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Synthesis of ^{18}F-labelled 2-(4′-fluorophenyl)-1,3-benzothiazole and evaluation as amyloid imaging agent in comparison with [^{11}C]PIB

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ABSTRACT

2-(4′-[^{18}F]fluorophenyl)-1,3-benzothiazole was synthesized as a fluorine-18 labelled derivative of the Pittsburg Compound-B (PIB), which has known affinity for amyloid β and promising characteristics as tracer for in vivo visualisation of amyloid deposits in patients suffering from Alzheimer's disease (AD). Both the nitro-precursor 2-(4′-nitrophenyl)-1,3-benzothiazole and the non-radioactive reference compound were synthesized using a 1-step synthesis pathway. Labelling was achieved by direct aromatic nucleophilic substitution of the nitro-precursor using [^{18}F]fluoride by heating for 20 min at 150 °C and with a radiochemical yield of 38%. The reference compound showed high affinity for amyloid in an in vitro competition binding study using human AD brain homogenates (K_i = 9.0 nM) and fluorescence imaging of incubated transgenic APP mouse brain slices confirmed binding to amyloid plaques. A biodistribution study in normal mice showed a high brain uptake at 2 min pi (3.20% ID/g) followed by a fast washout (60 min pi: 0.21% ID/g). A dynamic μPET study was performed in a transgenic APP and normal WT mouse, but, similar to [^{11}C]PIB, no difference was seen in tracer retention between both kind of mice. The new ^{18}F-labelled 2-phenylbenzothiazole showed excellent preclinical characteristics comparable with those of the ^{11}C-labelled PIB.

Alzheimer's disease (AD) is a slowly progressive and fatal neurodegenerative brain disorder associated with progressive episodic memory loss and decrease of cognitive functions and characterized by the presence of amyloid plaques and neurofibrillary tangles.[1] As the number of affected people, as well as the economical and social impact, will increase in the future due to the increasing average life span, the search for a radioactive tracer which allows in vivo diagnosis of AD in an early stage has become an important research object during the last years.[2] Several radioactive derivatives of thioflavin-T (ThT, Fig. 1A), a conjugated fluorescent compound used to stain amyloid plaques in vitro, have been reported as potential diagnostic tracers for this purpose.[3] The most promising of all reported compounds seems to be the carbon-11 labelled 6-OH-BTA or 6-hydroxy-2-(4′-N-[^{11}C]methylaminophenyl)-1,3-benzothiazole, also known as Pittsburgh Compound-B (PIB, Fig. 1B). PIB has already been tested intensively in several clinical studies showing clear differences between AD, mild cognitive impairment (MCI) and control subjects.[4] However, this agent is labelled with short-lived carbon-11 ($t_{1/2}$ = 20.39 min), which limits its availability to centres equipped with a cyclotron. This limitation may be overcome by introducing a fluorine-18 label which has a longer half-life ($t_{1/2}$ = 109.8 min) and thus may provide a positron emission tomography (PET) tracer that is useful for a widespread clinical application.

The aim of this study was the synthesis and biological evaluation of a ^{18}F-labelled derivative of ThT, useful for diagnosis of AD using PET. For this purpose we developed 2-(4′-[^{18}F]fluorophenyl)-1,3-benzothiazole (Fig. 1C). Its structure is based on that of PIB, in which the amine group on the 2-phenyl ring is replaced by a ^{18}F-atom and no OH-group is present on the benzothiazole part. No other substituents were introduced on the benzothiazole part or the 2-phenyl ring. Mathis et al. recently reported another ^{18}F-labelled PIB analogue and are evaluating this compound as potential amyloid imaging agent.[5]

The precursor for radiolabelling, 2-(4′-nitrophenyl)-1,3-benzothiazole (**1**), was synthesized using polyphosphoric acid (PPA) following the procedure as described by Shi et al. with small modifications with respect to temperature (150 °C instead of 220 °C as higher yields were obtained at lower temperature).[6] **1** was synthesized with a yield of 34%, starting from equal molar quantities of 2-aminothiophenol and 4-nitrobenzoic acid (Scheme 1).

