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Fluoro-pegylated (FPEG) Imaging Agents Targeting Aβ Aggregates

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Abstract

A novel approach of producing positron emission tomography (PET) imaging agents through the formation of bioconjugates based on pegylation-fluorination strategy resulting in fluoro-pegylated (FPEG) molecules is reported. This approach offers a simple and easy method by which to incorporate ^{18}F in the target molecule without appreciable increase in the lipophilicity. After ^{18}F labeling this convenient approach leads to PET imaging probes binding to Aβ aggregates in the brain (an important factor associated with Alzheimer's disease) using the known core structures, such as [2-(4-dimethylaminophenyl)-vinyl]-benzoxazol (**3′**) or 2-phenylbenzothiazole (**4**). This approach appears to be effective in some core structures, but it cannot be uniformly applied to all structures.

Keywaords

PET. F-18 radiochemistry; pegylation; brain uptake and Alzheimer's disease

Introduction

Alzheimer's disease (AD) is an important neurodegenerative disorder of the brain affecting millions of older Americans; and yet the diagnosis of this disease based on neurological observations is often difficult and un-reliable. The only definitive diagnosis of the disease is by pathological examination of postmortem staining of affected brain tissues. The presence of β-amyloid (Aβ) aggregates in the brain is generally accepted as the hallmark of AD(1–4). Recent success in developing radionuclide-labeled agents targeting the Aβ aggregates provides a window of opportunity to improve the diagnosis of AD(5–8). Preliminary reports of positron emission tomography (PET) imaging suggested that, 4-*N*-methylamino-4′-hydroxystilbene ([^{11}C]**1**, SB-13) (9), and [^{11}C] 2-(4′-(methylaminophenyl)-6-hydroxybenzothiazole ([^{11}C]**4**, also known as PIB)(10) (Figure 1) showed differential uptake and retention in the brain of AD patients as compared to controls. Since ^{11}C is a positron emitting isotope with $T_{1/2}$ = 20 min, which limits its usefulness for a wide spread clinical application, efforts are being made to develop comparable agents labeled with a longer half-life isotope - ^{18}F ($T_{1/2}$ = 110 min). Preliminary studies with [^{18}F]-2-(1-(2-(*N*-(2-fluoroethyl)-*N*-methylamino)naphthalen-6-yl) ethylidene)-malononitrile ([^{18}F]FDDNP)(7) showed differential uptake and retention in the brain of AD patients but this a highly lipophilic tracer, binding to both tangles and plaques making it difficult to measure the specific signal contribution from plaques. In 2002, Mathis *et. al*. reported a series of [^{18}F]-fluoroalkoxy-BTA-1 derivatives, which showed excellent binding affinities and brain penetrations(11). Two other potential series of Aβ specific imaging

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agents, 6-iodo-2-(4′-dimethylamino-)phenyl-imidazo[1,2-a]pyridine (**2**, IMPY)(12–14) and 2-(4-methylaminostyryl)-6-(2-fluoroethoxy)benzoxazole **(3,** BF-168)(15,16), have also shown high specific binding affinities to Aβ aggregates of AD brain homogenates (K_i < 10 nM; see Table 1).

We are interested in replacing the labeling isotope with ^{18}F, but the challenge is to maintain the Aβ aggregate-binding affinity and maintain suitable *in vivo* kinetics of the agents with sufficient selective uptake in the brain(6,17). Incorporating a radioactive fluoride atom (^{18}F) is typically accomplished using either electrophilic or nucleophilic conditions (18–20). Fluoride nucleophilic displacement reactions are advantageous as they often result in higher yields, higher specific activities and the fluoride can be produced more readily(19,20). [^{18}F] fluoride can be added via an SN2 type reaction with good leaving groups such as either the mesylate or tosylate precursor. One of the commonly used methods to append a fluorine atom involves adding a fluoroethyl or fluoropropyl group to the target compound. However, when these short fluoro alkyl chains were added to the core structures the results were sometimes not promising. This is often due to an increase in lipophilicity; the resulting ^{18}F labeled agents tend to have a higher non-specific binding to the Aβ aggregates. To circumvent these undesirable effects we have exploited a novel approach by using fluoro-pegylation (FPEG) of the core structures for ^{18}F labeling of stilbene derivatives(21). It is generally assumed that the *p-N*-methylaminophenyl or the *p-N,N*-dimethyl-aminophenyl group is critical for binding affinity; modification on the opposite end of these core structures may not alter the binding affinity(17).

Pegylation (PEG) is a common approach for changing *in vivo* pharmacokinetics of various biologically interesting proteins or peptides, through which the *in vivo* stability and pharmacokinetics can be improved leading to better therapeutics(22,23). Recently, the “pegylation” technique has been applied to modify pharmacokinetic properties of radiopharmaceuticals(24,25). Conjugating PEG macromolecules (high molecular weight PEGs, M.W. = 10,000–20,000) to labeled peptides may be efficacious in changing biodistribution *in vivo* and leading to improvements in specific localization of agents targeting peripheral tissues. However, it will be ineffective to use macromolecular PEG conjugated radiopharmaceuticals as imaging agents for the brain due to limitation of such macromolecules to cross the blood-brain barrier. In an attempt to modulate the lipophilicity of ^{18}F labeled stilbene derivatives (21,26), we have adopted a novel approach by adding a short length of FPEG (n = 2–5) and capping the end of the ethylene glycol chain with a fluorine atom(21). The resulting FPEG derivatives of SB-13 displayed excellent Aβ aggregate-binding affinities and high brain penetrations. The success of this FPEG approach has prompted us to expand this simple strategy to different Aβ-targeting small core structures (**1**–**4**).

In this report, we explored fluoropegylated compounds with IMPY (**2**), BF168 (**3**) and PIB (**4**) core structures with various chain lengths (n = 1~8) for biological evaluations. F-18 radiolabelings were attempted to several compounds with each cores ([^{18}F]**5a**–**c** for PIB, [^{18}F] **12c** for IMPY and [^{18}F]**8b** for BF168) and the initial biodistributions and autoradiograms of [^{18}F]**5a**–**c** were obtained. Reported herein is a structure-binding-activity study of attaching a short chain length FPEG (n ≤ 8) to these molecules for labeling with ^{18}F and concomitantly maintaining *in vitro* binding affinities and *in vivo* biological properties.

Experimental Procedures

General

All reagents in the syntheses were commercial products used without further purification unless otherwise indicated. ^{1}H NMR spectra were obtained on a Bruker DPX spectrometer (200 MHz) in $CDCl_3$ unless otherwise indicated. Chemical shifts are reported as δ values (parts per million)

relative to internal TMS. Coupling constants are reported in hertz. The multiplicity is defined by s (singlet), d (doublet), t (triplet), br (broad), m (multiplet). High resolution electron ionization (HREI) mass spectra were performed at the McMaster Regional Centre for Mass Spectrometry using a Micromass/Waters GCT instrument (GC-EI/CI Time of Flight Mass Spectrometer). Semi-preparative HPLC was performed using a Phenomenex Gemini C18 column [(5.0 × 250 mm, 5 μm); acetonitrile/water 70/30; flow rate 3 mL/min] and analytical HPLC conditions using a Phenomenex Gemini C18 analytical column [(5.0 × 250 mm, 5 μm); acetonitrile/water 80/20; flow rate 1 mL/min)].

Synthesis of 2-(4′-(methylaminophenyl)-6-hydroxybenzothiazole (PIB, 4) derivatives

Compound **4** (2-(4′-(methylaminophenyl)-6-hydroxybenzothiazole (PIB) was prepared using Mathis and co-workers approach (27). Monomethylation was accomplished via standard reported procedures (28) to yield **4** that was used in subsequent steps.

General procedure for the pegylation of 4—To a solution of **4** (1 eq) in anhydrous N, N-dimethylformamide (2 mL/0.1 mmol of **4**) in a microwavable vial (from Biotage) was added anhydrous cesium carbonate (2.5 eq) and the mixture stirred at room temperature under argon for 30 min. Pegylation agent (1.2 eq) followed by sodium iodide (1.5 eq) were then added to the vial. The vial was then sealed and subjected to microwave irradiation (Biotage Initiator system). The microwave conditions were 200 °C for 10 min with 10 sec pre-stirring. After cooling the reaction mixture to room temperature the vial was opened, the contents were transferred to a round-bottom flask and the volatiles were removed under a reduced pressure. The residue was extracted with ethyl acetate (3×10 mL) and the ethyl acetate layer was washed with water (1×10 mL) and brine (1×10 mL). The organic layer, after drying over anhydrous magnesium sulfate, was evaporated and the residue was purified on silica PTLC (PTLC) to afford the corresponding PEGylated derivatives.

Preparation of compounds 5(a–d)—Treatment of **4** with the fluoromesylates according to the general procedure afforded compounds **5(a–d)**.

2-[4′-(Methylamino)phenyl]-6-[2-(2-fluoroethoxy)-ethoxy]benzothiazole (5a): Yield: 84%.

(PTLC, 50 % ethyl acetate in hexane**)**. ^{1}H NMR (200 MHz, $CDCl_3$):δ 7.83–7.89 (3H, m), 7.33 (1H, d, *J* = 2.5 Hz), 7.06 (1H, dd, *J* = 8.9, 2.5 Hz), 6.63 (2H, d, *J* = 8.9 Hz), 4.60 (2H, dt, *J* = 47.6, 4.2 Hz), 4.21 (2H, t, *J* = 4.5 Hz), 3.89–3.94 (3H, m), 3.76 (1H, d, *J* = 4.2 Hz), 2.90 (3H, s). HRMS (EI) *m*/*z* calcd. for $[C_{18}H_{19}FN_2O_2S]^+$ 346.1151, found 346.1141.

