Supporting Information

A new highly deuterated [^{18}F]AV-45, [^{18}F]D15FSP, for imaging β-amyloid plaques in the brain

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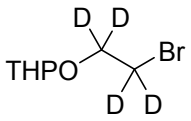
1. Synthesis of D15FSP (1)

1.1 Material

All reagents and solvents were purchased commercially (Aldrich, Acros, or Alfa Inc.) and were used without further purification, unless otherwise indicated. Solvents dried through a molecular sieve system (Pure Solve Solvent Purification System; Innovative Technology, Inc.). ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on a Bruker AVANCE II spectrometer at 400 MHz, 100 MHz and 376 MHz respectively, and referenced to NMR solvents as indicated. Chemical shifts are reported in ppm (δ), with a coupling constant, *J*, in Hz. The multiplicity is defined by s (singlet), d (doublet), t (triplet), br (broad), and m (multiplet). High-resolution mass spectrometry (HRMS) data were obtained with an Agilent (Santa Clara, CA) G3250AA LC/MSD TOF system. Thin-layer chromatography (TLC) analyses were performed using Merck (Darmstadt, Germany) silica gel $60F_{254}$ plates. Generally, crude compounds were purified by flash column chromatography (FC) packed with silica gel (Aldrich). High performance liquid chromatography (HPLC) was performed on an Agilent 1100 series system. Reactions of non-radioactive chemical compounds were monitored by thin-layer chromatography (TLC) analysis with pre-coated plates of silica gel 60 F_{254}.

1.2 2-(2-Bromoethoxy-1,1,2,2-d4)tetrahydro-2H-pyran (7)

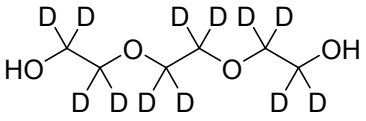


7

To a solution of D4-2-bromoethanol (258 mg, 2 mmol) in 10 mL tetrahydrofuran (THF) was added pyridinum *p*-toluenesulfonate (PPTS, 50 mg, 0.2 mmol) and 3,4-dihydo-2*H*-pyran (336 mg, 4 mmol). After stirring at rt for 3 h, the mixture was diluted with 50 mL EtOAc and washed with H_2O (20 mL × 2) as well as brine (20 mL). The organic layer was dried by Na_2SO_4 and filtered. The filtrate was

concentrated, and the residue was purified by FC (EtOAc/hexane = 5/95) to give 424 mg clear oil **7** (yield: 100%): ^{1}HNMR (400 MHz, $CDCl_3$) δ: 4.68-4.71 (m, 1H), 3.89-3.94 (m, 1H), 3.53-3.56 (m, 1H), 1.55-1.85 (m, 6H).

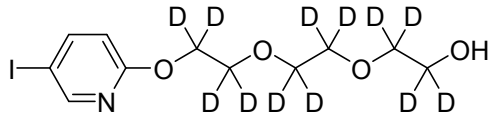
1.3 2,2'-((Ethane-1,2-diyl-d4)bis(oxy))bis(ethan-1,1,2,2-d4-1-ol) (9)



9

NaH (60% in mineral oil, 0.160 g, 4 mmol) was placed in a two-neck flask and washed with hexane. 5 mL DMF was added to form a suspension. A solution of D4-1,2-dihydroxyethane (62 mg, 1 mmol) in 5 mL DMF was added dropwise at 0 °C. After stirring at rt for 30 min, the mixture was cooled to 0 °C, and a solution of **7** (424 mg, 2 mmol) in 3 mL DMF was added dropwise, and the reaction mixture was stirred at rt overnight. The mixture was then poured into 50 mL cold sat. NH_4Cl, and extracted with DCM (50 mL × 2). The organic layer was washed with H_2O (30 mL) and brine (30 mL), dried over Na_2SO_4 and concentrated to give a yellowish oil compound **8**. The residue was then dissolved in 10 mL EtOH. *p*-Toluene sulfonic acid (172 mg, 1 mmol) was added and the solution was heated at 60 °C overnight. The solvent was removed under vacuum and the residue was purified by FC (EtOAc/hexane = 80/20) to give 136 mg clear oil **9** (yield: 41.9%): The ^{1}HNMR data was not collected because no hydrogen can be detected by NMR. The product was identified by co-spotting with triethylene glycol in TLC and used without further characterization.