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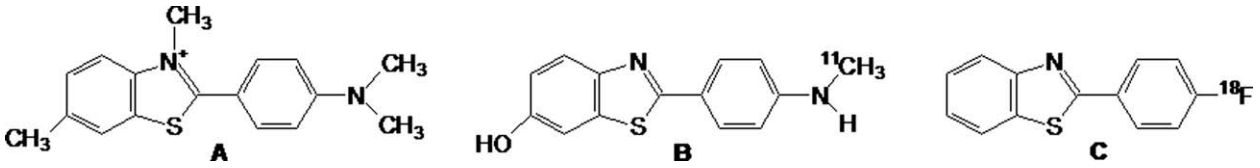
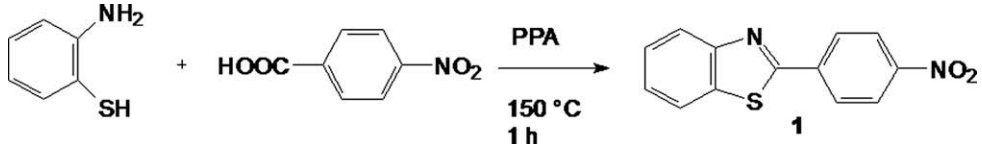


Figure 1. Structure of thioflavin-T (ThT, **A**), 6-hydroxy-2-(4′-*N*-[^{11}C]methylaminophenyl)-1,3-benzothiazole or PIB (**B**) and 2-(4′-[^{18}F]fluorophenyl)-1,3-benzothiazole (**C**).



Scheme 1. Synthesis of 2-(4′-nitrophenyl)-1,3-benzothiazole (**1**).

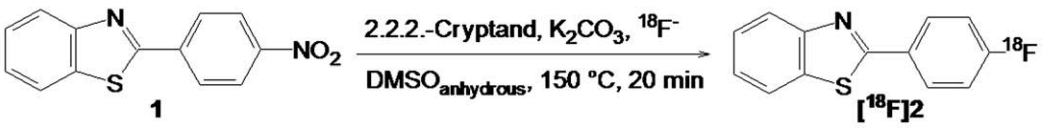
The non-radioactive 2-(4′-fluorophenyl)-1,3-benzothiazole (**2**) was prepared in a yield of 55% starting from 2-aminothiophenol and 4-fluorobenzoyl chloride (Scheme 2). A more convenient method (Dean-Stark trap instead of PPA) was used to synthesize **2**.[7] The identity of the non-radioactive compounds was confirmed by mass spectrometry (MS) and ^{1}H NMR.[8–10] As described in literature, both compounds have already been synthesized in different ways.[11,12]

A direct radiofluorination reaction was applied for aromatic nucleophilic substitution of a fluorine-18 atom for the nitro group of **1** (Scheme 3).[13] A solution of **1** in anhydrous DMSO was heated at 150 °C for 20 min in the presence of K_2CO_3, $^{18}F^-$ and 2.2.2-cryptand to achieve 2-(4′-[^{18}F]fluorophenyl)-1,3-benzothiazole (**[^{18}F]2**) with an average yield of 38% (not decay corrected, Fig. 2) and a specific activity of 48 ± 19 GBq/μmol. The separation of the 4'-[^{18}F]fluoro and 4′-nitro phenylbenzothiazole proved to be difficult but the use of reversed-phase high-performance liquid chromatography (RP-HPLC) with a mobile phase consisting of a mixture of ethanol, tetrahydrofuran and 0.05 M ammonium acetate buffer made it possible to separate the fluorine-18 labelled compound **[^{18}F]2** from the nitro-precursor **1**. The identity of the ^{18}F-labelled 2-phenylbenzothiazole **[^{18}F]2** was confirmed by co-injection of the non-radioactive reference compound **2** on RP-HPLC and comparison of the retention times. This radiolabelling is an example of a direct aromatic nucleophilic substitution with fluorine-18 of a 2-(4′-nitrophenyl)-1,3-benzothiazole in which the benzothiazole part acts as electron-withdrawing group. It is well known that electron-withdrawing substituents, such as a nitro or carbonyl group, present on the *ortho* or *para* position of a nitrophenyl ring, activate the nitro group and facilitate the exchange of the nitro group for a fluorine-18 atom.[14] In the case of **1**, the presence of the benzothiazole in *para* position of the nitro group apparently also activates the 4′-nitro group as relatively high yields of the corresponding 2-(4′-[^{18}F]fluorophenyl)-1,3-benzothiazole were obtained. This may allow the preparation of other similar compounds that can be radiolabelled using the same procedure, if the corresponding precursors can withstand the conditions of the nucleophilic substitution reaction.[15,16]

