Radiosynthesis and Evaluation of an ^{18}F-Labeled Positron Emission Tomography (PET) Radioligand for Metabotropic Glutamate Receptor Subtype 4 ($mGlu_4$)

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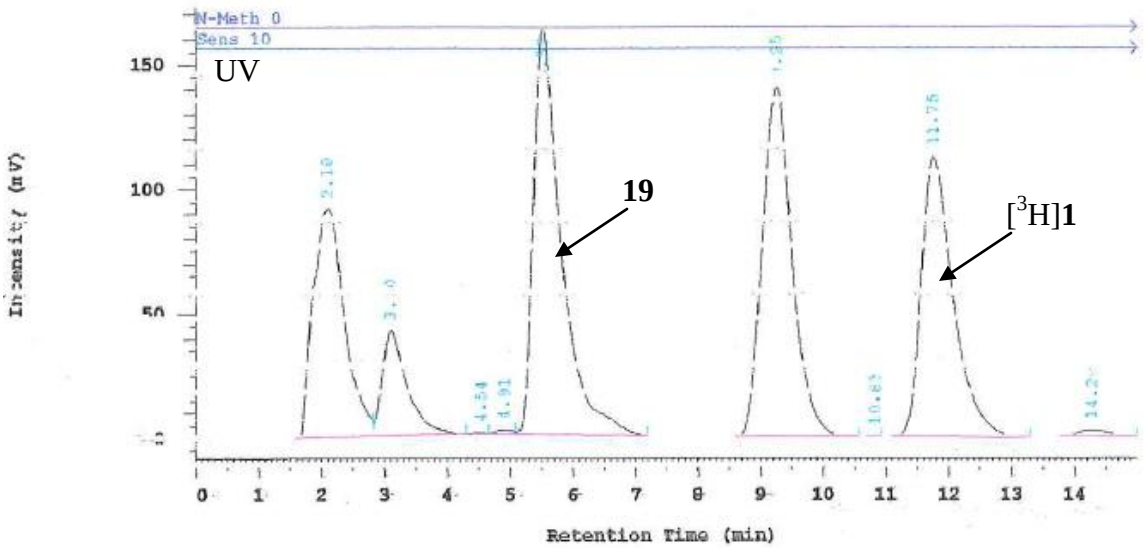
Supporting Information

A. Chromatography of [^{3}H]1 Radiosynthesis

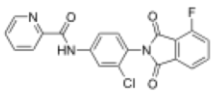


Gemini-NX C_{18} semipreparative column (250 mm × 10 mm, 5 μm)

55% Acetonitrile / 45% 0.1 M ammonium formate solution

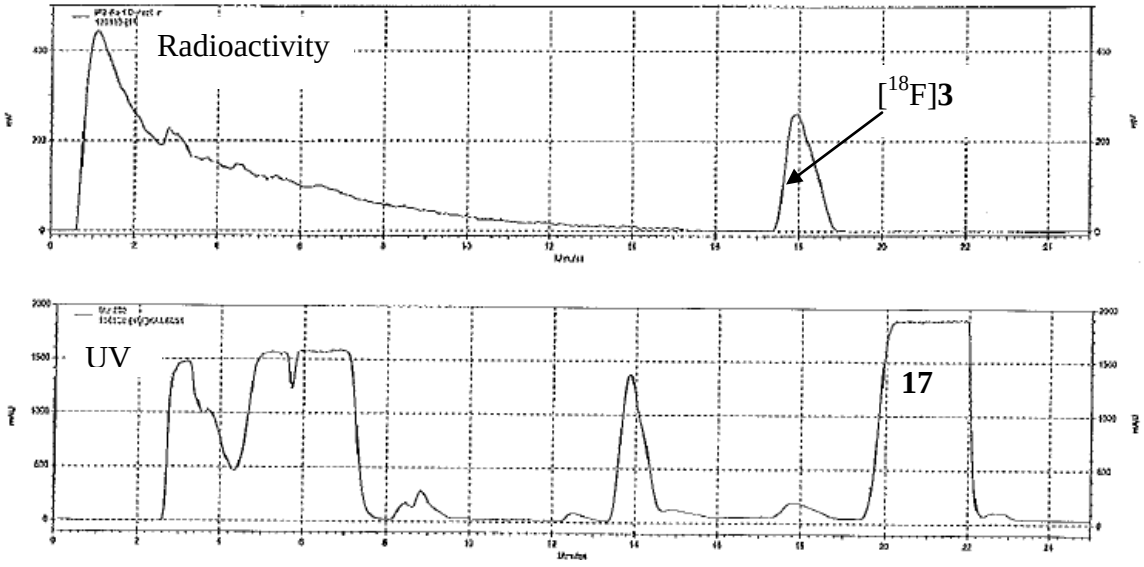
4.0 ml/min, 254 nm

B. In Vitro Selectivity of 3 to $mGlu_1$, $mGlu_5$ and $mGlu_8$.

Functional Assay: 10 μM		mGluR1a		mGluR5		mGluR8	
Comp#	Structure	Agonist-%MAX	Antigonist-%INH	Agonist-%MAX	Antigonist-%INH	Agonist-%MAX	Antigonist-%INH
3		0	15	-13	14	33	-44