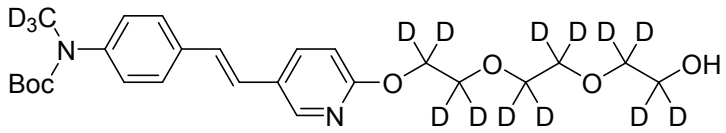
1.4 2-(2-(2-((5-iodopyridin-2-yl)oxy)ethoxy-1,1,2,2-d4)ethoxy-1,1,2,2-d4)ethan-1,1,2,2-d4-1-ol (10)



10

In a 20-mL Biotage microwave reaction vial, 2-Chloro-5-iodopyridine (166 mg, 0.7 mmol), **9** (136 mg, 0.84 mmol), cesium carbonate (Cs_2CO_3, 341 g, 1.05 mmol), and DMF (5 mL) were mixed and capped. After microwave heating at 150 °C for 55 min, the mixture was cooled to rt and diluted with 50 mL EtOAc and washed with H_2O (5 mL × 2) as well as brine (15 mL). The organic layer was dried by Na_2SO_4 and filtered. The filtrate was concentrated, and the residue was purified by FC (EtOAc/hexane = 7/3) to give 70 mg white solid **10** (yield: 28.6%): ^{1}HNMR (400 MHz, $CDCl_3$) δ: 8.31 (d, 1H, *J* = 2.4 Hz), 7.79 (dd, 1H, *J* = 2.4 Hz, *J* = 8.6 Hz), 6.65 (d, 1H, *J* = 8.6 Hz). HRMS (ESI) calculated for $C_{11}H_5D_{12}INO_4$ ($M+H^+$), 366.0955; found, 366.0967.

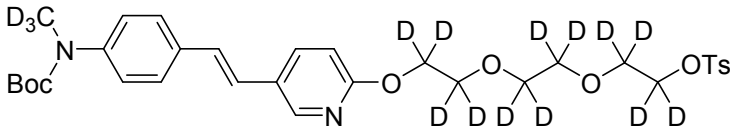
1.5 tert-Butyl (E)-(4-(2-(6-(2-(2-(2-hydroxyethoxy-1,1,2,2-d4)ethoxy-1,1,2,2-d4)ethoxy-1,1,2,2-d4)pyridin-3-yl)vinyl)phenyl)(methyl-d3)carbamate (12)



12

A mixture of **11** (56 mg, 0.24 mmol), **10** (70 mg, 0.2 mmol), potassium carbonate (K_2CO_3, 69 mg, 0.5 mmol), tetrabutylammonium bromide (129 mg, 0.4 mmol), and palladium acetate ($Pd(OAc)_2$, 4.48 mg, 0.02 mmol) in 5 mL DMF was deoxygenated by purging into nitrogen for 15 min and then heated at 65 °C for overnight. The mixture was cooled to RT, diluted with 50 mL EtOAc and washed with H_2O (15 mL × 2) as well as brine (15 mL). The organic layer was dried by Na_2SO_4 and filtered. The filtrate was concentrated, and the residue was purified by FC (EtOAc/hexane = 8/2) to give 60 mg white solid **12** (yield: 65%): ^{1}HNMR (400 MHz, $CDCl_3$) δ: 8.19 (d, 1H, *J* = 2.4 Hz), 7.80 (dd, 1H, *J* = 2.4 Hz, *J* = 8.6 Hz), 7.45 (d, 2H, *J* = 8.4 Hz), 7.23 (d, 2H, *J* = 8.4 Hz), 6.98(s, 2H), 6.80 (d, 1H, *J* = 8.6 Hz), 1.47 (s, 9H). HRMS (ESI) calculated for $C_{25}H_{20}D_{15}N_2O_6$ ($M+H^+$), 474.3437; found, 474.3461.

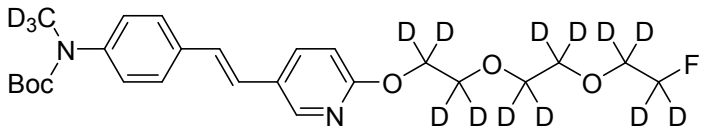
1.6 (E)-2-(2-(2-((5-(4-((tert-Butoxycarbonyl)(methyl-d3)amino)styryl-d3)pyridin-2-yl)oxy)ethoxy-1,1,2,2-d4)ethoxy-1,1,2,2-d4)ethyl-1,1,2,2-d4 4-methylbenzenesulfonate (4)