The in vitro affinity of the new agent **[^{18}F]2** for amyloid β plaques was determined by measuring the binding affinity of the reference compound **2** for post-mortem human brain homogenates of AD patients.[17] The K_i value, obtained from binding inhibition studies using [^{125}I]IMPY as radioligand, was 9.0 ± 2.0 nM. This affinity is comparable to that of ^{11}C-labelled PIB which has a K_i value of 2.8 ± 0.5 nM in the same assay. We can assume that the newly developed 2-(4′-[^{18}F]fluorophenyl)-1,3-benzothiazole binds to the same binding sites in the amyloid β plaques as do [^{125}I]IMPY and [^{11}C]PIB.

The in vitro affinity for amyloid of the new ^{18}F-labelled agent was also tested by incubating transgenic APP mouse brain slices with the non-radioactive reference compound **2**.[18,19] The same slices were immunohistochemically stained with the amyloid-binding monoclonal mouse antibody AβN25 to compare the regions with high amyloid load. Fig. 3 shows a superimposed image of both staining methods in the mouse subiculum, a highly plaque loaded structure located in the hippocampal region. The fluorescent signal from compound **2** clearly indicates the same amyloid plaques as the immunohistochemical signal from the staining with

Scheme 2. Synthesis of 2-(4′-fluorophenyl)-1,3-benzothiazole (**2**).



Scheme 3. Radiosynthesis of 2-(4′-[^{18}F]fluorophenyl)-1,3-benzothiazole (**[^{18}F]2**).

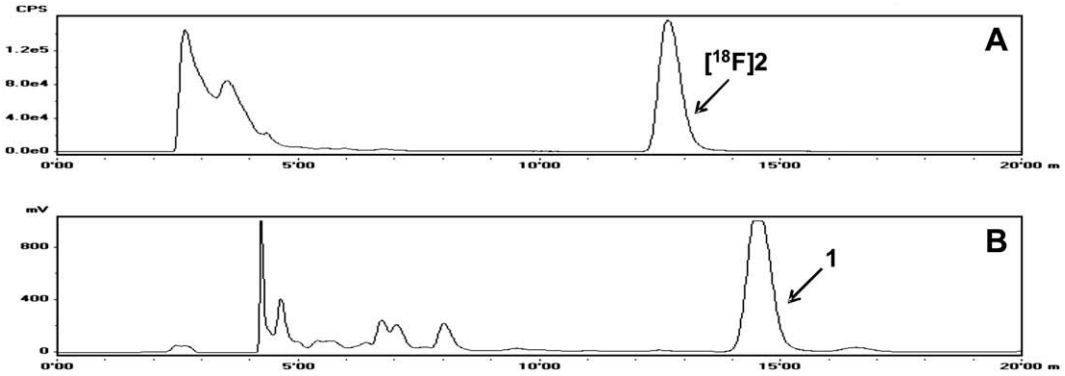


Figure 2. RP-HPLC chromatogram of the crude reaction mixture after labelling of **1** with ^{18}F with **A** = radiometric channel and **B** = UV channel (254 nm). The radioactive compound **[^{18}F]2** elutes at 13.5 min, while the precursor **1** elutes at 14.5 min.

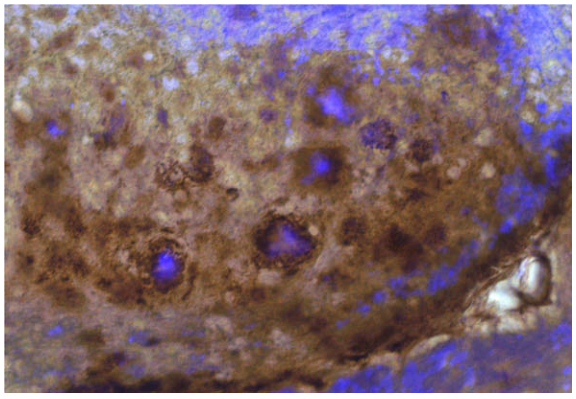


Figure 3. Vibratome section of a transgenic APP mouse brain subiculum stained with AβN25 (brown) in combination with 1 μM solution of 2-(4′-fluorophenyl)-1,3-benzothiazole **2** (blue).

