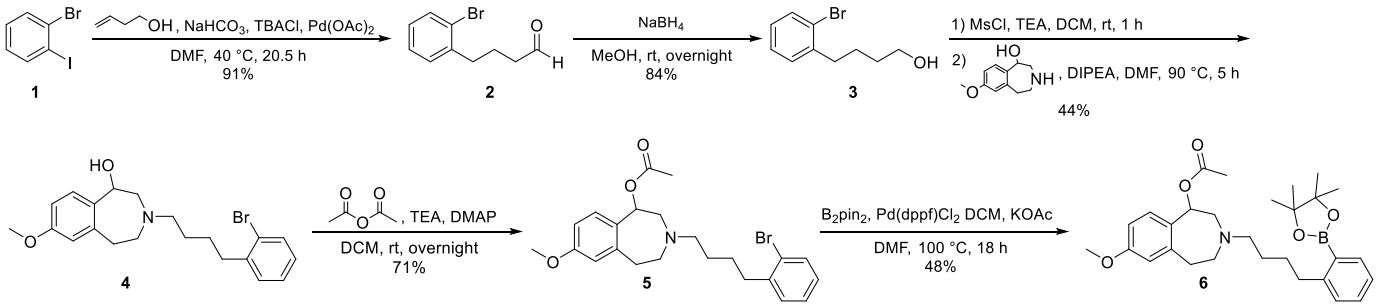
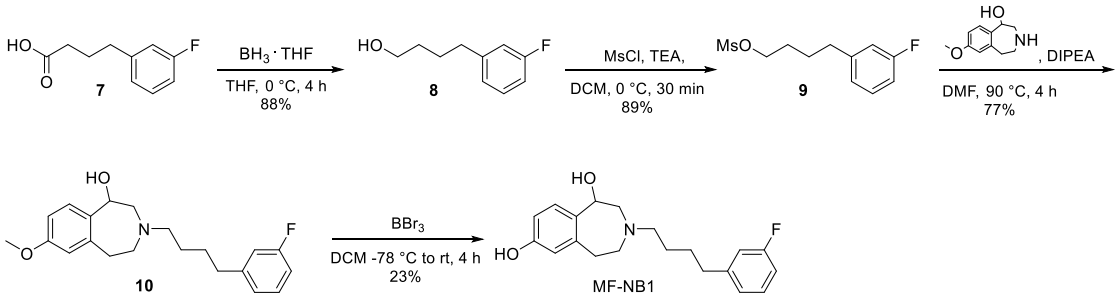
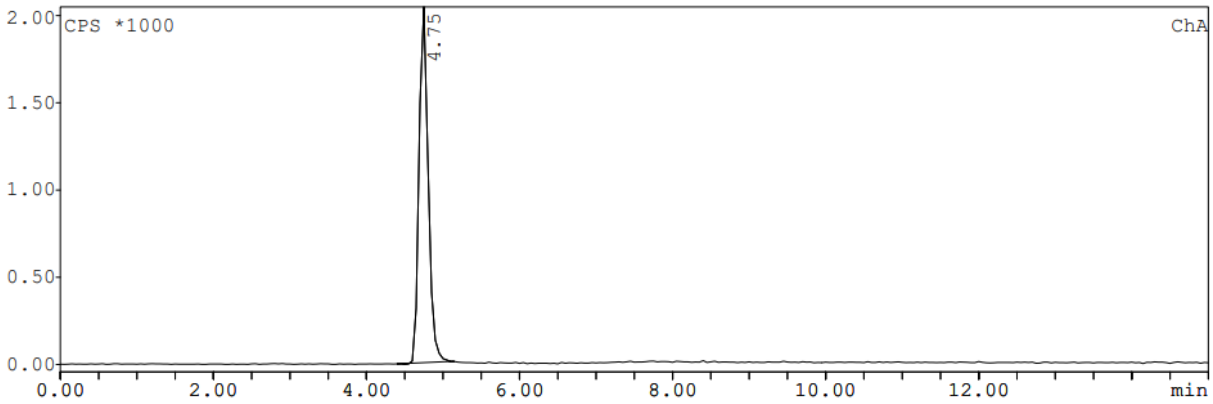


Supplemental Figure 1. Synthesis of boronic ester precursor **6** for synthesis of ^{18}F-OF-NB1.



