Supporting Information

4-[^{18}F]fluoro glutamic acid (BAY 85-8050) - a new amino acid radiotracer for PET imaging of tumors: Synthesis and *in vitro* characterization

Raisa N. Krasikova,[a] Olga F. Kuznetsova,[a] Olga S. Fedorova[a], Yuri N. Belokon[b], Viktor I. Maleev[b], Linjing Mu[c], Simon Ametamey[c], P. August Schubiger[c], Matthias Friebe[d], Mathias Berndt[d], Norman Koglin[d], Andre Mueller[d], Keith Graham[d], Lutz Lehmann[d], Ludger M. Dinkelborg[d]*

[a]Institute of Human Brain Russian Academy of Science, Acad. Pavlova 9, St.-Petersburg, 197376, Russia; [b]A.N. Nesmeyanov Institute of Organoelement Compounds Russian Academy of Science, B-334, Vavilova 28, Moscow, 119991, Russia; [c]Center for Radiopharmaceutical Science of ETH, PSI and USZ, Department of Chemistry and Applied Biosciences, ETH Zurich, 8093 Zurich, Switzerland; [d]Bayer Schering Pharma AG, Global Drug Discovery, 13342 Berlin, Germany

Additional Methods

Radiochemistry. Materials and Reagents. The commercially available reagents and solvents were used without further purification unless otherwise stated. ^{18}O-enriched water (97%) was purchased from the Global Scientific Technologies (Russia); anhydrous acetonitrile (DNA-quality) was bought from Merck (Germany); Sep-Pak Light Waters AccellTM Plus QMA cartridges were purchased from Waters (USA). The tetrabutylammonium hydrogen carbonate solution was prepared by bubbling CO_2 through a solution

of commercially available aqueous tetrabutylammonium hydroxide (20%) (Merck, Germany) until a pH of 8 was reached; the obtained stock solution was kept in a sealed vial at 4oC. The labeling precursor NiII-(*S*)-BPB-(*2S,4R*)-4-Br-Glu was prepared at the INEOS RAS according to a procedure developed earlier.[18] The reference materials, isolated (*2S,4S*)-**6** as well as (*2S,4R*)-**7**, and their mixture were synthesized at the INEOS RAS according to Belokon et al..[19]

HPLC, TLC and Other Equipment. The semi-preparative HPLC consisted of a Gilson Pump 305, an automatic sample injector (type VICI Valco) equipped with a 2 mL loop, and a Gilson 116 UV absorbance detector in series with a Beckman 170 radiodetector. The analytical HPLC was identical except that a Rheodyne type injector fitted with a 20 μL loop was used instead of an automatic sample injector. The data acquisition software Chrom & Spec was obtained from Ampersand (Russia). TLC analyses were run on pre-coated plates of silica gel F254 (Sorbfil, Lenchrom, Russia). Radioactivity spots were detected using an automatic radio-TLC scanner RT-10 (Raytest GmbH, Germany).

Radioisotope Production. N.c.a. [^{18}F]fluoride was produced via the ^{18}O(p,n)^{18}F nuclear reaction by the proton bombardment (17 MeV, 15 μA) of [^{18}O]H_2O (97% isotopic enrichment) in a small volume low pressure target of the MC-17 cyclotron (Scanditronix AB, Sweden). Radioactivity was measured with a dose calibrator PTW Curiementor-2 (Germany). The entire radiochemical synthesis was operated with the use of the remote-controlled synthesis apparatus for ^{18}F-nucleophilic fluorinations (home made at IHB RAS).

Preparation of the [Bu_4N]+[^{18}F]– (Fluorinating Agent). [^{18}F]fluoride generated in the water cyclotron target was collected on the Sep-Pak Light Waters AccellTM Plus QMA cartridge, pre-conditioned by washing with 10 mL of 0.5 M K_2CO_3 and 15 mL of water. The cartridge was flushed with helium for 5-15 min to remove residual [^{18}O]water. The [^{18}F]fluoride was than eluted from the QMA resin with 2 mL of a solution composed of MeCN (2 mL) and Tetrabutyl ammonium carbonate solution (0.075 mL, 20% aq., pH 8). The eluate was collected in a 5 mL conic vial. The solvents were gently removed to dryness in a stream of nitrogen gas at 130°C. Two portions of acetonitrile (0.5 mL

each) were added and evaporated step-wise to give the corresponding ^{18}F-reactive complex as a slightly brownish residue.

