)carbamate



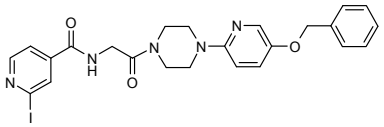
To a solution of 4.63 g (26.43 mmol) *t*-Butoxycarbonyl-glycine (Aldrich) in 500 mL THF and 5 mL triethyl amine (35.87 mmol) at –15°C, 3.43 mL (26.43 mmol) isobutyl chloroformate were added dropwise and the solution was maintained at this temperature for another 15 min. Then, 7.12 g of 1-(5-Benzyloxy-pyridin-2-yl)-piperazine and 18 mL triethyl amine (129 mmol) in 200 mL THF/dichloromethane (1:1) were added slowly to this cold solution, the temperature was kept below -10°C for another 15 min and was then allowed to reach room temperature. After stirring overnight the solvent was evaporated and the residue was taken up in ethyl acetate. This solution was washed successively with aqueous sodium carbonate, water, 1 M aqueous HCl solution, saturated aqueous sodium chloride solution, finally dried over magnesium sulfate and then evaporated. This residue was chromatographed on silica gel using a hexane/ethyl acetate gradient.

Yield: 8.04 g (70.6 %).

MS (ESIpos): m/z = 427 $[M+H]^+$

^{1}H-NMR (300MHz, CHLOROFORM-d): δ [ppm]= 1.46 (s, 9H), 3.36 - 3.45 (m, 2H), 3.51 (br. s., 4H), 3.70 - 3.81 (m, 2H), 4.02 (d, 2H), 5.05 (s, 2H), 5.53 (br. s., 1H), 6.65 (d, 1H), 7.23 (dd, 1H), 7.30 - 7.48 (m, 5H), 8.00 (d, 1H).

d) N-(2-{4-[5-(benzyloxy)pyridin-2-yl]piperazin-1-yl}-2-oxoethyl)-2-iodopyridine-4-carboxamide



8.0 g (18.76 mmol) of tert-butyl (2-{4-[5-(benzyloxy)pyridin-2-yl]piperazin-1-yl}-2-oxoethyl) carbamate were suspended in 160 mL 2N HCl in diethyl ether and stirred overnight at room temperature. The precipitate was filtered off and washed with ether and dried at 40°C *in vacuo*. Yield: 7.4 g (quantitative). The product was used in the next step without further purification.

MS (ESIpos): m/z = 327 $[M+H]^+$

To a solution of 274 mg (1.10 mmol) of 2-iodopyridine-4-carboxylic acid (Alfa Aesar) and 363 mg (1.0 mmol) of hydrochloride prepared above in 15 mL DMF were added 624 mg (1.2 mmol) Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (PyBOP) and 0.70 mL (4 mmol) N-ethyl-N,N-diisopropylamine and the reaction mixture was stirred overnight at room temperature. After evaporation of the solvent the residue was taken up in ethyl acetate. This solution was washed with water and saturated aqueous sodium chloride solution, dried over sodium sulfate and then evaporated. This residue was chromatographed on silica gel using a dichloromethane/methanol gradient and the appropriate fractions were combined and concentrated.

Yield: 320 mg (53.8 %).

MS (ESIpos): m/z = 558 $[M+H]^+$

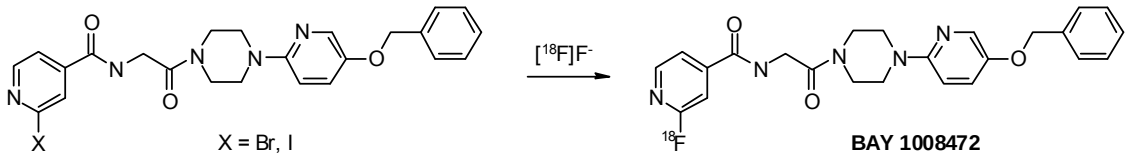
^{1}H-NMR (600MHz, DMSO-d_6): δ [ppm]= 2.52 (m, 2H), 2.60 (m, 2H), 2.74-2.76 (m, 4H), 3.36-3.37 (m, 2H), 4.24 (s, 2H), 6.02 (d, 1H), 6.46-6.62 (m, 6H), 6.96 (d, 1H), 7.12 (d, 1H), 7.38 (d, 1H), 7.69 (d, 1H), 8.17-8.22 (m, 1H).