2-[4′-(Methylamino)phenyl]-6-(2-(2-(2-fluoroethoxy)-ethoxy)-ethoxy)-benzothiazole (5b): Yield: 78%.

(PTLC, 60 % ethyl acetate in hexane). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.82–7.88 (3H, m), 7.32 (1H, d, *J* = 2.5 Hz), 7.05 (1H, dd, *J* = 8.8, 2.5 Hz) 6.63 (2H, d, *J* = 8.8 Hz), 4.56 (2H, dt, *J* = 47.6, 4.2 Hz), 4.19 (2H, t, *J* = 4.5 Hz), 3.65–3.88(8H, m), 2.89 (3H, s). HRMS (EI) *m*/*z* calcd. for $[C_{20}H_{23}FN_2O_3S]^+$ 390.1413, found 390.1386

2-[4′-(Methylamino)phenyl]-6-(2-(2-(2-(2-(2-(2-fluoroethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-benzothiazole (5c): Yield: 72%.

(PTLC: 80 % ethyl acetate in hexane). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.82–7.87 (3H, m), 7.33 (1H, d, *J* = 2.4 Hz), 7.05 (1H, dd, *J* = 8.8, 2.4 Hz), 6.63 (2H, d, *J* = 8.8 Hz), 4.54 (2H, dt, *J* = 47.6, 4.1 Hz), 4.18 (2H, t, *J* = 4.5 Hz), 3.65–3.90 (20H, m), 2.89 (3H, s). HRMS (EI) *m*/*z* calcd for $[C_{26}H_{35}FN_2O_6S]^+$ 522.2200, found 522.2175.

2-[4′-(Methylamino)phenyl]-6-(2-(2-(2-(2-(2-(2-(2-(2-fluoroethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-benzothiazole (5d): Yield: 71%.

(PTLC: ethyl acetate). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.81–7.87 (3H, m), 7.33 (1H, d, *J* = 2.4 Hz), 7.05 (1H, dd, *J* = 8.8, 2.4 Hz), 6.63 (2H, d, *J* = 8.8 Hz), 4.55 (2H, dt, *J* = 47.7, 4.2 Hz), 4.19 (2H, t, *J* = 4.4 Hz), 3.63–3.90(28H, m), 2.89 (3H, s). HRMS (EI) *m*/*z* calcd for $[C_{30}H_{43}FN_2O_8S]^+$ 610.2724, found 610.2705.

Preparation of compounds 6(a–c)—Treatment of **4** with hydroxymesylates according to the general procedure afforded compounds **6(a–c).**

2-(2-(2-(4-Methylamino-phenyl)-benzothiazol-6-yloxy)-ethoxy)-ethanol (6a): Yield: 82%.

(PTLC, 1 % methanol in dichloromethane). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.85–7.89 (3H, m), 7.33 (1H, d, *J* = 2.4 Hz), 7.06 (1H, dd, *J* = 8.8, 2.4 Hz), 6.64 (2H, d, *J* = 8.8 Hz), 4.20 (2H, d, *J* = 4.3 Hz), 3.90 (2H, d, *J* = 4.6 Hz), 3.69–3.78 (m, 4H), 2.90 (s, 3H). HRMS (EI) *m*/*z* calcd for $[C_{18}H_{20}N_2O_3S]^+$ 344.1195, found 344.1188

2-(2-(2-(2-(4-Methylamino-phenyl)-benzothiazol-6-yloxy]-ethoxy)-ethoxy)-ethanol (6b): Yield: 74%.

(PTLC, 1 % methanol in dichloromethane). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.83–7.88 (3H, m), 7.31 (1H, d, *J* = 2.5 Hz), 7.05 (1H, dd, *J* = 8.8, 2.5 Hz) 6.63 (2H, d, *J* = 8.8 Hz), 4.19 (2H, t, *J* = 4.5 Hz), 3.88 (2H, t, *J* = 4.6 Hz) 3.58–3.78 (8H, m), 2.90 (3H, s). HRMS (EI) *m*/*z* calcd for $[C_{20}H_{24}N_2O_4S]^+$ 388.1457, found 388.1444.

2-(2-(2-(2-(2-(2-(4-Methylamino-phenyl)-benzothiazol-6-yloxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-ethanol (6c): Yield: 66%.

(PTLC, 2 % methanol in dichloromethane). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.83–7.88 (s, 3H), 7.33 (1H, d, *J* = 2.41 Hz), 7.06 (1H, dd, *J* = 8.8, 2.5 Hz), 6.63 (2H, d, *J* = 8.8 Hz), 4.19 (2H, t, *J* = 4.5 Hz), 3.88 (2H, t, *J* = 4.8 Hz), 3.56 - 3.53 (20H, m), 2.90 (3H, s). HRMS (EI) *m*/*z* calcd for $[C_{26}H_{36}N_2O_7S]^+$ 520.2243, found 520.2282.

Preparation of compounds 7(a–c)—Alkylation of **4** with *tert*-butyldimethylsilyl protected mesylates as per the general procedure afforded compounds **7(a–c)**.

2-[4′-(Methylamino)phenyl]-6-(2-(2-*tert*-butyldimethylsilyloxy-ethoxy)-ethoxy)-benzothiazole (7a): Yield: 70%.

(PTLC, 50 % ethyl acetate in hexane). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.84–7.88 (3H, m), 7.32 (1H, d, *J* = 2.4 Hz), 7.06 (1H, dd, *J* = 8.8, 2.4 Hz), 6.64 (2H, d, *J* = 8.8 Hz), 4.20 (2H, d, *J* = 4.3 Hz), 3.90 (2H, d, *J* = 4.6 Hz), 3.64–3.78 (m, 4H), 2.90 (s, 3H), 0.88 (9H, s), 0.05 (6H, s).

2-[4′-(Methylamino)phenyl]-6-(2-(2-(2-*tert*-butyldimethylsilyloxy-ethoxy)-ethoxy)-ethoxy)-benzothiazole (7b): Yield: 62%.

(PTLC, 60 % ethyl acetate in hexane). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.82–7.87 (3H, m), 7.30 (1H, d, *J* = 2.5 Hz), 7.05 (1H, dd, *J* = 8.8, 2.5 Hz) 6.62 (2H, d, *J* = 8.8 Hz), 4.21 (2H, t, *J* = 4.5 Hz), 3.88 (2H, t, *J* = 4.6 Hz) 3.58–3.74 (8H, m), 2.90 (3H, s), 0.88 (9H, s), 0.05 (6H, s).

2-[4′-(Methylamino)phenyl]-6-(2-(2-(2-(2-(2-(2-*tert*-butyldimethylsilyloxy-ethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-benzothiazole (7c): Yield: 55%.

(PTLC, 80 % ethyl acetate in hexane). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.85–7.89 (3H, m), 7.32 (1H, d, *J* = 2.4 Hz), 7.05 (1H, dd, *J* = 8.4, 2.4 Hz), 6.64 (2H, d, *J* = 8.4 Hz), 4.19 (2H, t, *J* = 4.7 Hz), 3.88 (2H, t, *J* = 4.9 Hz), 3.44–3.77 (20H, m), 2.90 (3H, s), 0.88 (9H, s), 0.05 (6H, s).

General procedure for the preparation of compounds 10(a–c)

A. General procedure for Boc protection to form 7′(a–c): Compounds **7(a–c)** (1 eq.) were dissolved in anhydrous tetrahydrofuran (10 mL/mmol of **7**) and to the resulting solution ditert-butyldicarbonate (2 eq) and 4-dimethylaminopyridine (catalytic) were added and the mixture heated to reflux. After 16 h another batch of ditert-butyldicarbonate (1 eq) was added and the mixture was further refluxed for another 20 h. The reaction mixture was then cooled to room temperature and the solvent was removed under reduced pressure. The residue was taken in ethyl acetate (25 mL/mmol of **6**) washed successively with water (1×10 mL) and brine(1×10 mL) and dried over anhydrous magnesium sulfate. The residue after removing the solvent was purified by silica PTLC.

2-[4′-(N-tert-Butyloxycabonyl-N-methylamino)phenyl]-6-(2-(2-tert-butyldimethylsilyloxy-ethoxy)-ethoxy)-benzothiazole (7′a): Yield: 55%.

(PTLC, 20% ethyl acetate in hexane). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.91–8.01 (3H, m), 7.34–7.38 (3H, m), 7.08 (1H, dd, *J* = 8.8, 2.4 Hz), 4.22 (2H, d, *J* = 4.3 Hz), 3.89 (2H, d, *J* = 4.6 Hz), 3.60–3.76 (m, 4H), 3.02 (s, 3H), 1.47 (9H, s), 0.88 (9H, s), 0.05(6H, s).

2-[4′-(N-tert-Butyloxycarbonyl-N-methylamino)phenyl]-6-(2-(2-(2-tert-butyldimethylsilyloxy-ethoxy)-ethoxy)-ethoxy)-benzothiazole (7′b): Yield: 48%.

(PTLC, 30 % ethyl acetate in hexane). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.90–7.99 (3H, m), 7.34–7.37 (3H, m), 7.06 (1H, dd, *J* = 8.6, 2.5 Hz) 4.20 (2H, t, *J* = 4.5 Hz), 3.88 (2H, t, *J* = 4.6 Hz) 3.54–3.69 (8H, m), 3.01 (3H, s), 1.46 (9H, s), 0.88 (9H, s), 0.05 (6H, s).

2-[4′-(N-tert-Butyloxycarbonyl-N-methylamino)phenyl]-6-(2-(2-(2-(2-(2-(2-tert-butyldimethylsilyloxy-ethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-benzothiazole (7′c): Yield: 40%.