4

To a solution of **12** (60 mg, 0.13 mmol) and triethylamine (52.5 mg, 0.52 mmol) in 4 mL DCM was added *p*-toluenesulfonyl chloride (49.4 mg, 0.26 mmol) and 4-(dimethylamino)pyridine (DMAP, 2 mg) at 0 °C. The solution was allowed to warm to rt. After 6 h, the mixture was diluted with 20 mL DCM, washed with H_2O (10 mL × 2) as well as brine (10 mL). The organic layer was dried by Na_2SO_4 and concentrated to give an oil that was purified by FC (EtOAc/hexane = 1/1) to give 52 mg white solid **4** (63.7%): ^{1}HNMR (400 MHz, $CDCl_3$) δ: 8.19 (d, 1H, *J* = 2.4 Hz), 7.80-7.83 (m, 3H), 7.46 (d, 2H, *J* = 8.6 Hz), 7.34 (d, 2H, *J* = 8.4 Hz), 7.24 (d, 2H, *J* = 8.6 Hz), 6.98 (s, 2H), 6.80 (d, 1H, *J* = 8.6 Hz), 2.45 (s, 3H), 1.49 (s, 9H). HRMS (ESI) calculated for $C_{32}H_{26}D_{15}N_2O_8S$ ($M+H^+$), 628.3525; found, 628.3551.

1.7 tert-Butyl (E)-(4-(2-(6-(2-(2-(2-fluoroethoxy-1,1,2,2-d4)ethoxy-1,1,2,2-d4)ethoxy-1,1,2,2-d4)pyridin-3-yl)vinyl)phenyl)(methyl-d3)carbamate (13)

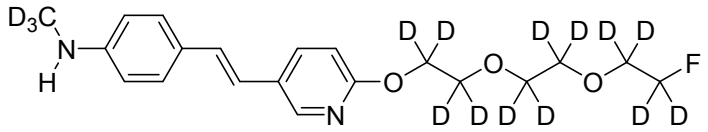


13

A mixture of **4** (32 mg, 0.051 mmol) and tetrabutylammonium fluoride (TBAF, 0.1 mL, 1.0 M in THF) in 1.5 mL THF was stirred at 70 °C overnight. The reaction mixture was evaporated in vacuo, and the residue was purified by FC (EtOAc/MeOH = 9/1) to give 15 mg colorless oil **13** (yield: 61.9%): ^{1}HNMR (400 MHz, $CDCl_3$) δ: 8.19 (d, 1H, *J* = 2.4 Hz), 7.81 (d, 1H, *J* = 8.6 Hz), 7.46 (d, 2H, *J* = 8.6 Hz), 7.24 (d, 2H, *J* = 8.6 Hz), 6.98 (s, 2H), 6.80 (d, 1H, *J* = 8.6 Hz), 1.48 (s, 9H).

^{19}FNMR (376 MHz , $CDCl_3$) δ: -225 ppm (br). HRMS (ESI) calculated for $C_{25}H_{19}D_{15}FN_2O_5$ ($M+H^+$), 476.3393; found, 476.3381.

1.8 (E)-4-(2-(6-(2-(2-(2-Fluoroethoxy-1,1,2,2-d4)ethoxy-1,1,2,2-d4)ethoxy-1,1,2,2-d4)pyridin-3-yl)vinyl)-N-(methyl-d3)aniline (1)

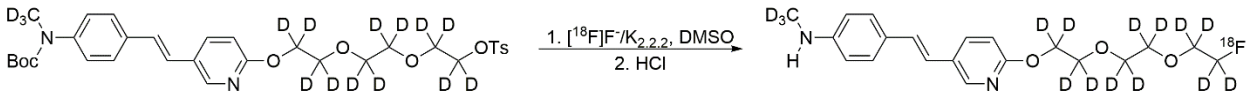


1

A solution of **13** (10 mg, 0.021 mmol) in 1 mL trifluoroacetic acid (TFA) was stirred at rt for 1 h. The reaction mixture was evaporated in vacuo, and the residue was purified by FC (DCM/MeOH =92.5/7.5) to give 5 mg yellowish oil **1** (yield: 63.5%) ^{1}HNMR (400 MHz, $CDCl_3$) δ: 8.15 (d, 1H, J = 2.2 Hz), 7.77 (dd, 1H, J = 2.4 Hz, J = 8.8 Hz), 7.36 (d, 2H, J = 8.4 Hz), 6.92 (d, 1H, J = 16.3 Hz), 6.82(d, 1H, J = 16.3 Hz), 6.78 (d, 1H, J = 8.7 Hz), 6.61 (d, 2H, J = 8.4 Hz). ^{19}FNMR (376 MHz , $CDCl_3$) δ: -225 ppm (br). (HRMS (ESI) calculated for $C_{20}H_{11}D_{15}FN_2O_3$ ($M+H^+$), 376.2869; found, 376.2881.