Functional assays were performed using a single concentration (10 μM) of **3** to determine agonist or antagonist activity.[1,2] The tests were carried out by the NIMH PDSP program at the University of North Carolina at Chapel Hill.

C. UV and Radioactivity Traces of the Radiolabeling Reaction Mixture by the Semi-Preparative HPLC



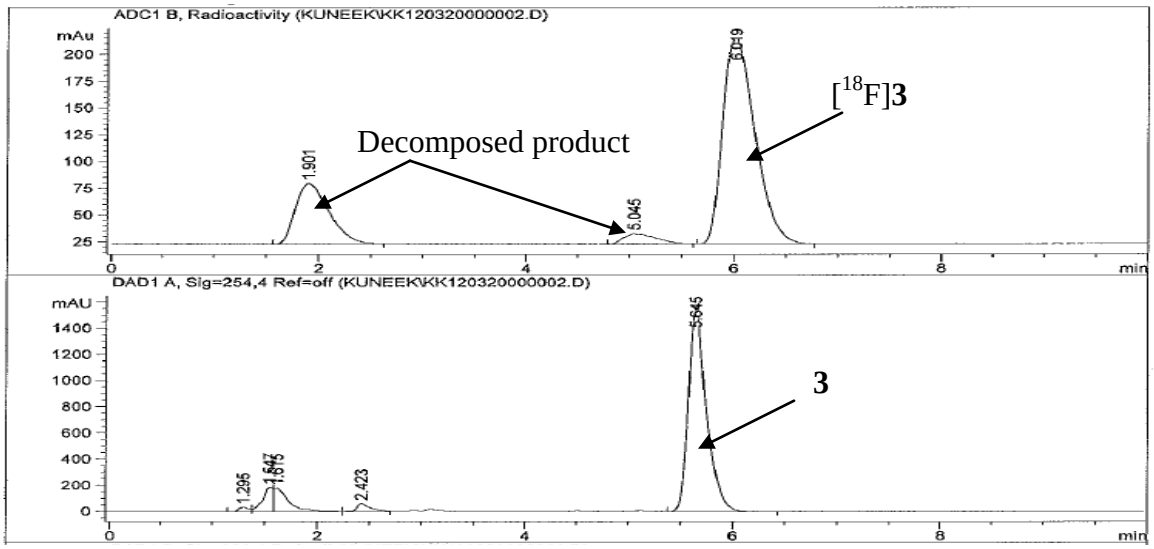
Eclipse XDB-Phenyl semipreparative column (250 mm × 10 mm, 5 μm)

0.1% formic acid solution of water and acetonitrile (52:48)

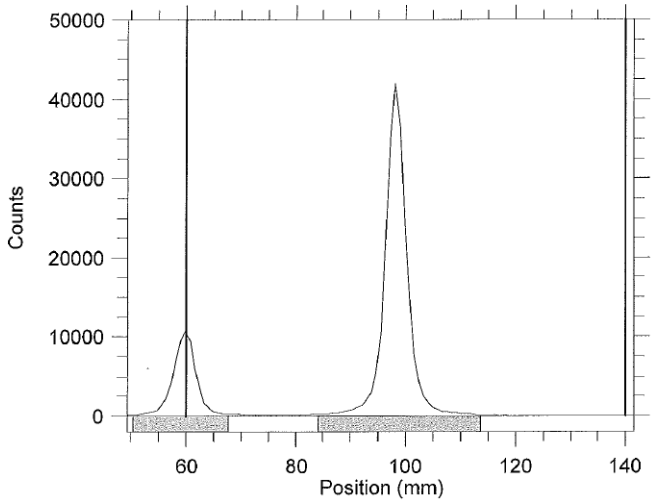
Flow rate: 4 ml/min

D. Analysis of [^{18}F]3 Formulated in 10% Ethanol Solution in Saline.

(a)