The ^{19}F reference compound was synthesized according to this procedure using 2-fluoro-pyridine-4-carboxylic acid (Aldrich). Analytical data for N-(2-{4-[5-(benzyloxy)pyridin-2-yl] piperazin-1-yl}-2-oxoethyl)-2-fluoropyridine-4-carboxamide (**1**):

MS (ESIpos): m/z = 449 $[M+H]^+$

^{1}H-NMR (400MHz, DMSO-d_6): δ [ppm]= 3.37 (br. s., 2H), 3.44 (br. s., 2H), 3.52 - 3.66 (m, 4H), 4.22 (d, 2H), 5.07 (s, 2H), 6.83 (d, 1H), 7.27 - 7.47 (m, 6H), 7.53 (s, 1H), 7.70 - 7.81 (m, 1H), 7.95 (d, 1H), 8.39 (d, 1H), 9.01 (t, 1H).

Radiosynthesis of BAY 1008472 ([^{18}F]1)



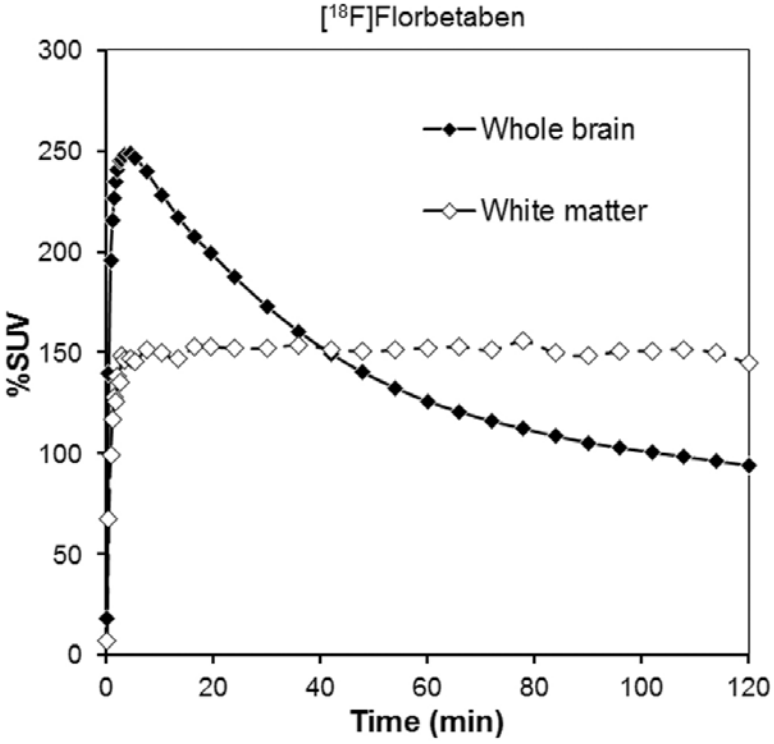
The radiolabeling of BAY 1008472 was accomplished initially by a nucleophilic [^{18}F]fluorination of the corresponding bromo-precursor under classical conditions with potassium carbonate and Kryptofix in DMSO at 180°C. Since the labeling results were rather poor (~6-8% radiochemical yield corrected for decay), the labeling was systematically optimized. The usage of tetrabutyl ammonium hydroxide instead of potassium carbonate and Kryptofix more than doubled the radiochemical yield (16-35%) as well as the specific radioactivity. The subsequent use of the corresponding iodo-precursor instead of the bromo-precursor gave radiochemical yields as high as 59% corrected for radioactive decay and a specific radioactivity (SA) of up to 140 GBq/μmol. The iodo-precursor was separated from the radiolabeled product quantitatively by preparative HPLC. The chemical purity of BAY 1008472 was determined to be always greater than 90%. The SA of the product was measured by analytical HPLC. SA was calibrated for UV absorbance (λ=254 nm) response per mass of ligand and calculated as the radioactivity of the radioligand (GBq) divided by the amount of the associated carrier substance (μmol).