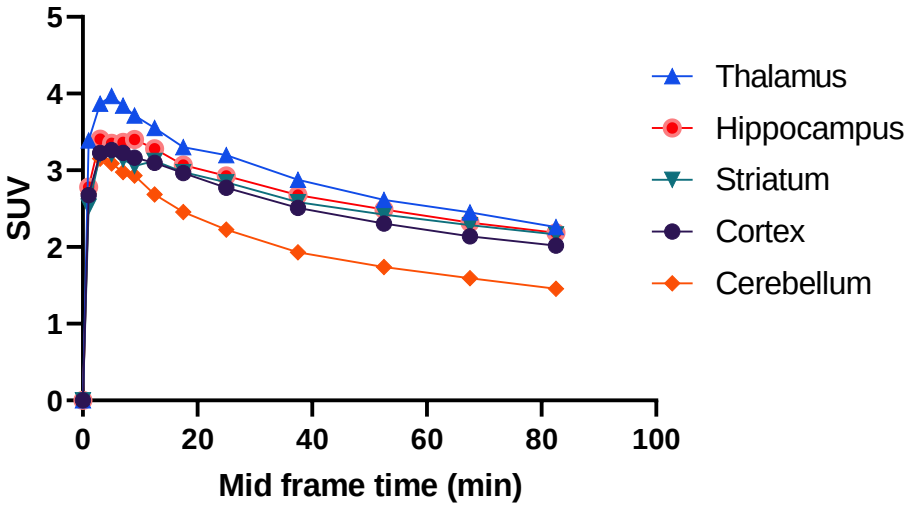
Supplemental Figure 2. Synthesis of MF-NB1.



Supplemental Figure 3. Quality control of ^{18}F-OF-NB1 after formulation. In brief, ^{18}F-fluoride ions were produced by the bombardment of 98% enriched ^{18}O-water by means of the ^{18}O(p,n)^{18}F nuclear reaction in a Cyclone 18/9 cyclotron