AβN25. Comparable images were also obtained after incubation with the non-radioactive analogue of PIB (data not shown).

To estimate the ability of the new compound to pass the blood–brain barrier (BBB) by passive diffusion, the lipophilicity of RP-HPLC purified **[^{18}F]2** was determined by partitioning between 1-octanol and 0.025 M phosphate buffer pH 7.4 (n = 6).[20] The log partition coefficient (P) value was 2.86 ± 0.05, which is comparable with that of PIB (2.48 ± 0.06) and within the optimal range (log$P$ between 1 and 3) for passive diffusion of a compound through the BBB.[21]

Biodistribution of **[^{18}F]2** was studied in normal mice, which were sacrificed at 2 or 60 min pi.[18,22] The results are shown in Table 1. The initial brain uptake was high at 2 min pi (3.20% ID/g) and was followed by a rapid washout (at 60 min pi: 0.21% ID/g). Similar values were observed for [^{11}C]PIB (2 min pi: 3.60% ID/g and 60 min pi: 0.60% ID/g), although the washout from normal brain (% ID cerebrum 2 min pi/% ID cerebrum 60 min pi) seems faster for the ^{18}F-labelled compound (14.7 vs 6.0). The tissue distribution in other organs was quite similar to that of [^{11}C]PIB (data not shown). Clearance of the activity from the blood is relatively efficient and parallels clearance from the brain. The uptake in the kidneys and excretion in the urine is moderate. Hepatobiliary excretion to the intestines is pronounced and rather fast. The total activity in the hepatobiliary system is 30.3% ID at 2 min pi and 49.4% ID at 60 min pi.

2-(4′-[^{18}F]fluorophenyl)-1,3-benzothiazole **[^{18}F]2** was also injected intravenously in a transgenic APP and normal WT mouse to perform a dynamic μPET study during 60 min.[18,23] A different anaesthetic was used for the μPET studies as compared to the biodistribution study (isoflurane vs Hypnorm®), but we previously found that none of these anaesthetics has an influence on both brain uptake and brain washout of phenylbenzothiazoles. However, comparison of μPET and biodistribution results should be interpreted with caution. The amount of activity present in the frontal cortex, expressed as standard uptake value (SUV), showed a high brain uptake and fast washout. Normally we do not expect a washout in the transgenic APP mouse but the difference in brain washout between the APP and WT mouse was rather baseline (Fig. 4). The same results were obtained when both mice were injected with ^{11}C-labelled PIB. The absence of difference in [^{11}C]PIB-retention between normal WT and transgenic APP mice is in accordance with earlier published data, showing differences between human and mouse amyloid.[24,25]

In conclusion, 2-(4′-[^{18}F]fluorophenyl)-1,3-benzothiazole **[^{18}F]2** showed excellent characteristics comparable with those of [^{11}C]PIB, namely good affinity for amyloid plaques present in human AD brain homogenates and transgenic APP mouse brain sections, favourable log$P$ value for BBB passage and a high initial brain uptake in normal mice followed by a fast washout. The brain

Table 1
Tissue distribution expressed as percentage of the injected dose (% ID) and percentage of the injected dose per gram tissue (% ID/g) after iv injection of 2-(4′-[^{18}F]fluorophenyl)-1,3-benzothiazole **[^{18}F]2** in normal mice at 2 and 60 min pi (n = 4 at each time point)

	2 min pi (% ID)	2 min pi (% ID/g)	60 min pi (% ID)	60 min pi (% ID/g)
Urine	0.6 ± 0.2	–	15.5 ± 2.7	–
Kidneys	11.4 ± 1.6	17.9 ± 1.7	5.9 ± 1.5	8.2 ± 1.8
Liver	21.8 ± 1.8	10.2 ± 1.6	11.7 ± 1.5	5.3 ± 0.6
Lungs	3.9 ± 1.0	13.8 ± 2.3	0.4 ± 0.0	1.5 ± 0.3
Intestines	8.5 ± 0.6	–	37.7 ± 4.3	–
Blood	7.8 ± 0.7	2.9 ± 0.2	1.8 ± 0.3	0.6 ± 0.1
Cerebrum	1.03 ± 0.13	3.20 ± 0.38	0.07 ± 0.01	0.21 ± 0.03
Cerebellum	0.33 ± 0.08	3.35 ± 0.38	0.02 ± 0.01	0.21 ± 0.05