(PTLC, 50 % ethyl acetate in hexane). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.92–8.01 (3H, m), 7.36–7.40 (3H, m), 7.05 (1H, dd, *J* = 8.4, 2.4 Hz), 4.20 (2H, t, *J* = 4.7 Hz), 3.88 (2H, t, *J* = 4.9 Hz), 3.44–3.77 (20H, m), 3.02 (3H, s), 1.47 (9H, s), 0.88 (9H, s), 0.05 (6H, s).

B. General procedure for deprotection followed by preparation of the mesylate derivatives 10(a–c): *tert*-Butyl carbonate (BOC) protected compounds **7′(a–c)** were dissolved in anhydrous tetrahydrofuran (3 mL/0.1 mmol of **7′**) and the resulting solution was cooled to 0 °C. Tetrabutylammonium fluoride (2 eq, 1M in tetrahydrofuran) was added to the ice cold solution and stirred at the same temperature for 15 min followed by room temperature for 2 h. The solvent was then removed, the residue dissolved in ethyl acetate (30 mL) and washed with water (1×10 mL), brine (1×10 mL) and dried over anhydrous magnesium sulfate. The resultant solution was then concentrated and redissolved in anhydrous dichloromethane (1 mL/0.1 mmol of **7′**). Anhydrous triethylamine (4 eq) was then added and the mixture cooled in an ice-acetone bath (~ −5 °C). Methanesulfonyl choride (3 eq) was added and the mixture stirred at the same temperature for 15 min. The reaction mixture was brought to room temperature gradually and stirred for an additional 2 h. It was then quenched with ice and extracted with dichloromethane (3×5 mL). The organic layer after drying over anhydrous magnesium sulfate was purified by silica PTLC.

2-(4′-(N-tert-Butyloxycabonyl-N-methylamino)phenyl)-6-(2-(2-methylsulfonyloxy-ethoxy)-ethoxy)benzothiazole (10a): Yield: 92%.

(PTLC, 1 % methanol in dichlormethane, 92 %). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.91–8.01 (3H, m), 7.34–7.38 (3H, m), 7.08 (1H, dd, *J* = 8.8, 2.4 Hz), 4.39–4.44 (2H, m), 4.19–4.23 (2H, m), 3.84–3.89 (4H, m), 3.30 (3H, s), 3.05 (3H, s), 1.47 (9H, s).

2-(4′-(N-tert-Butyloxycarbonyl-N-methylamino)phenyl]-6-(2-(2-(2- methylsulfonyloxy-ethoxy)-ethoxy)ethoxy)benzothiazole (10b): Yield: 95%.

(PTLC, 1 % methanol in dichloromethane, 95 %). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.90–8.01 (3H, m), 7.33–7.38 (3H, m), 7.09 (1H, dd, *J* = 8.8, 2.5 Hz), 4.35–4.39 (2H, m), 4.17 – 4.22 (2H, m), 3.86–3.91 (2H, m), 3.69–3.80 (6H, m), 3.31 (3H, s), 3.05 (3H, s), 1.47 (9H, s)

2-[4′-(N-tert-Butyloxycarbonyl-N-methylamino)phenyl]-6-(2-(2-(2-(2-(2-(2-methylsulfonyloxy-ethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-benzothiazole (10c): Yield: 90%.

(PTLC, 2 % methanol in dichloromethane). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.91–8.01 (3H, m), 7.34–7.38 (3H, m), 7.11 (1H, dd, *J* = 8.8, 2.4 Hz), 4.33–4.38 (2H, m), 4.18– 4.23 (2H, m), 3.87–3.92 (2H, m), 3.62–3.78 (18 H, m), 3.31 (3H, s), 3.06 (3H, s), 1.47 (9H, s).

Preparation of [2-(4-dimethylaminophenyl)-vinyl]-benzoxazol derivatives

2-(2-(4-dimethylaminophenyl)vinyl)-benzoxazol-6-ol (3′)—2-Methyl-benzoxazol-6-ol (prepared following Schreiner and coworkers method (29)) (1.7 mmol) was dissolved in anhydrous tetrahydrofuran (8 mL) and cooled to 0 °C. Trimethylsilyl chloride (1.8 mmol) and diisopropylethylamine (1.84 mmol) were then added and the resultant solution stirred for 2 h at room temperature. After cooling to −78 °C, sodium hexamethyldisilazane (11.7 mmol, 1.0 M solution in tetrahydrofuran) was added slowly over 1.5 h and then stirred at −78 °C for an additional hour. 4-(dimethylamino)-benzaldehyde was then added and the reaction allowed to warm to room temperature overnight. The reaction was then poured into a 1M solution of sodium hydrogen sulfate and extracted with ethyl acetate. The organic layer was then washed with brine, dried over magnesium sulfate and concentrated to yield a yellow solid that was purified using column chromatography (3% methanol in dichloromethane). Yield: 45%. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.57 (2H, d, *J* = 8.9 Hz), 7.55 (1H, d, *J* = 16.0 Hz), 7.43 (1H, d, *J* = 8.5 Hz), 6.99 (1H, d, *J* = 2.1 Hz), 6.89 (1H, d, *J* = 16.0 Hz), 6.78 (1H, dd, *J* = 8.5, 2.1 Hz), 6.73 (2H, d, *J* = 8.9 Hz), 2.98 (6H, s). HRMS (EI) *m*/*z* calcd. for $[C_{17}H_{16}N_2O_2]^+$ 280.1212, found 280.1205.

General procedure for *pegylation* of 3′—To a solution of (**3**′) (1 eq) in anhydrous *N*,*N*-dimethylformamide (2 mL) in a microwavable vial (from Biotage) was added potassium carbonate (3.0 eq) and pegylating agent (1.2 –1.5 eq). The vial was sealed and subjected to microwave irradiation (Biotage Initiator system) at 200 °C for 10 min with 10 sec pre-stirring and with fixed hold time “on”. After cooling the reaction mixture to room temperature the vial was opened, the contents poured into water and extracted with ethyl acetate (3×10 mL). The ethyl acetate layer was washed with water (2×10 mL) and brine (2×10 mL). The organic phase was then dried over anhydrous sodium sulfate and evaporated. The residue was purified by silica PTLC to afford the corresponding PEGylated derivatives (**8a**–**d**).

6-(2-Fluoroethoxy)-[2-(4-dimethylaminophenyl)-vinyl]-benzoxazol (8a): Yield: 68%.

^{1}H NMR (200 MHz, $CDCl_3$): δ 7.64 (1H, d, *J* = 16.2 Hz), 7.54 (1H, d, *J* = 8.7 Hz), 7.47 (2H, d, *J* = 8.8 Hz), 7.06 (1H, d, *J* = 2.3 Hz), 6.93 (1H, dd, *J* = 8.7, 2.3 Hz), 6.80 (1H, d, *J* = 16.2

Hz), 6.72 (2H, d, *J* = 8.8 Hz), 4.78 (2H, dt, *J* = 47.4, 4.0 Hz), 4.26 (2H, dt, *J* = 27.7, 4.0 Hz), 3.02 (6H, s). HRMS (EI) *m*/*z* calcd. for $[C_{19}H_{19}FN_2O_2]^+$ 326.1434, found 326.1431.

6-(2-(2-(2-Fluoroethoxy)-ethoxy)-ethoxy)-[2-(4-dimethylaminophenyl)-vinyl]-benzoxazol (8b): Yield: 71%.

^{1}H NMR (200 MHz, $CDCl_3$): δ 7.63 (1H, d, *J* = 16.2 Hz), 7.52 (1H, d, *J* = 8.8 Hz), 7.47 (2H, d, *J* = 9.0 Hz), 7.06 (1H, d, *J* = 2.1 Hz), 6.92 (1H, dd, *J* = 8.8, 2.1 Hz), 6.80 (1H, d, *J* = 16.2 Hz), 6.72 (2H, d, *J* = 9.0 Hz), 4.57 (2H, dt, *J* = 47.6, 4.1 Hz), 4.19 (2H, t, *J* = 4.5 Hz), 3.92-3.67 (10H, m), 3.02 (6H, s). HRMS (EI) *m*/*z* calcd. for $[C_{23}H_{27}FN_2O_4]^+$ 414.1955, found 414.1946.

6-(2-(2-(2-(2-(2-(2-Fluoroethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-[2-(4-dimethylaminophenyl)-vinyl]-benzoxazol (8c): Yield: 66%.

^{1}H NMR (200 MHz, $CDCl_3$): δ 7.63 (1H, d, *J* = 16.2 Hz), 7.51 (1H, d, *J* = 8.1 Hz), 7.46 (2H, d, *J* = 8.7 Hz), 7.05 (1H, d, *J* = 2.1 Hz), 6.91 (1H, dd, *J* = 8.1, 2.1 Hz), 6.80 (1H, d, *J* = 16.2 Hz), 6.71 (2H, d, *J* = 8.7 Hz), 4.54 (2H, dt, *J* = 47.5, 3.8 Hz), 4.17 (2H, t, *J* = 5.1 Hz), 3.90-3.65 (20H, m), 3.02 (6H, s). HRMS (EI) *m*/*z* calcd. for $[C_{29}H_{39}FN_2O_7]^+$ 546.2741, found 546.2740.

6-(2-(2-(2-(2-(2-(2-(2-Fluoroethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-[2-(4-dimethylaminophenyl)-vinyl]-benzoxazol (8d): Yield: 95%.