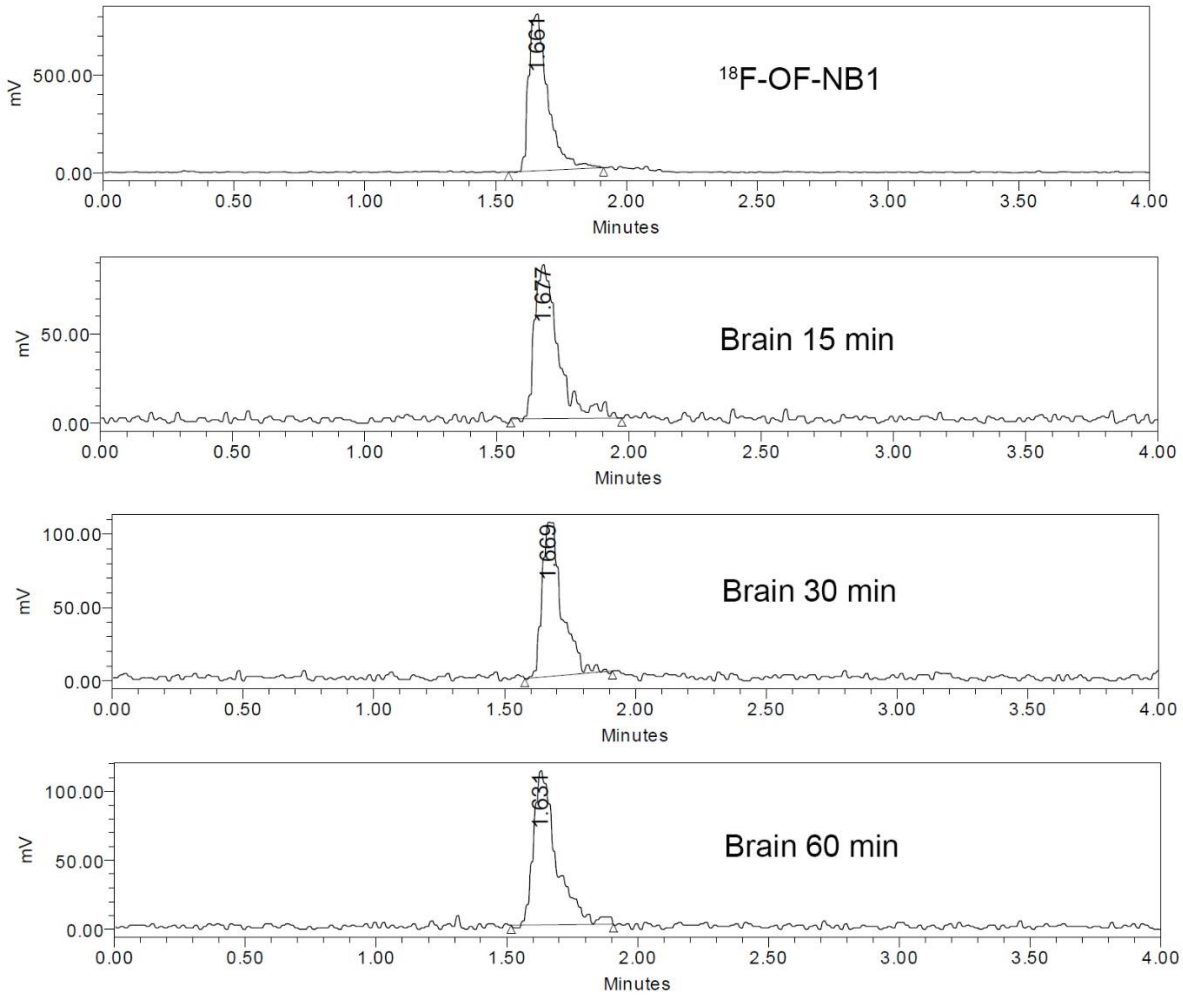
(18-MeV; IBA) resulting in a starting activity of nearly 60 GBq in 0.94 mL of ^{18}O-enriched H_2O. [^{18}F]fluoride ions were captured on an anion-exchange cartridge (Waters SepPak Accell QMA cartridge, light carbonate, preconditioned) and were then eluted with a mixture of Kryptofix 222 (6.3 mg/mL, 16.7 µmol/mL; Merck), $K_2C_2O_4$ (1.0 mg/mL, 5.4 µmol/mL), and K_2CO_3 (0.1 mg/mL, 0.7 µmol/mL) dissolved in a solution of MeCN/H_2O (4:1, 6 x 0.15 mL), followed by azeotropic drying with acetonitrile (3 x 0.8 mL) in a borosilicate glass Reacti-Vial (Wheaton Industries, 6 mL) sealed with a screw plastic cap in which a Teflon (DuPont)-faced rubber septum was fitted. The Reacti-Vial was subsequently left to cool down for 2 min and purged with air (20 mL), after which the residue was redissolved in a solution of 6–8 mg (18–24 µmol) of boronic ester **6** and 14 mg (21 µmol) of $Cu(OTf)_2(py)_4$ in 0.3 mL of dry dimethylacetamide. The mixture was stirred at 120 ˚C for 20 min before it was diluted with 35 mL of H_2O and the subsequent trapping of the intermediate on a C18 Plus cartridge (Waters, pre-conditioned with 5 mL MeCN and 5 mL H_2O). The cartridge was washed with water (5 mL) followed by the elution of radiolabeled protected intermediate **11** with 2 mL of EtOH which was evaporated at 95 °C for 6 min and subsequently azeotropically dried with MeCN (2 x 0.8 mL). The Reacti-Vial was cooled down in an ice bath before 0.5 mL of dichloromethane were added, followed by 1 mL of BBr_3 (1 M in DCM) and stirring at room temperature for 15 min. The dichloromethane was evaporated and the reaction was then quenched by the addition of aq. 0.1% H_3PO_4/MeCN (5:1, 3 mL). Afterwards, the products were purified by semi-preparative HPLC on a Merck-Hitachi L2130 HPLC system mounted with a radiation detector VRM 202 (Comecer, Netherlands). The purification was performed using an Agilent Eclipse XDB-C18 reversed phase column (5 µm, 9.4 x 250 mm) and a gradient system comprising MeCN (solvent B) and aq. H_3PO_4 (0.1%, solvent C) with a flow rate of 4 mL/min and UV detection wavelength λ of 230 nm: 0.0-5.0 min, 20% B; 5.1-25.0 min, 20-40% B; 25.1-28.0 min, 40% B; 28.1-30.0 min, 40-60% B; 30.1-33.0 min, 60-90% B; 33.1-36.0 min, 90% B; 36.1-38.0 min, 90-20% B; 38.1-42.0 min, 20% B. The collected fraction was diluted with 35 mL of H_2O and the product was trapped on a C18 Light cartridge (Waters, pre-conditioned with 2 mL EtOH and 2 mL water). The cartridge was washed with 2 mL of water followed by elution of the radiotracer with 1 mL of EtOH. The product was formulated with 5% EtOH in water for injection to afford ^{18}F-OF-NB1. The identity of the radiotracer was confirmed by co-injection with its corresponding non-radioactive reference compound whereby an aliquot taken from the final formulation was injected into an Agilent 1100 HPLC system equipped with a Raytest Gabi Star radiodetector as well as an analytical Atlantis T3 C18 reversed-phase column (3 µm, 4.6 x 150 mm). A flow rate of 1 mL/min and detection wavelength λ of 230