2. Radiochemistry

2.1 Preparation of [^{18}F]D15FSP ([^{18}F]1) – HPLC profiles and determination of molar activities (A_m)



4 [^{18}F]**1**, [^{18}F]D15FSP

Drying and activation of [^{18}F]fluoride : An activated QMA light carb was loaded with [^{18}F]fluoride (0.94 ± 0.54 GBq) and eluted with 0.7 mL $K_{2.2.2}/K_2CO_3$ solution (40 mg K_2CO_3, 222 mg $K_{2.2.2}$, 18.4

mL ACN, 3.6 mL water) into a 1 mL conical vial. The solution was dried azeotropically twice with 1 mL acetonitrile at 90 ºC.

Labeling: Precursor **4** was dissolved in 0.5 mL DMSO and added to dried fluoride prepared above. The vial was crimped and heated at 110 ºC for 15 minutes.

Deprotection: The resulting mixture from reaction listed above was cooled to room temperature for 1 minute. 300 µL HCl (aqueous10%) was added. The mixture was heated at 100 ºC for 10 minutes to remove the N-Boc protecting group.

Workup: The reaction medium was added to 8 mL ice cold water/100 µL 3N NaOH. All the mixture was loaded on activated Oasis cartridge. The cartridge was rinsed with 2 × 3 mL water and the product was eluted with 1 mL ACN.

Purification: Eluted acetonitrile solution was diluted with 2 mL 10 mM AFB (ammonium formate buffer, pH 6.5) and injected into preparative HPLC: Gemini C18, 250 × 10 mm, ACN/10 mM AFB (pH 6.5) 60/40, 4 mL/min, 350 nm. Fraction at 9 - 11 min was collected. The fraction was diluted with 50 mL water and pushed through Vydac C4 mini column, washed with 3 mL water. The final product, [^{18}F]D15FSP ([^{18}F]**1**), was eluted with 1 mL EtOH.

Result: The final product, [^{18}F]D15FSP ([^{18}F]**1**), showed RCP: 96.6 ± 1.7% (n = 3). RCY: 53.0 ± 7.8% (decay corrected). Molar activity (A_m): 23.0 ± 7.4 GBq/µmol).

Table S1. Individual data of radiolabeling of [^{18}F]D15FSP

Run#	Starting Activity (MBq)	RCP (%)	RCY (decay corrected) (%)	Molar activity (A_m, GBq/µmol)
1	799	95	48	30.8
2	1668	99	64	25.2
3	367	96	47	13.0