^{1}H NMR (200 MHz, $CDCl_3$): δ 7.62 (1H, d, *J* = 16.2 Hz), 7.50 (1H, d, *J* = 8.6 Hz), 7.48 (2H, d, *J* = 8.9 Hz), 7.08 (1H, d, *J* = 2.2 Hz), 6.92 (1H, dd, *J* = 8.6, 2.2 Hz), 6.80 (1H, d, *J* = 16.2 Hz), 6.73 (2H, d, *J* = 8.9 Hz), 4.53 (2H, dt, *J* = 47.7, 4.0 Hz), 4.17 (2H, t, *J* = 4.39 Hz), 3.87-3.59 (30H, m), 3.02 (6H, s). HRMS (EI) *m*/*z* calcd. for $[C_{33}H_{47}FN_2O_9]^+$ 634.3266, found 634.3242.

Preparation of 2-(2-(2-(2-(4-(dimethylamino)styryl)benzoxazol-6-yloxy)ethoxy) ethoxy)ethanol (9)—To a solution of (**3′**) (1 eq) in anhydrous *N*,*N*-dimethylformamide (2 mL) in a microwavable vial (from Biotage) was added potassium carbonate (3.0 eq) and 2-(2-(2-chloroethoxy)ethoxy)ethanol (1.5 eq). The vial was sealed and subjected to microwave irradiation (Biotage Initiator system) at 200 °C for 10 min with 10 sec pre-stirring and with fixed hold time “on”. After cooling the reaction mixture to room temperature the vial was opened, the contents poured into water and extracted with ethyl acetate (3×10 mL). The ethyl acetate layer was washed with water (2×10 mL) and brine (2×10 mL). The organic phase was then dried over anhydrous sodium sulfate, and evaporated. The residue was purified by silica PTLC (25% hexanes in ethyl acetate) to afford the corresponding hydroxy PEGylated derivative (**9**) in 80 % yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.61 (1H, d, *J* = 16.2 Hz), 7.52 (1H, d, *J* = 8.8 Hz), 7.48 (2H, d, *J* = 9.0 Hz), 7.05 (1H, d, *J* = 2.2 Hz), 6.92 (1H, dd, *J* = 8.8, 2.2 Hz), 6.80 (1H, d, *J* = 16.2 Hz), 6.72 (2H, d, *J* = 9.0 Hz), 4.17 (2H, t, *J* = 4.4 Hz), 3.88 (2H, t, *J* = 4.4 Hz), 3.76-3.59 (8H, m), 3.00 (6H, s).

Preparation of 2-(2-(2-(2-(4-(dimethylamino)styryl)benzoxazol-6-yloxy)ethoxy) ethoxy)ethyl methanesulfonate (11)—Compound **9** was dissolved in dichloromethane followed by the addition of triethylamine (4.0 eq). Methanesulfonyl chloride was then added via a syringe and the resultant solution stirred for 3 h at room temperature. The solution was then poured into water and extracted with dichloromethane, washed with brine and dried over sodium sulfate. The residue was purified via silica PTLC (25% hexanes in ethyl acetate) to afford the mesylated precursor (**11**) in 75% yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.63 (1H, d, *J* = 16.2 Hz), 7.52 (1H, d, *J* = 8.8 Hz), 7.48 (2H, d, *J* = 9.0 Hz), 7.05 (1H, d, *J* = 2.1 Hz), 6.92 (1H, dd, *J* = 8.8, 2.1 Hz), 6.80 (1H, d, *J* = 16.2 Hz), 6.72 (2H, d, *J* = 9.0 Hz), 4.37 (2H, t, *J* = 4.4 Hz), 4.19 (2H, t, *J* = 4.4 Hz), 3.87 (2H, t, *J* = 4.3 Hz), 3.79-3.61 (6H, m), 3.05 (3H, s), 3.02 (6H, s).

Synthesis of 6-iodo-2-(4′-dimethylamino)phenyl-imidazo[1,2-a]pyridine (IMPY) (2) derivatives

Preparation of **2** (IMPY core) has been described elsewhere (30). The general procedure for the synthesis of 6-FPEG substituted-IMPY conjugates was accomplished using the following procedure:

Method A—Conventional synthesis: The mixture of **2** (prepared as reported previously (30)), fluoro-polyglycols (2–5 eq.), CuI (10%mol), Cs_2CO_3 (2 eq.), 1,10-phenanthroline (20% mol) in toluene (1mL/0.1 mmol **2**) was stirred in a sealed tube for 48 h. The solvent was then removed and the residue purified by silica PTLC (ethyl acetate or 95% dichloromethane- 5% methanol as the developing solvent) to give the desired product.

Method B—Microwave synthesis: The mixture of reactants and reagents described above in a sealed tube was put in the microwave oven - condition: 170°C, 60 min, normal absorption level (Yields were similar to those used for the conventional synthesis).

6-(2-Fluoroethoxy)-2-(4-dimethylamino-)phenyl-imidazo[1,2-a]pyridine (12a): Yield: 17%. (Method A)

^{1}H NMR (200 MHz, $CDCl_3$): δ 7.78 (2H, d, *J* = 8.8 Hz), 7.68 (1H, d, *J* = 2.2 Hz), 7.67 (1H, s), 7.50 (1H, d, *J* = 9.7 Hz), 6.96 (1H, dd, *J* =9.7, 2.2 Hz), 6.74 (2H, d, *J* = 8.8 Hz), 4.75 (2H, dt, *J* = 47.7, 4.1 Hz), 4.16 (2H, dt, *J* = 25.9, 4.1 Hz), δ 2.99 (6H, s). HRMS (EI) *m*/*z* calcd. for $[C_{17}H_{19}FN_3O]^+$ $(M+H)^+$ 300.1512, found 300.1500.

6-(2-(2-Fluoroethoxy)-ethoxy)-2-(4-dimethylamino-)phenyl-imidazo[1,2-a]pyridine (12b): Yield: 59%. (Method A)

^{1}H NMR (200 MHz, $CDCl_3$): δ 7.78 (2H, d, *J* = 8.8 Hz). 7.66 (1H, d, *J* = 2.2 Hz), 7.64 (1H, s), 7.46 (1H, d, *J* = 9.7 Hz), 6.94 (1H, dd, *J* =9.7, 2.2 Hz), 6.76 (2H, d, *J* = 8.8 Hz), 4.59 (2H, dt, *J* = 47.6, 4.1 Hz), 4.08 (2H, t, *J* = 4.2 Hz), 3.88 (2H, t, *J* = 4.2 Hz), 3.80 (2H, dt, *J* = 25.9, 4.1 Hz), 2.98 (6H, s). HRMS (EI) *m*/*z* calcd. for $[C_{19}H_{23}FN_3O_2]^+$ $(M+H)^+$ 344.1774, found 344.1768.

6-(2-(2-(2-Fluoroethoxy)-ethoxy)-ethoxy)-2-(4-dimethylamino-)phenyl-imidazo[1,2-a] pyridine (12c): Yield: 60%. (Method A)

^{1}H NMR (200 MHz, $CDCl_3$): δ 7.77 (2H, d, *J* = 8.8 Hz), 7.65 (1H, d, *J* = 2.2 Hz), 7.63 (1H, s), 7.45 (1H, d, *J* = 9.7 Hz), 6.93 (1H, dd, *J* =9.7, 2.2 Hz), 6.75 (2H, d, *J* = 8.8 Hz), 4.54 (2H, dt, *J* = 47.7, 4.1 Hz), 4.06 (2H, t, *J* = 4.6 Hz), 3.82 (2H, t, *J* = 4.6 Hz), 3.70-3.59 (6H, m), 2.97 (6H, s). HRMS (EI) *m*/*z* calcd. for $[C_{21}H_{27}FN_3O_3]^+$ $(M+H)^+$ 388.2036, found 388.2032.

6-(2-(2-(2-(2-(2-(2-Fluoroethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-2-(4-dimethylamino-) phenyl-imidazo[1,2-a]pyridine (12d): Yield: 18%. (Method A)

^{1}H NMR (200 MHz, $CDCl_3$): δ 7.78 (2H, d, *J* = 8.8 Hz), 7.71 (1H, d, *J* = 1.9 Hz), 7.67 (1H, s), 7.48 (1H, d, *J* = 9.7 Hz), 6.93 (1H, dd, *J* = 9.7, 2.2 Hz), 6.76 (2H, d, *J* = 8.8 Hz), 4.53 (2H, dt, *J* = 47.7, 4.1 Hz), 4.09 (2H, t, *J* = 4.6 Hz), 3.85 (2H, t, *J* = 4.6 Hz), 3.89-3.64 (18H, m), 2.98 (6H, s). HRMS (EI) *m*/*z* calcd. for $[C_{27}H_{39}FN_3O_6]$ $(M+H)^+$ 520.2823, found 520.2808.

6-(2-(2-(2-(2-(2-(2-(2-Fluoroethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-ethoxy)-2-(4-dimethylamino-) phenyl-imidazo[1,2-a]pyridine (12e): Yield: 58%. (Method A)

^{1}H NMR (200 MHz, $CDCl_3$): δ 7.75 (2H, d, *J* = 8.8 Hz), 7.68 (1H, d, *J* = 1.9 Hz), 7.62 (1H, s), 7.52 (1H, d, *J* = 9.7 Hz), 6.95 (1H, dd, *J* = 9.7, 2.2 Hz), 6.71 (2H, d, *J* = 8.8 Hz), 4.50 (2H, dt, *J* = 47.7, 4.0 Hz), 4.07 (2H, t, *J* = 4.6 Hz), 3.64–3.85 (28H, m), 2.95 (6H, s). HRMS (EI) *m*/*z* calcd. for $[C_{31}H_{47}FN_3O_8]^+$ $(M+H)^+$ 608.3347, found 608.3329.