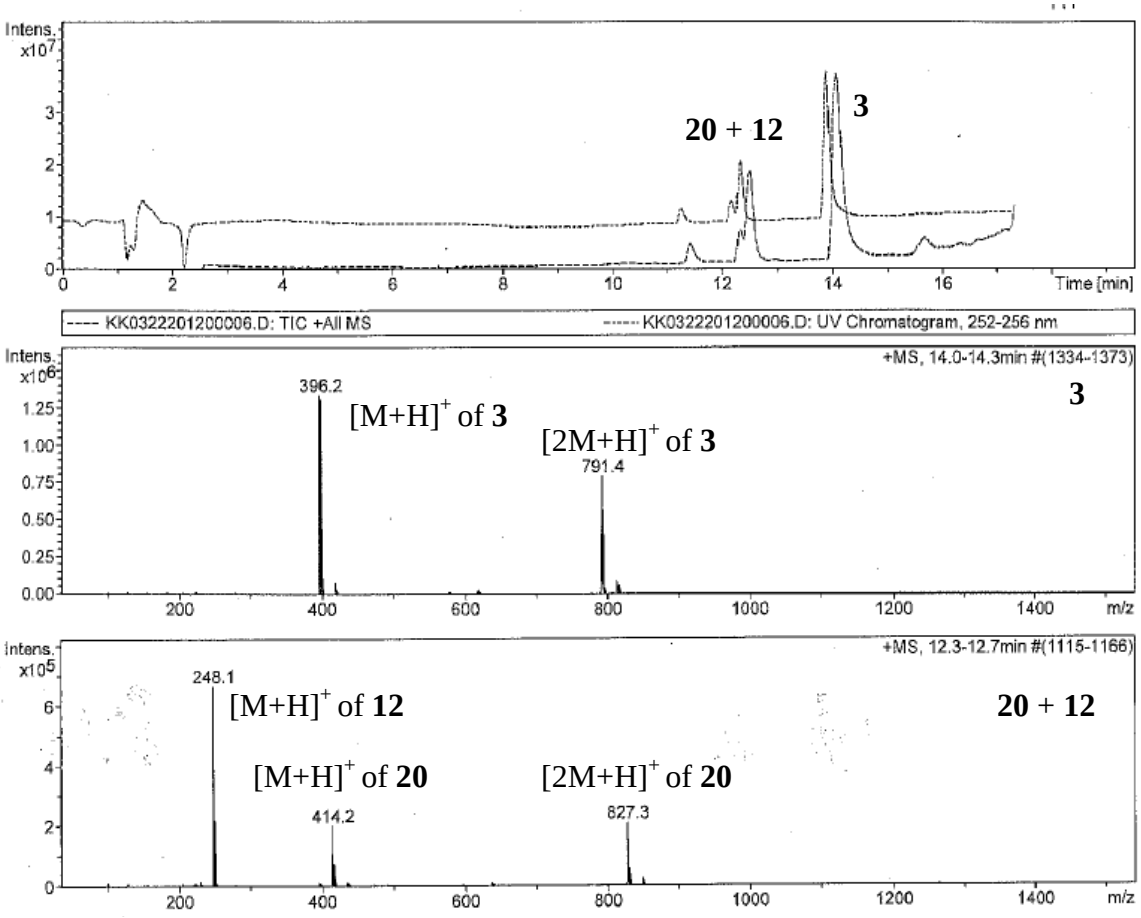
(b)

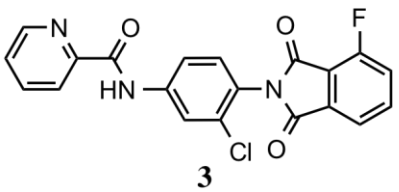


Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	50.3	67.6	59.6	-0.005	59756.0	59756.0	20.77	21.14
Rgn 2	84.1	113.7	98.2	0.477	222855.0	222855.0	77.46	78.86
2 Peaks					282611.0	282611.0	98.24	100.00

(a) Analytical HPLC profile of [^{18}F]**3** co-injected with nonradioactive standard **3** by a neutral method. (b) The radio-TLC result obtained after the radioactive aliquot was formulated in 10% ethanol in saline, cospotted with nonradioactive **3** on TLC and developed with a solution of ethyl acetate and hexane (1:1).