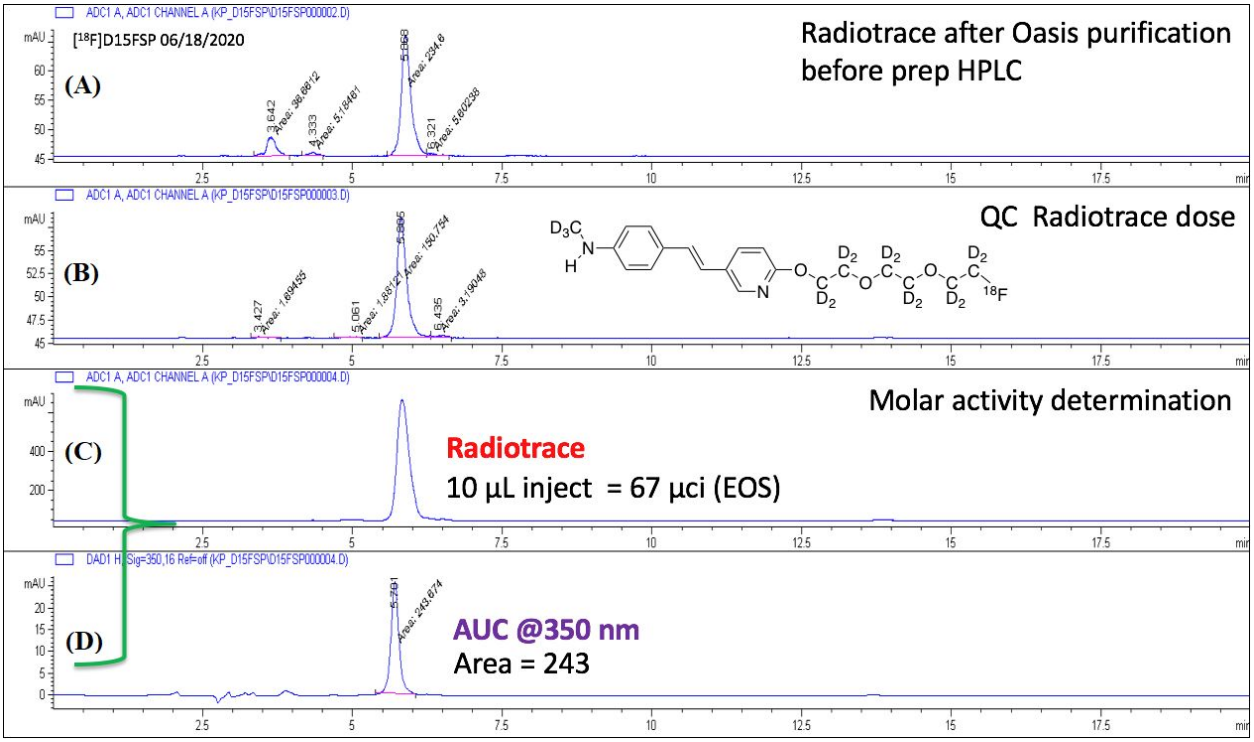


Fig. S1 HPLC profiles of the radiotrace of [^{18}F]D15FSP ([^{18}F]**1**) before (A) and after HLC purification (B). Traces (C) and (D) are radioactive and the corresponding UV profile, respectively used for molar activity determination

3. Biological evaluation studies

All reagents and solvents were purchased commercially and were used without further purification unless otherwise indicated. Solvents were dried through a molecular sieve system (Pure Solve Solvent Purification System; Innovative Technology, Inc.).

3.1 In vitro competitive binding assay

Competitive binding assays were performed with the mixture of 100 µL AD brain homogenates (~20 µg), 100 µL [^{125}I]IMPY, a known Ab binding ligand [1-2], (~100,000 cpm), and 50 µL of competing compounds ("cold" D15FSP (**1**), D3FSP (**2**) or AV-45 (**3**), 10^{-5} to 10^{-10} M) in PBS buffer. Non-specific binding was defined in the presence of 5 µM AV-45 (**3**). After incubating at 37 °C for 60 min, the bound and the free radioactivity were separated by vacuum filtration through Whatman

GF/B filters using a Brandel M-24R cell harvester, followed by washing with PBS buffer three times. The radioactivity on the filters was counted with a gamma counter (Wizard^2, Perkin-Elmer). Data were analyzed using the nonlinear least-square curve fitting program LIGAND to determine IC_{50}. K_i was calculated by Cheng-Prusoff equation using 5.3 nM as K_d of [^{125}I]IMPY.

3.2 In vitro autoradiography of AD brain sections

Frozen brains from AD and control subjects were cut into 20 μm sections. After drying in the air, the sections were incubated with ~10^5 cpm [^{18}F]D15FSP ([^{18}F]**1**) or [^{18}F]AV-45 ([^{18}F]**3**) in 1 mL 40% ethanol for 60 min at 25 ℃. The sections were then washed with 40% ethanol (5 min), saturated Li_2CO_3 in 40% ethanol (5 min), 40% ethanol (5 min), followed by rinsing with water for 30 s. After drying, the sections were exposed to BAS-MS imaging plate (Fuji film) for 30 min. Digitized images were acquired with Typhoon FLA 7000 (GE Healthcare). For blocking studies, the sections were incubated with a mixture of 5 μM cold compounds and ~10^5 cpm [^{18}F]D15FSP ([^{18}F]**1**) in 1 mL 40% ethanol for 60 min at 25 ℃. The wash, exposure, and imaging procedures were same as described above.

3.3 In vivo biodistribution in CD-1 mice

Animal studies were undertaken in compliance with University of Pennsylvania IACUC guidelines related to the conduct of animal experiments. CD-1 mice (25–32 g, 5-6 weeks, male) were injected with [^{18}F]D15FSP (~370 kBq/mouse), 150 μL formulated in 10% ethanol and 90% saline directly into the tail vein. The mice were sacrificed by cardiac puncture under isoflurane anesthesia at various time points (2, 30, 60, and 120 min) after the injection. Organs of interest were removed and weighed, and the radioactivity was counted with a γ-counter. The injected percentage dose per organ was calculated by a comparison of the tissue counts to suitably diluted aliquots of the injected material. Each time point consisted of a group of 3 animals.