Synthesis of 2-(2-(2-(2-(4-(dimethylamino)phenyl)imidazo[1,2-*a*]pyridin-6-yloxy)ethoxy)ethoxy)ethanol (13)

Method 1: The mixture of **2** (prepared as reported previously (30)) (36 mg, 0.1 mmol), triethylene glycol (135 mg, 1mmol), CuI (19 mg, 0.1 mmol), Cs_2CO_3 (65 mg, 0.2 mmol) and 1,10-phenanthroline 927 mg, 0.15 mmol) in toluene (1 mL) was reacted in MW (170°C, absorption: normal) for 1h. The solvent was removed and the residue was purified by silica PTLC (CH_2Cl_2:MeOH = 95:5) to give 28 mg of product (73.6%).

Method 2: The above mixture was stirred at 120°C in a sealed tube for 48h. Same work up as described in Method 1 gave the desired product in 36% yield.

^{1}H NMR (200 MHz, $CDCl_3$): δ 7.75 (2H, d, *J* = 7.8 Hz), 7.67 (2H, s), 7.58 (2H, d, *J* = 9.2 Hz), 6.96 (2H, d, *J* = 8.9 Hz), 6.69 (2H, d, *J* = 7.8 Hz), 4.07 (2H, t, *J* = 4.5 Hz), 3.84 (2H, t, *J* = 4.5 Hz), 3.71–3.76 (6H, m), 3.61 (2H, t, *J* = 4.5 Hz), 2.95 (6H, s).

Synthesis of 2-(2-(2-(2-(4-(dimethylamino)phenyl)imidazo[1,2-*a*]pyridin-6-yloxy)ethoxy)ethoxy)ethyl 4-methylbenzenesulfonate (14)—To a solution of **13** (45 mg, 0.12 mmol) dissolved in pyridine (1 mL) was added tosyl chloride (45 mg, 0.24 mmol) in solid form at 0°C followed by DMAP (10 mg) and the resultant solution stirred at room temperature for 3 h. The reaction mixture was then poured into ice water and the mixture extracted with a mixed solvent (CH_2Cl_2: MeOH = 95:5). The combined organic solvent was dried over Na_2SO_4, filtered and the filtrate concentrated to yield the crude product which was purified by silica PTLC (CH_2Cl_2: MeOH = 95:5) to give 29 mg of product (46%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.77 (2H, d, *J* = 8.7 Hz), 7.70 (1H, d, *J* = 2.2 Hz), 7.67 (1H, s), 7.48 (2H, d, *J* = 9.6 Hz), 7.29 (2H, d, *J* = 8.2 Hz), 6.94 (2H, dd, *J* = 9.7, 2.1 Hz), 6.76 (2H, d, *J* = 8.8 Hz), 4.16 (2H, t, *J* = 4.7 Hz), 4.07 (2H, t, *J* = 4.6 Hz), 3.83 (2H, t, *J* = 4.6 Hz), 3.53–3.69 (6H, m), 2.98 (6H, s), 2.40 (3H, s).

Radiochemistry

General procedure for ^{18}F labeling of 10a—[^{18}F]Fluoride was produced by the JSW type BC3015 cyclotron at the Cyclotron Facility, University of Pennsylvania using ^{18}O (p,n)^{18}F reaction. An [^{18}O]-enriched aqueous solution of [^{18}F]Fluoride was passed through a Sep-Pak Light quaternary methyl ammonium (QMA) cartridge (Waters) and the cartridge dried by airflow. The ^{18}F activity was then eluted using 1.2 mL of a Kryptofix→222/potassium carbonate solution, which is made up of 22 mg of Kryptofix→ 222 and 4.6 mg of potassium carbonate in acetonitrile/water (1.77/0.23). The solvent was removed under a stream of nitrogen at 120 °C and the residue azeotropically dried twice with 1 mL of anhydrous acetonitrile also at 120 °C. The mesylate precursor (**10a**) (0.5, 1, 3, and 6 mg) was then dissolved in 0.2 mL of dimethyl sulfoxide and added to the reaction vessel containing the dry ^{18}F. The reaction was subsequently carried out under different temperatures for different time periods as shown in Table 2. 10% aqueous HCl (0.5 mL) was then added and the resultant mixture heated for an additional 10 min. Following completion of the reaction, water (2 mL) was added and the resultant solution loaded onto a Waters Oasis HLB cartridge (3cc) previously washed with 2 × 3 mL ethanol and 2 × 3 mL of water. The cartridge was washed with 4 mL of water and the crude product eluted with 2 mL of acetonitrile. The Waters C18 Sep Pak could be used alternatively. However the recovery yield of radioactivity by acetonitrile was lower. Acetonitrile solution was then injected onto a semi-preparative HPLC for purification. The

retention time of the major hydrolysis by-product (Rt = 3.6 min) was well resolved from the desired ^{18}F labeled product (Rt = 5.2 min), which was isolated in >99 % radiochemical purity and the specific activity was estimated to be 500–1000 Ci/mmol after purification (Specific activity was estimated by comparing UV peak intensity of purified [^{18}F]**5a** with reference non-radioactive compound of known concentration). Radiochemical yields are summarized in Table 2.

General procedure for ^{18}F labeling of 10b, 10c, 11, and 14—Compounds **10b**, **10c**, **11**, and **14** were labeled with ^{18}F using the similarly described procedure for **10a**. Subsequent Boc deprotection were carried out for **10b** and **10c**. For all these compounds, reaction was carried out at 120 °C for 4 min, which is the optimum condition with regard to time and temperature

The crude reaction was purified via HPLC using the same conditions (except **11**, which used the same columns with solvent composition acetonitrile/water 60/40 at the same flow rate). Radiochemical yields ranged between 11–35% and all were isolated in good radiochemical purity (>90%) with the exception of [^{18}F]**8b** wherein a second peak was evident within minutes of isolation. Specific activities for [^{18}F]**5b, c** and [^{18}F]**12c** ranged between 800–1500 Ci/mmol.

Preparation of brain tissue homogenates

Postmortem brain tissues were obtained from AD patients at autopsy, and neuropathological diagnosis was confirmed by current criteria (NIA-Reagan Institute Consensus Group, 1997). Homogenates were then prepared from dissected gray matters from AD patients in phosphate buffered saline (PBS, pH 7.4) at the concentration of approximately 100 mg wet tissue/ml (motor-driven glass homogenizer with setting of 6 for 30 sec). The homogenates were aliquoted into 1 ml-portions and stored at −70°C for 6–12 month without loss of binding signal.

Binding Studies

As reported previously, [^{125}I]IMPY (12), with 2,200 Ci/mmol specific activity and greater than 95% radiochemical purity, was prepared using the standard iododestannylation reaction and purified by a simplified C-4 mini column (12). Binding assays were carried out in 12 × 75 mm borosilicate glass tubes. The reaction mixture contained 50 μl of brain homogenates (20–50 μg), 50 μl of [^{125}I]IMPY (0.04–0.06 nM diluted in PBS) and 50 μl of inhibitors (10^{-5}–10^{-10} M diluted serially in PBS containing 0.1 % bovine serum albumin, BSA) in a final volume of 1 ml. Nonspecific binding was defined in the presence of IMPY (600 nM) in the same assay tubes. The mixture was incubated at 37°C for 2 h and 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 2 × 3 ml washes of PBS at room temperature. Filters containing the bound ^{125}I ligand were assayed for radioactivity content in a gamma counter (Packard 5000) with 70% counting efficiency. Under the assay conditions, the specifically bound fraction was less than 15% of the total radioactivity. The results of inhibition experiments were subjected to nonlinear regression analysis using EBDA by which K_i values were calculated.

Film Autoradiography

Brain sections from AD subjects were mounted onto glass slides and incubated with F-18 tracers (300,000 – 600,000 cpm/200 μL) for 1 h at room temperature. The sections were then washed in saturated Li_2CO_3 in 40% EtOH (two two-min washes) and in 40% EtOH (two min) followed by rinsing with water for 30 sec. After drying, the F-18 labeled sections were exposed to Kodak MR film overnight.

Partition Coefficient

Partition coefficients were measured by mixing the [^{18}F]labeled sample with 3 g each of 1-octanol and buffer (0.1 M phosphate, pH 7.4) in a test tube. The two layers in the test tube were vortexed for 3 min followed by centrifugation for 5 min at room temperature. The radioactivities of two weighed samples (0.5 g each) from the 1-octanol and buffer layers were counted in a well counter. The partition coefficient was determined by calculating the ratio of cpm/g of 1-octanol to that of buffer. Sample from the 1-octanol layer was re-partitioned with buffer solution and each layer was counted again. This procedure was repeated until consistent value of partition coefficient values was obtained (usually the 3rd or 4th partition). The measurements were repeated three times.

Biodistribution Studies in Normal Mice

While under isoflurane anesthesia, 0.15 mL of a saline solution containing the [^{18}F] tracers (10–20 uCi) was injected directly into the lateral tail vein of male ICR mice. The mice (n = 3 for each time point) were sacrificed by cervical dislocation. At 2, 30, 60 and 120 min post-injection, organs of interest were removed, weighed and assayed for radioactivity content with an automatic gamma counter. The percentage dose per organ was calculated by a comparison of the tissue counts to counts of 1% of initial dose (100 times diluted aliquots of the injected material). Total activities of blood and bone were calculated under the assumption that they were 7% and 14% of the body weight, respectively. The % dose/g of samples was calculated by comparing the sample counts with the count of the diluted initial dose.