were in use with a gradient system comprising 0.1% TFA in H_2O (solvent A), MeCN (solvent B); 0.0-10.0 min, 40-60% B; 10.01-11.00 min, 60-40% B; 11.01-15.0 min, 40% B.



Supplemental Figure 4. Representative regional distribution of ^{18}F-OF-NB1 across the brain of a Wistar rat. The following equations were used simultaneously to determine the receptor occupancy from $SUV_{0\text{-}90\ min}$: - 1) AUC = (b_{max} - b_{min}) * D_{50} / (D + D_{50}) + b_{min}. 2) RO = (b_{max} - AUC) / (b_{max} - b_{min}) * 100, where AUC is defined as the area under the curve of the whole respective PET scan, D is the administered dose while RO is the receptor occupancy in %. B_{max} and b_{min} are the maximum and minimum binding values, respectively. For the autoradiography, adult rat brain tissues embedded in optimal cutting temperature medium were developed as 10 µm thick coronal sections on a cryostat (Cryo-Star HM 560 MV; Microm, Thermo Scientific, Wilmington, DE, USA). Slices were adsorbed on SuperFrost Plus glass slides (Menzel, Braunschweig, Germany) and subsequently preserved at -20 °C. The slices were thawed for 15 min on ice and subsequently preconditioned in an aqueous incubation buffer consisting of 30 mM HEPES, 0.56 mM $MgCl_2$, 110 mM NaCl, 5 mM KCl, 3.3 mM $CaCl_2$ and 0.1 % fatty acid free bovine serum albumin (pH 7.4, 0 °C) for 10 min. The slides were allowed to dry for 2-3 min at room temperature and then incubated with 3 nM of ^{18}F-OF-NB1, either alone or premixed with GluN2B subunit or σ1R blockers for 20 min at room temperature in a humidified chamber. The liquid was decanted and the slices were left for 5 min in the incubation buffer at 0 °C. The slices were washed (2 x 3 min) in a washing buffer (Same composition as the incubation buffer but without the fatty acid free

bovine serum albumin) and (2 x 5 sec) in distilled water at 0 °C. The slices were subsequently dried for 5 min at room temperature and then exposed to a phosphor imager plate (Fuji, Dielsdorf, Switzerland) for 35-40 minutes. The films were scanned by a BAS5000 reader (Fuji) and the data was analyzed using AIDA 4.50.010 software (Raytest Isotopenmessgeräte GmbH, Straubenhardt, Germany).