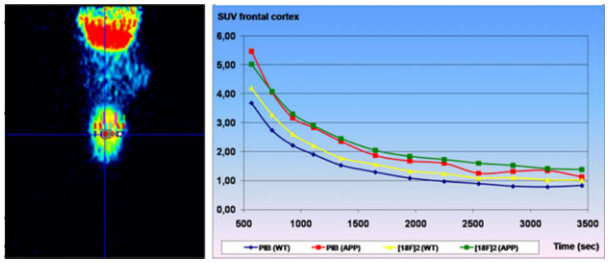


Figure 4. Dynamic μPET study of [^{11}C]PIB and 2-(4′-[^{18}F]fluorophenyl)-1,3-benzothiazole **[^{18}F]2** in a normal WT and transgenic APP mouse (left coronal image at 60 s pi was similar for both compounds in both animals).

washout of **[^{18}F]2** observed in normal mice is more than two times faster than that observed for [^{11}C]PIB. These promising results make **[^{18}F]2** a potential amyloid imaging agent for in vivo visualisation of amyloid plaques with a similar diagnositic power as [^{11}C]PIB and the potential of a widespread clinical application.

References and notes

1. Selkoe, D. J. *Physiol. Rev.* **2001**, *81*, 741.
2. Blennow, K.; de Leon, M.; Zetterberg, H. *Lancet* **2006**, *368*, 387.
3. Furumoto, S.; Okamura, N.; Iwata, R.; Yanai, K.; Arai, H.; Kudo, Y. *Curr. Top. Med. Chem.* **2007**, *7*, 1773.
4. Klunk, W. E.; Engler, H.; Nordberg, A.; Wang, Y.; Blomqvist, G.; Holt, D.; Bergström, M.; Savitcheva, I.; Huang, G.; Estrada, S.; Ausén, B.; Debnath, M.; Barletta, J.; Price, J. C.; Sandell, J.; Lopresti, B.; Wall, A.; Koivisto, P.; Antoni, G.; Mathis, C.; Langström, B. *Ann. Neurol.* **2004**, *55*, 306.
5. Mathis, C.; Lopresti, B.; Mason, N.; Price, J.; Flatt, N.; Bi, W.; Ziolko, S.; DeKosky, S.; Klunk, W. *J. Nucl. Med.* **2007**, *48*, 56P.
6. Shi, D.-F.; Bradshaw, T. D.; Wrigley, S.; McCall, C. J.; Lelieveld, P.; Fichtner, I.; Stevens, M. F. G. *J. Med. Chem.* **1996**, *39*, 3375.
7. Mathis, C. A.; Bacskai, B. J.; Kajdasz, S. T.; McLellan, M. E.; Frosch, M. P.; Hyman, B. T.; Holt, D. P.; Wang, Y.; Huang, G.-F.; Debnathd, M. L.; Klunk, W. E. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 295.
8. Accurate mass determination was performed on a time-of-flight mass spectrometer (LCT, Micromass, Manchester, UK) equipped with an orthogonal electrospray ionisation interface, operated in positive mode (ES^+). Formic acid was added to enhance electrospray ionisation. The drift of the mass spectrometer was corrected by co-injection of a compound with known mass. After acquisition of the data with Masslynx software (version 3.5), the spectrum was corrected using this compound as lock mass.
9. *Compound* ***1***: ^{1}H NMR ($CDCl_3$): δ 7.47 (1H, dd, 6-H); δ 7.57 (1H, dd, 5-H); δ 7.97 (1H, d, 4-H); δ 8.14 (1H, d, 7-H); δ 8.27 (2H, d, 2′-H 6′-H); δ 8.36 (2H, d, 3′-H 5′-H). Accurate MS–ES^+ *m*/*z* $[M+H]^+$ 257.0356 (calcd for $C_{13}H_8N_2O_2S$ 257.0379). Mp 226.1–227.4 °C.
10. *Compound* **2**: ^{1}H NMR ($CDCl_3$): δ 7.16 (2H, dd, 3′-H 5′-H); δ 7.38 (1H, t, 5-H); δ 7.50 (1H, t, 6-H); δ 7.90 (1H, d, 7-H); δ 8.06 (2H, d, 2′-H 6′-H); δ 8.10 (1H, d, 4-H). Accurate MS–ES^+ *m*/*z* $[M+H]^+$ 230.2855 (calcd for $C_{14}H_{10}NFS$ 230.2856). Mp 97.2–98.5 °C.
11. Kamal, A.; Ahmed, S. K.; Reddy, K. S.; Khan, M. N. A.; Shetty, R. V.; Siddhardha, B.; Murty, U. S. N.; China, A.; Nagaraja, V. *Lett. Drug Des. Discov.* **2007**, *4*, 550.
12. Mu, X.-J.; Zou, J.-P.; Zeng, R.-S.; Wu, J.-C. *Tetrahedron Lett.* **2005**, *46*, 4345.