Results and Discussion

One of the major advantages of our FPEG approach is incorporation of the fluoro tag at the end of a polyethylene glycol chain. The preparation of these compounds is readily achieved in a relatively simple and straightforward manor. In this report, we describe the FPEG derivatives of 3 cores: PIB (**4**), [2-(4-dimethylaminophenyl)-vinyl]-benzoxazol (**3′**), and IMPY (**2**), previously reported as imaging agents for Aβ aggregates(10,12–16). Syntheses of core compounds **2** and **4** and polyethylene glycol precursors were accomplished following literature procedures with minor modifications(21,26). Compound **3′**, was prepared from 6-hydroxy-2-methylbenzoxazole followed by *in situ* trimethylsilyl protection of the phenolic OH, deprotonation and condensation with N, N′-dimethylaminobenzaldehyde as described during the synthesis of similar compounds by Schreiner and co-workers (Scheme 1)(29). Conjugation of the free phenolic hydroxy groups to compounds **3′** and **4** with various oligoethylene glycol precursors was accomplished under microwave irradiation in good yields (68–95%)(Scheme 1 A and B). Utilizing the same methodology the radiofluorination precursors can be generated quickly and efficiently, conveniently allowing the radioactive fluoride to be added in the last step of the synthesis. Preparation of the mesylate precursor was generated following synthesis of the hydroxy derivative using a similar microwave procedure (Scheme 2 C). It was important to also prepare and identify the hydroxy derivatives of FPEG as they compete for binding to beta amyloid plaques and they are the major byproducts during radiolabeling.

The synthetic versatility of this strategy was further demonstrated with conjugates of compound **2** wherein FPEG was conjugated to **2** via a copper catalyzed coupling reaction with the aryl iodide and corresponding fluoro/hydroxy PEG derivative (Scheme 3). The desired FPEG derivatives were prepared in moderate to good yields (17–60%).

In vitro binding studies to evaluate the binding affinities of the FPEG conjugates (**5a**–**d**, **8a**–**d** and **12a**–**e**) to Aβ aggregates were carried out in AD brain homogenate with [^{125}I]IMPY (**2**) as the ligand (31) (Table 1). Previous experience in preparing FPEG-stilbene (**1**) derivatives suggested that FPEG n = 3 was an ideal compromise between the addition of PEG groups to

reduce lipophilicity and the need for a small neutral molecule to maintain good brain penetration (21). However, when similar n = 3 derivatives (**5b**, **8b** and **12c**) were prepared and evaluated *in vitro* it was evident this was not always the case. Only **5b** and **8b** retained high binding affinities for the n = 3 FPEG conjugates with K_i values of 3.8 and 14.5 nM, respectively. Compound **12c** was not nearly as potent with a K_i value of 30 nM. In fact further derivatization of **12** only increased the K_i (96 and 387 nM for **12d** and **12e**, respectively)(i.e. reducing the binding affinity), while the other core compounds remained highly potent in their binding affinities. Based on these initial *in vitro* results, FPEG conjugation clearly failed for conjugates **12a-e** (IMPY (**2**) derivatives) but was quite successful with the other two core compounds (PIB conjugates **5a-d** and BF conjugates **8a**–**d**).

^{18}F labeling was performed on either the mesyl or tosyl precursors undergoing a nucleophilic displacement reaction with the fluoride anion. Radiolabeling with ^{18}F was successfully performed on all core structures from precursors **10a**–**c** (Scheme 3), **11** and **14** to generate [^{18}F]**5a**–**c**, [^{18}F]**8b** and [^{18}F]**12c**, respectively. We labeled FPEG conjugates n = 3 initially based on our past experience and success using this approach (21,26). Both core conjugates [^{18}F]**12c** (IMPY, **2**) and [^{18}F]**5b** (PIB, **4**) were labeled in moderate yields (30–35%) and with good radiochemical purities (> 90%) from the tosyl and mesyl precursors, respectively. Retention times of the ^{18}F labeled products (Rt ([^{18}F]**12c**) = 6.5 min and Rt([^{18}F]**5b**) = 8.0 min) were well separated from the major hydrolysis products (Rt = 4.0 and 4.5 min). The preparation was about 80–90 min and the specific activity for all prepared ^{18}F compounds was estimated to be 500–1500 Ci/mmol at the end of synthesis. Radiolabeled [^{18}F]**8b,** which was prepared from the mesylate precursor in moderate radiochemical yield (23 %), unfortunately could not be prepared in good radiochemical purity due to the instability of this compound. The formation of a second peak was evident within minutes of labeling upon standing at room temperature in ethanol or the acetonitrile/water HPLC solvent mixture. These results are consistent with those found by Shimadzu *et. al.* during their labeling of a similar substrate (32). They attributed the formation of a second peak to the facile formation of *E* and *Z* isomers (32). Based on the promising *in vitro* binding affinities of **5a**–**d** and initial labeling results of [^{18}F]**5b** we then prepared both [^{18}F]**5a** and [^{18}F]**5c** using the standard approach in good yields (35 and 11%, respectively) and purity (> 98%).

For many years use of mesylate precursors for radiofluorination chemistry has been demonstrated (19), however the utility of these precursors for radiolabeling FPEG conjugates is relatively new. In order to study the radiofluorination reaction with FPEG, we prepared a large batch of precursor **10a** and evaluated the effects of precursor mass, temperature, reaction time and sep-pak purification strategies.

Initially, using 1 mg of precursor **10a** dissolved in 250 μL of DMSO, the reaction temperature was varied from 75°C to 120°C using standard oil bath heating for 4 minutes. Deprotection of the Boc protecting group was then achieved by adding 10% HCl and heating for 10 minutes. Water was then added (2 mL) and the solution loaded onto an Oasis HLB sep-pak cartridge. Following washing with water, the crude labeled product was eluted with 2 mL of acetonitrile and injected onto the HPLC. Labeling yields were highest at 120°C (Table 2). Next, the amount of precursor (**10a**) was varied from 0.5 mg to 6 mg with oil bath heating at 120°C for 4 minutes. Boc deprotection was then accomplished as described above leading to radiochemical yields ranging from 30–50%, with the highest between 1 and 3 mg. The final study performed with traditional oil bath heating evaluated the effect of increasing the reaction times from 4 minutes to 16 minutes using 1 mg of precursor and heating at 120°C. We found that reaction times from 8–16 minute all led to high radiochemical yields of greater than 59%. The radiochemical purity for all reactions was greater than 98%.

Microwave heating methods were also studied in the same manor, evaluating the effect of precursor mass, increased temperatures and reaction times. In general, the precursor mass did not have an affect when ranging between 0.25–3 mg. Increasing the reaction times from 1–4 minutes showed significant improvement on the radiochemical yield (up to 60% with 4 minutes at 80 W, 100°C using 1 mg of precursor dissolved in 250 μL of DMSO), while increasing the temperature at a constant power level seemed to decrease the yields (Table 2).

From these studies it is evident that both traditional oil bath and microwave heating strategies can prepare radiolabeled **[^{18}F]5a** (FPEG PIB, n=2) conjugates in good radiochemical yields (60–64%). The optimized oil bath conditions are 1–3 mg of precursor heated at 120°C for 12 minutes, followed by the standard deprotection. Traditional oil bath heating is still preferred in our hands as it has led to more reproducible results.

The lipophilicity (logP) of the labeled tracers ([^{18}F]**5a–c**, **8b** and **12c)** was evaluated as LogP values between 0–3 can often indicate good blood-brain barrier penetration. The logP value of [^{18}F]**12c** was 2.69 and that of the parent compound, IMPY (**2**) was 2.19(33), suggesting that addition of the FPEG unit does not significantly change the lipophilicity of the compound. Furthermore, all ^{18}F-labeled tracers were within an appropriate range (LogP values between 2.69–3.04, Table 1) and similar to those of the stilbene-FPEG conjugates (LogP = 2.05–2.53) (21).

The binding of these radiofluorinated probes to Aβ plaques in AD brain sections was evaluated by *in vitro* autoradiography and labeling of Aβ plaques in confirmed AD brain sections was observed for [^{18}F]**5a–c**. [^{18}F]**5a** especially displayed a superior Aβ plaque labeling showing a strong signal and a low background (Figure 2). The results strongly confirm the binding of these ^{18}F tracers to the Aβ aggregates in the brain of AD patients. Similarly, [^{18}F]**8b** was able to label the plaques using the mixture of isomers (data not shown). However, unlike the other conjugates, [^{18}F]**12c** did not display a significant labeling under similar labeling conditions (data not shown).

Biodistribution studies in normal mice with [^{18}F]**5a–c** (displayed in Table 3) and [^{18}F]**12c** were performed. [^{18}F]**8b** was not evaluated for the distribution study based on the higher molecular weight, lower brain penetration of the parent core (**3**, BF-168) (15,16) and the facile racemization seen during labeling. We use normal mice as a convenient animal model to evaluate the brain penetration of these ^{18}F tracers. Normal mice are not expected to have Aβ plaques in the brain, and therefore an ideal compound will have a high initial brain uptake that washes out quickly because there are no plaques to cause retention of Aβ aggregate specific probes. [^{18}F]**5a–c** were all able to penetrate the blood-brain barrier with [^{18}F]**5a** showing the highest initial brain uptake (10.27 %dose/g at 2 min post *iv* injection) and a fast washout (3.94 %dose/g at 60 min post *iv* injection). [^{18}F]**5b** also displayed a good initial brain uptake and washout (5.53 and 2.18 %dose/g at 2 and 60 min post *iv* injection, respectively). The brain uptakes of these compounds ([^{18}F]**5a**,**b)** are similar to those of the stilbene series (6.6–8.1% dose/g at 2 min post *iv* injection and 1.2–2.62% dose/g at 60 min post *iv* injection, (21)). However, the washout rate of this series of compounds is slower compared to the stilbene derivatives. Rapid initial brain uptake in normal mouse brain coupled with a fast washout are highly desirable properties for Aβ plaque-targeting imaging agent as these would lead to high signal to background ratios. Unlike [^{18}F]**5a** and **b**, [^{18}F]**5c** showed a much lower initial brain uptake (1.68 %dose/g), indicating low penetration of the compound through the intact blood-brain barrier. The poor penetration of [^{18}F]**5c** (FPEG n=6) led us to not pursue the studies using the further extended chain length of n = 8 (**5d**). It was also observed that *in vivo* defluorination was likely occurring with [^{18}F]**5a** because the bone uptake increased with time (6.35 %dose/g at 2 h), but was minimal with [^{18}F]**5b** and [^{18}F]**5c**. In addition, the biodistribution of [^{18}F]

12e was performed and showed low brain uptake and slow washout (results not shown) further confirming that FPEG conjugation with the IMPY core is not successful.