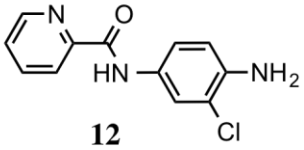
E. Stability Test of 3 in the Neutral Media





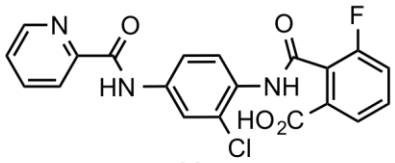
3

Exact Mass: 395.05



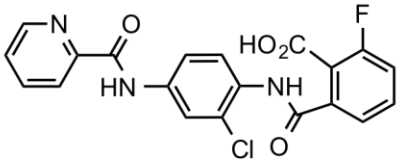
12

Exact Mass: 247.05



20a

Exact Mass: 413.06

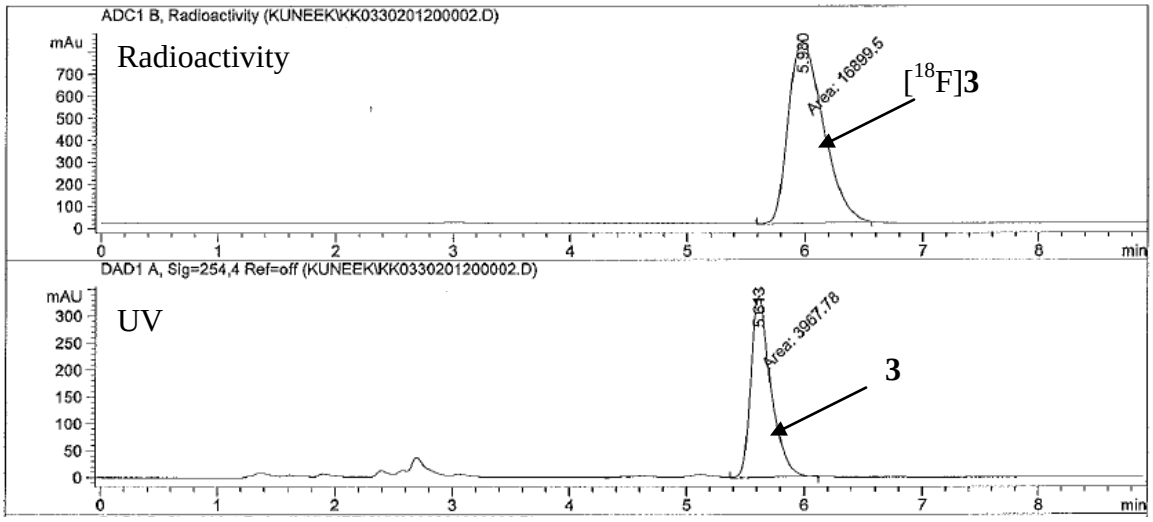


20b

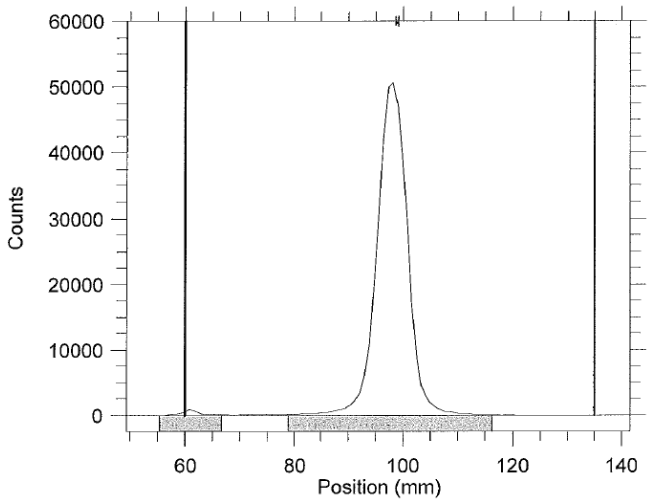
Exact Mass: 413.06

F. Analysis of [^{18}F]3 Formulated with 10% Ethanol Solution in 0.1 M Citrate Buffer (pH 4).

(a)



(b)



Reg	(mm) Start	(mm) Stop	(mm) Centroid	RF	Region Counts	Region CPM	% of Total	% of ROI
Rgn 1	55.5	66.8	60.9	0.012	3995.0	3995.0	1.03	1.04
Rgn 2	78.9	116.3	97.9	0.505	379987.0	379987.0	98.18	98.96
2 Peaks					383982.0	383982.0	99.22	100.00

(a) Analytical HPLC profile of [^{18}F]**3** co-injected with nonradioactive standard **3** by an acidic method. (b) The radio-TLC result obtained after the radioactive aliquot was formulated in a 0.1 M citrate buffer solution (pH 4) with 10% ethanol, cospotted with the nonradioactive **3** on TLC and developed with a solution of ethyl acetate and hexane (1:1).

References

(1) Shi, Q.; Savage, J. E.; Hufeisen, S. J.; Rauser, L.; Grajkowska, E.; Ernsberger, P.; Wroblewski, J. T.; Nadeau, J. H.; Roth, B. L. L-Homocysteine sulfinic acid and other acidic homocysteine derivatives are potent and selective metabotropic glutamate receptor agonists. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 131-142.

(2) Roth, B. L.: National Institute of Mental Health Psychoactive Drug Screening Program Assay Protocol Book