13. [^{18}F]Fluoride was produced via a [^{18}O(p,n)^{18}F] reaction by irradiation of 0.5 ml of 97% enriched $H_2{}^{18}O$ (Rotem HYOX^{18}, Rotem Industries, Beer Sheva, Israel) in a niobium target using 18-MeV protons from a Cyclone 18/9 cyclotron (Ion Beam Applications, Louvain-la-Neuve, Belgium). The aqueous solution of [^{18}F]F^- was transferred to a synthesis module where [^{18}F]F^- was separated from $H_2{}^{18}O$ using a Sep-Pak® Light Waters Accell™ Plus CM cartridge (Waters). [^{18}F]F^- was then eluted from the cartridge into a reaction vial with a solution containing 2.5 mg potassium carbonate and 27.9 mg 2.2.2-cryptand dissolved in 0.75 ml of water/acetonitrile (5:95 v/v). After evaporation of the solvent from the reaction vial under a stream of helium at 115 °C for 7 min, [^{18}F]F^- was further dried by azeotropic distillation of traces of water using 1 ml of anhydrous acetonitrile (115 °C, 5 min). A solution of 1.5 mg 2-(4′-nitrophenyl)-1,3-benzothiazole in 0.5 ml anhydrous DMSO was added to the radioactive residue and the mixture was heated at 150 °C for 20 min in a closed vial to provide the crude radiolabeled compound. The mixture was cooled down to room temperature and after dilution with an equal volume of 0.05 M ammonium acetate purified with RP-HPLC using an XTerra™ MS C18 3.5 μm column (2.1 mm × 50 mm, Waters), eluted isocratically with a mixture of 0.05 M ammonium acetate, tetrahydrofuran and ethanol (50:37:13, v/v) at a flow rate of 1 ml/min. The fraction containing the isolated radioactive compound was diluted with an equal volume of water and then applied on an activated Sep-Pak® Plus C18 cartridge (Waters, first activated by successive washing with 5 ml ethanol and 10 ml water) that was rinsed with 10 ml water and then eluted with 1 ml ethanol. The purity of the labeled tracer was analysed using an XTerra RP_{18} 5 μm 4.6 × 250 mm column (Waters) eluted with an isocratic mixture of 50% 0.05M ammonium acetate and 50% ethanol/tetrahydrofuran (75:25, v/v) at a flow rate of 1 ml/min (Rt **[^{18}F]2** = 28.5 min).
14. Kilbourn, M. R. *Fluorine-18 Labeling of Radiopharmaceuticals. Nuclear Science Series*; National Academy Press: Washington, DC, 1990.
15. Serdons, K.; Terwinghe, C.; Vermaelen, P.; Kung, H.; Bormans, G.; Verbruggen, A. *J. Nucl. Med.* **2006**, *47*, 31P.
16. Chitneni, S. K.; Serdons, K.; Evens, N.; Fonge, H.; Celen, S.; Deroose, C. M.; Debyzer, Z.; Mortelmans, M.; Verbruggen, A. M.; Bormans, G. M. *J. Chromatogr. A* **2007**, *1189*, 323.
17. Kung, M. P.; Hou, C.; Zhuang, Z. P.; Skovronsky, D.; Kung, H. F. *Brain Res.* **2004**, *1025*, 89.
18. All animal experiments were approved by the local Ethical Committee.
19. Vibratome sections (40 μm) of the brain of transgenic mice overexpressing human APP (amyloid precursor protein) were incubated for 1 h at room temperature with the non-radioactive compound **2** at a concentration of 1 μM. After incubation, the sections were rinsed with tap water and treated with 0.2% NaOH in 80% ethanol to reduce background staining. The slices were rinsed again with tap water and coverslipped using a Mowiol-DABCO solution. For immunohistochemical staining, the same vibratome sections were treated with a 0.1% Triton X-100 solution in PBS (PBST) and 10% foetal calf serum to block non-specific binding. The slices were subsequently incubated overnight with the amyloid-binding monoclonal antibody AβN25 (Oncogene Research Products, San Diego, CA, USA) which stains both dense and fibrillar plaques. The sections were treated again with PBST and rinsed with PBS before fixation with Mowiol-DABCO solution. Fluorescence microscopy was performed using a Leica DMR microscope equipped with a digital Leica DC480 camera and a UV filter set with following specifications: excitation: 340–380 nm bandpass filter, dichromatic mirror 400 nm; emission: 425 nm longpass filter. The images were collected and processed with Leica IM500 image processing software.