Our approach towards preparing ^{18}F labeled FPEG tracers is based on four critically important attributes: 1. The end-caped fluoro-pegylation with a short PEG chain (n=2–5) connecting to the core structure will lead to a small and neutral molecule that can penetrate intact blood-brain barrier. 2. The FPEG derivatives retain high binding affinities with K_i values comparable to those of the parent compounds in most cases but is not universally applicable. 3. The fluorine atom at one end of the pegylation chain can be readily labeled through the use of a simple nucleophilic substitution reaction of either O-mesylate or O-tosylate group by ^{18}F fluoride in moderate to good radiochemical yields. It is also revealed that precise optimization of reaction conditions (amount of precursor, reaction time and temperature) is needed to maximize the radiochemical yield. The resulting ^{18}F labeled FPEG compounds are relatively stable *in vivo* after an i.v injection. These unique features may provide a suitable approach for the preparation of other novel ^{18}F labeled tracers.

In summary, we present here examples of a novel approach towards preparing PET imaging agents via a fluorinated pegylation approach. These examples illustrate a simple, efficient, and highly flexible methodology. This approach has proven effective for two core structures, but unfortunately it was not universally applicable. However, the full power of this approach towards the development of other novel ^{18}F labeled imaging agents is yet to be explored and it will be interesting and exciting to see its application to other new imaging agents.

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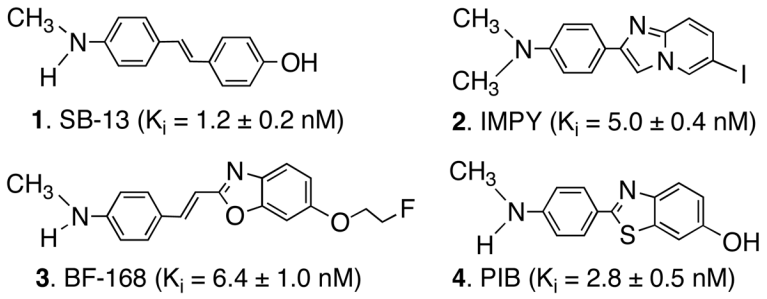


Figure 1.

Structures of four imaging agents (**1**–**4**) targeting Aβ aggregates in the brain. The K_i values indicate the *in vitro* binding affinity to Aβ aggregates(15,27,31).

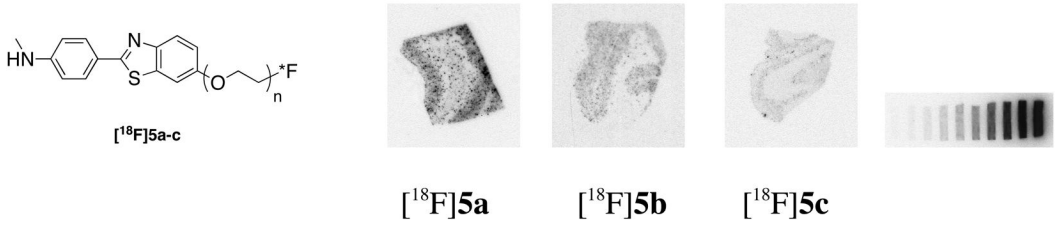


Figure 2.
In vitro autoradiography of brain cortical sections from confirmed AD patients labeled with [^{18}F]**5a–c.** The Aβ plaques, known to be a hallmark of AD, were distinctively labeled with all ^{18}F tracers.

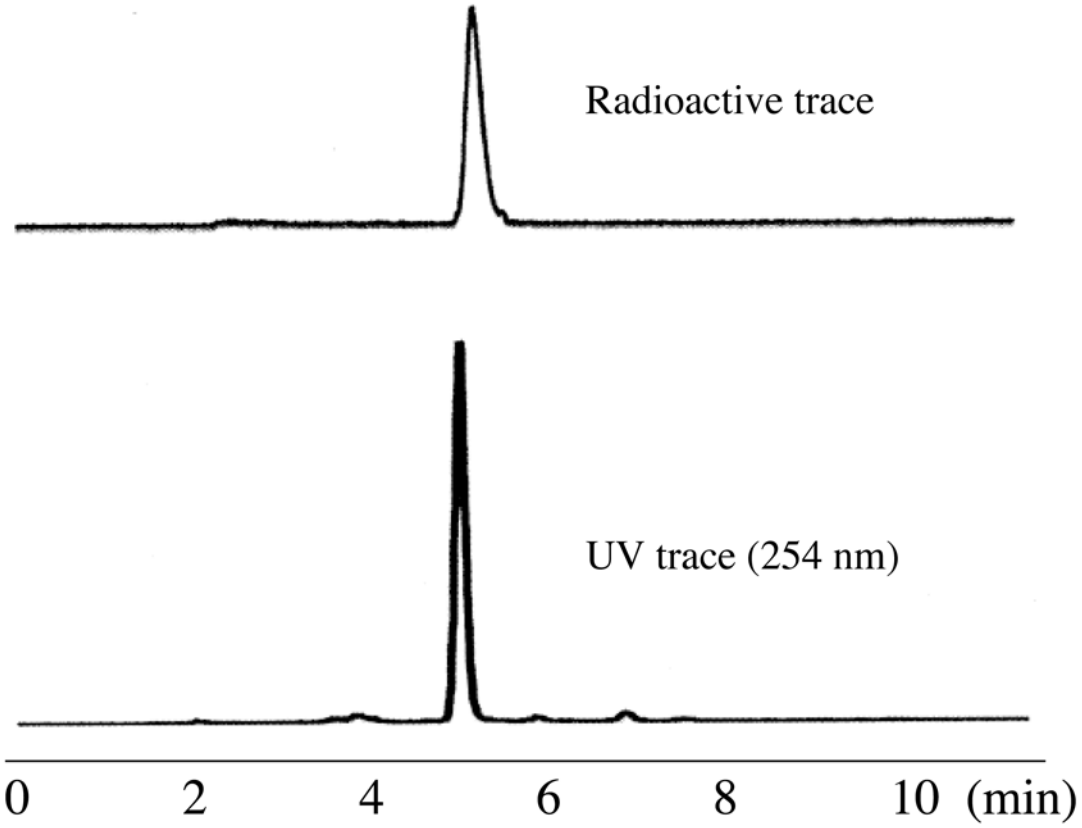
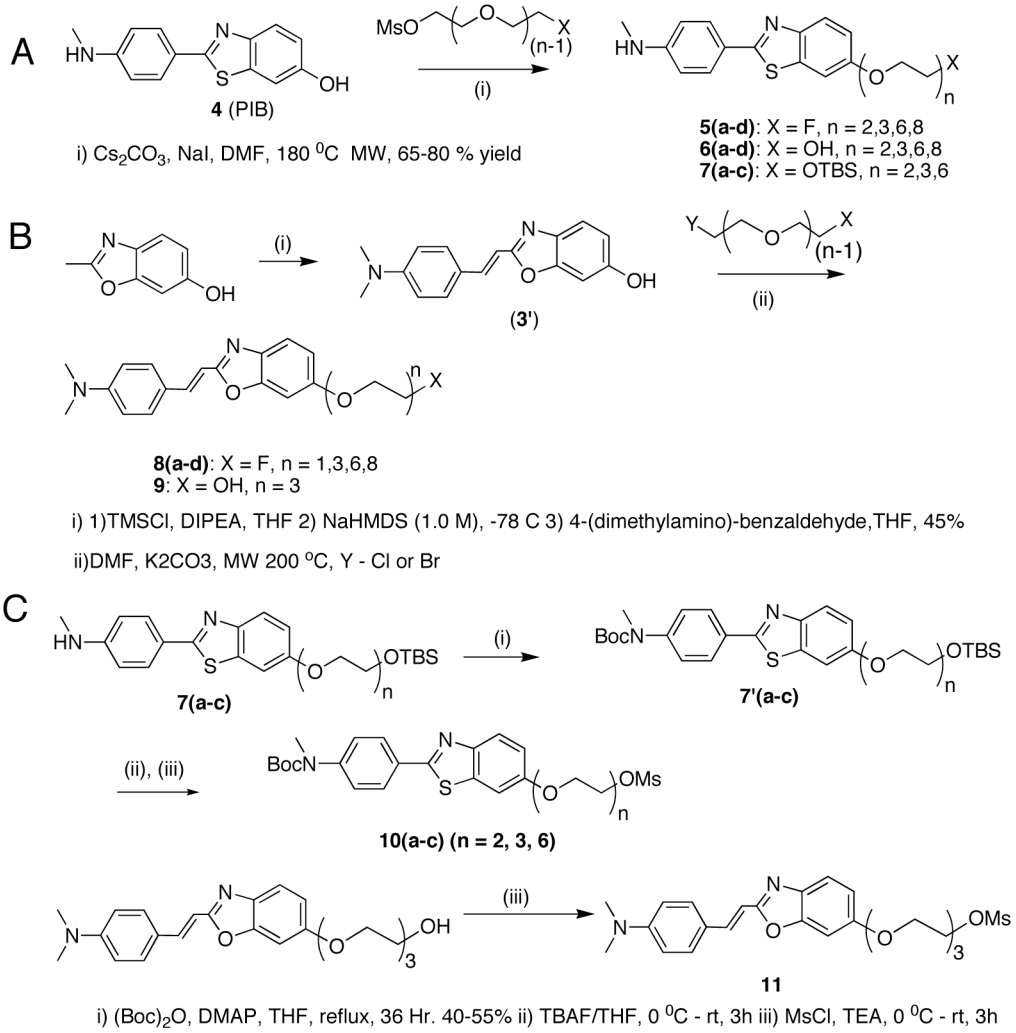
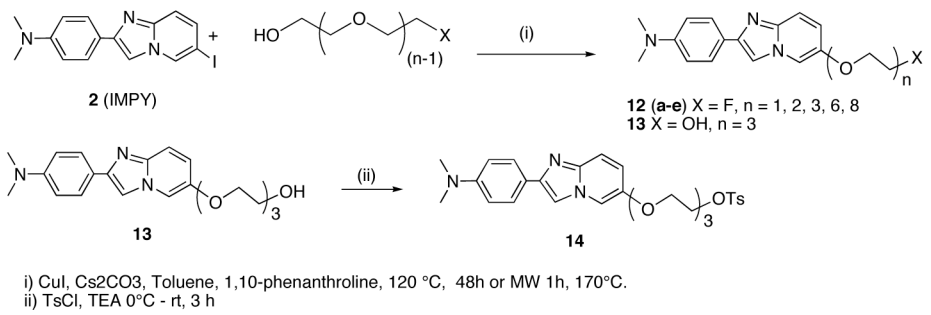