Quality Control. The radiochemical and chemical purity of the final preparation were determined by reversed phase HPLC. A Zorbax NH2, (4.6 x 250 mm) column was isocratically developed using 10 mM NaH_2PO_4, pH 3.5/MeOH (85/15, v/v) at a flow rate of 1.5 mL/min and the UV-signal was followed at 210 nm. The identity of **5** was confirmed by co-injection of the [^{19}F] sample. Retention time tR of 4-[^{19}F]F-Glu was 20.30 min and R_t of **5** was 20.62 min (UV detector preceding the radiodetector in a line-setting). The stereoisomeric composition of **5** was determined by chiral HPLC (Crownpak CR (+), Daicel, 150 x 4 mm, HClO4 pH1, 0.8 mL/min; 210 nm). Retention times for *2S,4R* and *2S,4S*-isomers were 3.14 and 4.0 min, respectively.

Biological evaluation. The non-small cell lung cancer cell line A549 and the colon tumor cell line HT29 were both obtained from ATTC (USA). A549 cells were maintained in Dulbeccos modified Eagles medium (D-MEM)/ F-12 (Invitrogen, Germany) + 10 % FCS, HT29 cells were maintained in D-MEM (High glucose) (Invitrogen, Germany) + 10 % FCS. For tracer uptake studies A549 and HT29 cells were seeded in 48 well plates at a concentration of 25,000 cells/mL 2-3 days prior to the uptake assay and grown under standard conditions (37°C, 5% CO2) until sub-confluency. The cell culture medium was removed prior to the assay and the cells were washed twice with PBS buffer. The tracer compound (**5** or **1**) was added to the assay buffer (PBS/ 0.1 % w/v BSA) using 250 kBq/well. In parallel, **5** was co-incubated with L-glutamic acid (1 mM), whereas **1** was co-incubated with D-glucose (5 mM) for studying the specificity of tracer uptake. Tracer uptake was stopped after 10, 20, and 30 min of incubation by removal of the assay buffer. Cells were quickly washed twice with PBS and lysed by the addition of 1N NaOH. Cell lysate was removed from the plates and radioactivity was determined in a gamma counter (Wizard 3, Perkin Elmer, USA).

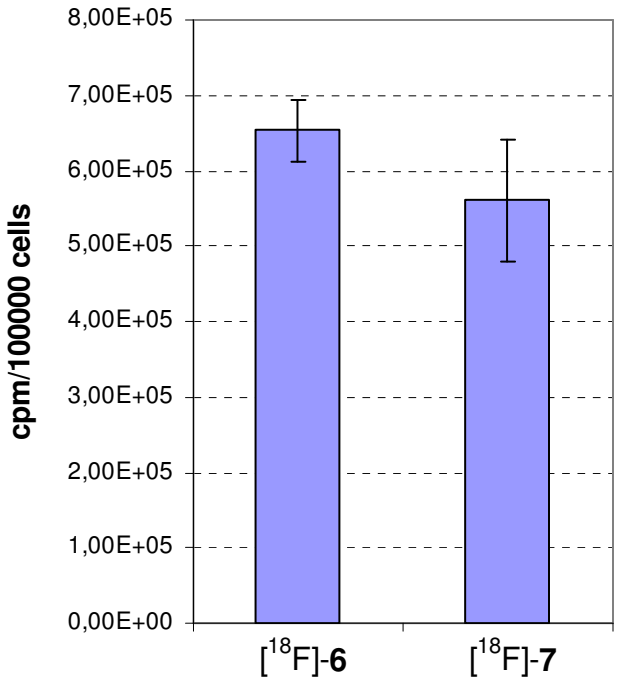

Figure S1. *In vitro* uptake of [^{18}F]-**6** and [^{18}F]-**7** in A549 cells. Cells were incubated for 30 min with 250 kBq of either [^{18}F]-**6** or [^{18}F]-**7**. No significant difference in uptake of the C4-isomers was observed.