20. Serdons, K.; Verduyckt, T.; Cleynhens, J.; Bormans, G.; Verbruggen, A. *J. Label. Compd. Radiopharm.* **2008**, *51*, 357.
21. Dishino, D.; Welch, M.; Kilbourn, M.; Raichle, M. *J. Nucl. Med.* **1983**, *24*, 1030.
22. A solution of **[^{18}F]2** obtained after RP-HPLC purification was diluted using 0.9% NaCl in water for injection to a concentration of 3.7 MBq/ml. The concentration of ethanol did not exceed 10% and the concentration of THF did not exceed 0.02%, as determined by gas chromatography. The biodistribution was studied in male NMRI mice (body mass 30–40 g). An aliquot of 0.1 ml of the diluted tracer solution was injected into the mice via a tail vein, after intraperitoneal injection of 0.1 ml Hypnorm® (fentanyl citrate 63 μg/ml and fluanisone 2 mg/ml). The mice were sacrificed by decapitation at 2 or 60 min pi. Blood was collected in a tared tube and weighed. All organs and other body parts were dissected and weighed, and their radioactivity was counted in a gamma counter. Results were corrected for background radioactivity and are expressed as percentage of the injected dose (% ID) or as percentage of the injected dose per gram tissue (% ID/g). For calculation of total radioactivity in blood, blood mass was assumed to be 7% of the total body mass.
23. Dynamic μPET imaging was performed with a Focus 220 microPET scanner (Siemens Medical Solutions USA, Inc), which has a transaxial resolution of 1.35 mm in full-width at half-maximum. Before being imaged, a transgenic APP mouse (50 months old) and a normal WT mouse (2 months old) were anaesthesised with isoflurane (1.5–2.5%) in oxygen at a flow rate of 1–2 l/min and were injected via a tail vein with 2.11 and 6.57 MBq **[^{18}F]2**, respectively. The mice were breathing spontaneously throughout the entire experiment. Dynamic μPET images were acquired for 60 min (4 × 15 s, 4 × 60 s, 5 × 180 s, 8 × 300 s) and reconstruction was done using filtered back projection with a RAMP 0.5 filter. Data were analysed using PMOD2.7, volumes of interest (VOIs) were defined on the summed images, time–activity curves (TACs) were drawn and values were expressed as standard uptake values (SUV). The same mice were also injected with 2.40 and 8.47 MBq [^{11}C]PIB, respectively (1 week interval).
24. Klunk, W. E.; Lopresti, B. J.; Ikonomovic, M. D.; Lefterov, I. M.; Koldamova, R. P.; Abrahamson, E. E.; Debnath, M. L.; Holt, D. P.; Huang, G.-F.; Shao, L.; DeKosky, S. T.; Price, J. C.; Mathis, C. A. *J. Neurosci.* **2005**, *25*, 10598.
25. Toyama, H.; Ye, D.; Ichise, M.; Liow, J.-S.; Cai, L.; Jacobowitz, D.; Musachio, J. L.; Hong, J.; Crescenzo, M.; Tipre, D.; Lu, J.-Q.; Zoghbi, S.; Vines, D. C.; Seidel, J.; Katada, K.; Green, M. V.; Pike, V. W.; Cohen, R. M.; Innis, R. B. *Eur. J. Nucl. Med. Mol. Imaging* **2005**, *32*, 593.