Table S2. Distribution of radioactivity after the injection of [^{18}F]D15FSP in normal male CD-1 mice expressed as % injected dose/g (%ID/g)

	2 min	30 min	60 min	120 min
Blood	2.21 ± 0.16	2.16 ± 0.16	1.98 ± 0.24	1.51 ± 0.28
Heart	5.41 ± 0.28	2.00 ± 0.21	1.63 ± 0.07	1.28 ± 0.14
Muscle	2.16 ± 0.08	1.16 ± 0.14	0.88 ± 0.08	0.71 ± 0.07
Lung	4.61 ± 0.73	2.43 ± 0.22	2.11 ± 0.18	1.46 ± 0.26
Kidney	9.72 ± 0.83	8.09 ± 1.12	5.11 ± 0.45	3.00 ± 0.49
Spleen	2.47 ± 0.18	1.57 ± 0.39	1.03 ± 0.08	1.20 ± 0.27
Pancreas	3.85 ± 0.52	2.03 ± 0.35	1.39 ± 0.12	1.55 ± 0.24
Liver	5.50 ± 2.17	9.52 ± 0.67	7.37 ± 1.31	7.29 ± 0.79
Stomach	2.32 ± 0.90	6.80 ± 1.33	4.00 ± 0.72	2.83 ± 1.01
Small intestine	3.59 ± 0.62	7.99 ± 0.95	10.15 ± 3.30	8.96 ± 2.11
Large intestine	1.43 ± 0.11	2.46 ± 1.47	3.38 ± 1.01	11.43 ± 4.86
Brain	5.99 ± 0.14	1.30 ± 0.19	1.11 ± 0.03	1.18 ± 0.11
Bone	0.97 ± 0.13	0.69 ± 0.05	0.79 ± 0.07	1.44 ± 0.03

Values are the average ± SD of three animals.

3.4 Estimated human dosimetry of [^{18}F]D15FSP

Human radiation dosimetry was estimated based on biodistribution of [^{18}F]D15FSP for iv injection in normal male mice. The distribution of the radioactivity concentrations were measured in various organs of mice at 2, 30, 60 and 120 min after injection of [^{18}F]D15FSP. The radiation dose estimates were calculated for human organs, based on an extrapolation of the animal data to humans using OLINDA (v.1.0 (2003)/EXM software.

Table S3. Estimated human dosimetry data of [^{18}F]D15FSP in mSv/MBq

	Organ Absorbed Dose

Target Organ	mSv/MBq
Adrenals	1.51E-02
Brain	5.89E-03
Breasts	8.20E-03
Gallbladder wall	2.00E-02
Lower large intestine wall	4.07E-02
Small intestine	4.43E-02
Stomach wall	2.06E-02
Upper large intestine wall	3.55E-02
Heart Wall	3.55E-02
Kidneys	5.39E-02
Liver	5.09E-02
Lungs	9.42E-03
Muscle	8.60E-03
Ovaries	1.59E-02
Pancreas	3.28E-02
Red Marrow	1.05E-02
Osteogenic cells	2.05E-02
Skin	6.72E-02
Spleen	1.21E-02
Testes	8.15E-03
Thymus	1.07E-02
Thyroid	8.23E-03
Urinary bladder wall	1.11E-02
Uterus	1.43E-02
Total Body	1.22E-02
Effective Dose	1.89E-02 mSv/MBq

References:

1. Kung, M.; Hou, C.; Zhuang, Z.; Zhang, B.; Skovronsky, D.; Gur, T.; Lee, V.; Trojanowski, J.; Kung, H., IMPY: An improved thioflavin-T derivative for in vivo Labeling of b-amyloid plaques. *Brain Res*. **2002,** *956* (2), 202-210.
2. Choi, S.; Golding, G.; Zhuang, Z.; Zhang, W.; Lim, N.; Hefti, F.; Benedum, T.; Kilbourn, M.; Skovronsky, D.; Kung, H., Preclinical properties of ^{18}F-AV-45: a PET agent for Aβ plaques in the brain. *J. Nucl. Med.* **2009,** *50*, 1887-1894.