Figure 3.
HPLC profiles of [^{18}F]**5a** (top) and **5a** (bottom)
HPLC condition: Agelent 1100 series; Phenomenex Gemini C-18 analytical column CH_3CN/Water 8/2 1 mL/min. t_R = 4.80 min (UV), 5.03 min (_). The slight difference in retention time between UV and _ is due to the sequential configuration of detector system.

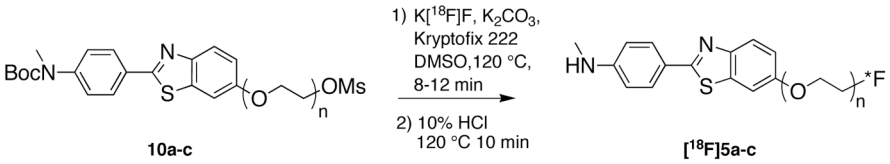


Scheme 1.
Syntheses of FPEG conjugates and radiolabeling precursors



Scheme 2.

Synthesis of FPEG-IMPY conjugates **12a–e**, **13** and the corresponding precursor **14** for radiolabeling



Scheme 3.
^{18}F radiolabeling of **10a–c**

Table 1

Inhibition constants (K_i, nM) of compounds on [^{125}I]IMPY binding to AD brain homogenates*

Compounds	K_i (nM)	logP+	Compounds	K_i (nM)	logP+
(1) SB-13	**1.2 ± 0.2**+	2.36	**(2) IMPY**	**5.0 ± 0.4**∞	2.19
1a (SB n=2)+	2.9 ± 0.2	2.53	**12a** (IMPY n=1)	16 ± 2.0	-
1b (SB n=3)+	6.7 ± 0.3	2.41	**12b** (IMPY n=2)	31 ± 9.0	-
1c (SB n=4)+	4.4 ± 0.8	2.05	**12c** (IMPY n=3)	30 ± 2.5	2.69
1d (SB n=5)+	6.8 ± 0.8	2.27	**12d** (IMPY n=6)	96 ± 14	-
			12e (IMPY n=8)	387 ± 12	-
(4) PIB	**2.8 ± 0.5**Δ	1.3	**(3) BF-168**	**6.4 ± 1.0**§	-
5a (PIB n=2)	2.2 ± 0.5	3.04	**8a** (BF n=1)	12 ± 0.5	-
5b (PIB n=3)	3.8 ± 0.5	3.04	**8b** (BF n=3)	14.5 ± 5.0	2.93
5c (PIB n=6)	4.7 ± 0.9	2.99	**8c** (BF n=6)	10.0 ± 0.2	-
5d (PIB n=8)	9.0 ± 1.8	-	**8d** (BF n=8)	6.0 ± 0.6	-

*Values (Ki, nM) are the mean ± SEM of three independent experiments, each in duplicate.

+(21);

Δ(6);

§(15);

∞(33).

+logP = log of partition coefficient between 1-Octanol and buffer.

Table 2

Optimization studies for compound [^{18}F]**5a**, examining the effect of precursor mass, temperature and time for traditional heating verses microwave heating strategies.

Entry	Heating Method	Precursor Mass (mg)	Time (min)	Temp. (°C)	Radiochemical Yield (%)
1	Oil	0.5	4	120	32
2	Oil	1	4	120	50
3	Oil	3	4	120	50
4	Oil	6	4	120	30
5	Oil	1	8	120	59
6	Oil	1	12	120	64
7	Oil	1	16	120	62
8	Oil	1	4	75	17
9	Oil	1	4	90	26
10	Oil	1	4	105	45
11	MW (80W)	0.25	1	100	24
12	MW (80W)	0.5	1	100	30
13	MW (80W)	1	1	100	41
14	MW (80W)	3	1	100	24
15	MW (80W)	1	2	100	42
16	MW (80W)	1	3	100	40
17	MW (80W)	1	4	100	60
18	MW (80W)	1	1	90	54
19	MW (80W)	1	1	110	52
20	MW (80W)	1	1	130	48
21	MW (80W)	1	1	150	43

Table 3

Biodistribution in normal mice of [^{18}F] tracers (%dose/g, avg of 3 mice ± SD)

A. [^{18}F]5a				
Organ	**2 min**	**30 min**	**1 h**	**2 h**
Blood	3.37 ± 0.46	3.60 ± 0.13	4.55 ± 0.40	4.38 ± 0.14
Heart	8.32 ± 0.37	3.49 ± 0.27	3.81 ± 0.25	3.59 ± 0.03
Muscle	0.82 ± 0.12	2.83 ± 0.22	2.76 ± 0.17	2.36 ± 0.11
Lung	7.79 ± 0.34	3.91 ± 0.28	3.84 ± 0.55	3.63 ± 0.15
Kidney	13.02 ± 1.04	4.54 ± 0.62	3.98 ± 0.24	3.18 ± 0.16
Spleen	6.92 ± 0.79	3.93 ± 0.29	3.87 ± 0.61	3.32 ± 0.16
Liver	19.02 ± 1.06	7.98 ± 0.60	6.35 ± 0.46	5.05 ± 0.43
Skin	1.08 ± 0.22	3.14 ± 0.22	3.15 ± 0.28	2.62 ± 0.16
Brain	10.27 ± 1.30	4.59 ± 0.47	3.94 ± 0.04	3.86 ± 0.35
Bone	1.69 ± 0.21	2.28 ± 0.20	3.17 ± 0.39	6.35 ± 1.32
B. [^{18}F]**5b**				
Organ	2 min	30 min	1 h	2 h
Blood	6.29 ± 1.19	3.41 ± 0.07	3.91 ± 0.23	4.04 ± 0.45
Heart	6.26 ± 1.12	3.22 ± 0.33	3.06 ± 0.15	2.50 ± 0.09
Muscle	1.40 ± 0.11	1.92 ± 0.34	1.58 ± 0.13	1.38 ± 0.11
Lung	7.35 ± 1.50	3.94 ± 0.29	3.63 ± 0.25	3.24 ± 0.10
Kidney	9.02 ± 0.77	5.27 ± 0.77	3.97 ± 0.25	2.97 ± 0.07
Spleen	5.24 ± 0.67	2.66 ± 0.14	2.84 ± 0.13	2.46 ± 0.05
Liver	21.84 ± 1.56	13.75 ± 1.88	11.22 ± 0.82	9.13 ± 1.11
Skin	2.09 ± 0.22	2.27 ± 0.17	1.92 ± 0.13	1.57 ± 0.06
Brain	5.53 ± 0.56	2.33 ± 0.15	2.18 ± 0.09	1.96 ± 0.13
Bone	2.13 ± 0.16	1.48 ± 0.03	1.82 ± 0.04	2.41 ± 0.28
C. [^{18}F]**5c**				
Organ	2 min	30 min	1 h	2 h
Blood	3.54 ± 0.12	2.52 ± 0.29	2.46 ± 0.29	1.54 ± 0.19
Heart	9.37 ± 0.18	2.11 ± 0.27	1.82 ± 0.28	1.19 ± 0.15
Muscle	1.60 ± 0.98	1.87 ± 0.38	1.52 ± 0.24	0.93 ± 0.07
Lung	4.68 ± 0.27	2.41 ± 0.37	2.08 ± 0.32	1.24 ± 0.16
Kidney	23.00 ± 0.89	5.01 ± 0.84	3.50 ± 0.86	1.27 ± 0.08
Spleen	4.74 ± 0.23	2.05 ± 0.18	1.87 ± 0.26	1.13 ± 0.16
Liver	12.43 ± 1.11	3.94 ± 0.29	2.86 ± 0.38	1.67 ± 0.32
Skin	0.95 ± 0.12	2.14 ± 1.03	1.42 ± 0.14	0.91 ± 0.10
Brain	2.57 ± 0.12	1.69 ± 0.23	1.80 ± 0.25	1.29 ± 0.17
Bone	1.68 ± 0.60	1.20 ± 0.29	1.68 ± 0.17	2.31 ± 0.37