Supplemental Figure 5. *Ex vivo* metabolite study with ^{18}F-OF-NB1 in male Wistar rats. Radio-UPLC analysis of brain homogenate samples after 15, 30 and 60 min post-injection showed the exclusive presence of the parent intact tracer. In brief, the brain extracts were dissolved in 2 mL phosphate buffered saline (PBS) followed by homogenization and subsequent addition of 2 mL acetonitrile. Afterwards, the mixture was vortexed, centrifuged at 5000g for 5 min (4 °C) and the resultant supernatant was filtered. The blood samples were collected in heparin-coated tubes followed by transfer of 0.1 mL of the sample to Eppendorf tubes containing acetonitrile. The Eppendorf tubes were vortexed and centrifuged at 5000g for 5 min at 4 °C resulting in plasma supernatant. The supernatant was

collected into new Eppendorf tubes containing acetonitrile and subsequently followed by a second centrifugation. The resultant supernatant was filtered. The processed brain and blood samples were analyzed by radio-ultra-performance liquid chromatography (UPLC).

Supplemental Table 1. Brain biodistribution of ^{18}F-OF-NB1 in Wistar rats (baseline injections n=4, eliprodil 2 mg/kg blockade n=4), reported as averaged % normalized injected dose per gram body weight ± SD. For blockade experiments, 2 mg/kg of eliprodil constituted in a solution of aqueous glucose (5%), NaCl (0.45%) and citric acid (1 mM) was administered prior to tracer injection. Organs were extracted, weighed and radioactivity was measured in a gamma-counter (Perkin Elmer, Schwerzenbach, Switzerland). For statistical analysis, an independent two-tailed paired Student's test assuming normal distribution of the dataset was employed to determine statistical probability values.

Organ	45 min post-injection	45 min post-injection + 2 mg/kg eliprodil	Specificity %
Bulbus olfactorius	0.24 ± 0.04	0.11 ± 0.007	53
Hippocampus	0.24 ± 0.02	0.17 ± 0.012	31
Thalamus	0.29 ± 0.04	0.17 ± 0.011	42
Cerebellum	0.23 ± 0.02	0.11 ± 0.007	52
Brain stem	0.28 ± 0.04	0.12 ± 0.009	55
Colliculus superior/inferior	0.29 ± 0.03	0.12 ± 0.009	57
Cortex	0.32 ± 0.04	0.16 ± 0.010	52
Striatum	0.29 ± 0.03	0.15 ± 0.008	48

Supplemental Table 2. *Ex vivo* whole body biodistribution of ^{18}F-OF-NB1 in Wistar rats (baseline injections n=4, eliprodil 2 mg/kg blockade n=4), reported as averaged % normalized injected dose per gram body weight ± SD.

Organ	**45 min post-injection**	**45 min post-injection + 2 mg/kg eliprodil**	**Specificity %**
Spleen	0.27 ± 0.02	0.22 ± 0.017	19
Liver	0.10 ± 0.02	0.13 ± 0.017	No specificity
Kidney	0.62 ± 0.05	0.25 ± 0.018	59
Adrenal gland	1.4 ± 0.21	0.42 ± 0.054	69
Lungs	0.30 ± 0.04	0.22 ± 0.041	28
Bone	0.08 ± 0.01	0.07 ± 0.007	13
Heart	0.08 ± 0.01	0.06 ± 0.003	27
Fat	0.03 ± 0.005	0.04 ± 0.008	No specificity
Intestine	0.64 ± 0.08	0.77 ± 0.270	No specificity
Testicles	0.07 ± 0.01	0.07 ± 0.004	0.05
Blood	0.01 ± 0.001	0.01 ± 0.001	No specificity
Urine	0.55 ± 0.18	0.70 ± 0.082	No specificity
Muscle	0.08 ± 0.01	0.07 ± 0.006	16
Pancreas	0.72 ± 0.03	0.61 ± 0.035	16
Skin	0.04 ± 0.005	0.04 ± 0.003	3