Precursor	Labeling conditions	Radiochem. yield [%] (d.c.)	Specific activity [GBq/μmol]
Br-precursor	K_2CO_3, Kryptofix, 180°C, DMSO, 30min	6-8	10-20
Br-precursor	TBAOH, 180°C, DMSO, 30min	16-35	10-35
I-precursor	TBAOH, 180°C, DMSO, 30min	35-59	13-140

Specifically, the radiosynthesis of BAY 1008472 (N-(2-{4-[5-(benzyloxy)pyridine-2-yl]piperazin-1-yl}-2-oxoethyl-2-[^{18}F]fluoroisonicotinamide) was performed on a remotely controlled GE tracerlab FX_N synthesis module. [^{18}F]fluoride was trapped on an anion exchange cartridge (QMA SepPak light, Waters). The activity was eluted with tetrabutylammonium hydroxide (TBAOH) solution (1.5 mL MeCN, 0.3 mL H_2O + 8 µL TBAOH sol. (40%)) into the reaction vessel. The solvent was removed by heating at 120°C under a stream of nitrogen and vacuum. Anhydrous MeCN (1 mL) was added and evaporated as before. 5 mg iodo precursor (N-(2-{4-[5-(benzyloxy)pyridine-2-yl]piperazin-1-yl}-2-oxoethyl-2-iodoisonicotinamide) in 0.5 mL dimethyl sulfoxide were added to the dried residue and the resulting solution was stirred for 10-20 min at 140-180 °C. After cooling to room temperature, the solution was diluted with 4.5 mL of the mobile phase and subsequently purified by preparative HPLC (ACE 5-C18-HL 250mmx10mm; isocratic, 23% acetonitrile in water with 0.1 % trifluoroacetic acid, flow: 4 mL/min; t_R~33 min). The collected product was diluted with 40 mL water and immobilized on a Sep-Pak plus short tC18 cartridge (Waters), which was washed with 10 mL water and eluted with 1 mL ethanol into the product vial to deliver the fluorine-18 labeled product BAY 1008472 in an overall synthesis time of ~90 min and in a radiochemical yield of 35-59% corrected for decay (radiochemical purity >99% (HPLC). The desired fluorine-18 labeled product BAY 1008472 (t_R=3.2 min) was analyzed using analytical HPLC: ACE3-C18 50 mm x 4,6 mm; solvent gradient: start 5 % acetonitrile – 95 % acetonitrile in 0.1% trifluoroacetic acid in 7 min., flow: 2mL/min and confirmed by co-injection with the corresponding non-radioactive fluorine-19 fluoro-standard (N-(2-{4-[5-(benzyloxy)pyridine-2-yl]piperazin-1-yl}-2-oxoethyl-2-fluoroisonicotinamide) on the analytical HPLC (t_R=3.0 min). The specific radioactivity was determined to be between 13 and 140 GBq/µmol depending on the used starting activity.

The (radio-)syntheses of the other fluorine-18 ligands and of compounds **2**-**32** is described in the international patent applications WO 2010/028776 and WO 2011/09559.

SUPPLEMENTAL FIGURE 2 Time activity curves for whole brain and white matter after administration of [^{18}F]Florbetaben to the same monkey that was used in the studies with [^{18}F]**1** and [^{18}F]**2**



SUPPLEMENTAL FIGURE 3 A) Plasma stability of BAY 1008472 in Rhesus monkey PET study **B,C)** Detection of plasma metabolites by HPLC combined with radio-detection. Chromatograms correspond to plasma samples taken 4 min and 30 min post injection of BAY 1008472. Please note that only a polar metabolite fraction was found.