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Synthesis of a [^{18}F]fluorobenzothiazole as potential amyloid imaging agent

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This study describes the synthesis of a fluoroethylated derivative of [*N*-methyl-^{11}C]2-(4′-methylaminophenyl)-6-hydroxybenzothiazole ([^{11}C]6-OH-BTA-1; Pittsburgh Compound B (PIB)), an already established amyloid imaging agent. The [^{11}C]methylamino group of [^{11}C]6-OH-BTA-1 was formally replaced by a fluoroethyl group in a cold synthesis via N-alkylation of *N*-Boc-2-(4′-aminophenyl)-6-(methoxyethoxymethoxy)benzothiazole with fluoroethyl tosylate. Subsequent deprotection gave the target compound 2-[4′-(2-fluoroethyl)aminophenyl]-6-hydroxybenzothiazole (FBTA). In a radioligand competition assay on aggregated synthetic amyloid fibrils using *N*-[^{3}H-methyl]6-OH-BTA-1, 100 nM FBTA inhibited binding with 93 ± 1 and 83 ± 1% efficiency for Aβ_{1-40} and Aβ_{1-42}, respectively. For the radiosynthesis a precursor carrying a tosylethyl moiety was prepared allowing the introduction of [^{18}F]fluoride via nucleophilic substitution with [^{18}F]tetra-*n*-butyl-ammonium fluoride (TBAF). Subsequent removal of all protecting groups was performed in a one-pot procedure followed by semi-preparative HPLC, delivering the target compound [^{18}F]FBTA in good radiochemical yield of 21% on average and radiochemical purity of ⩾98% at EOS. *In vitro* autoradiography on human *postmortem* AD brain tissue slices showed intense cortical binding of [^{18}F]FBTA (1 nM), which was displaced in presence of 6-OH-BTA-1 (1 μM). Brain up-take was evaluated in wild-type (wt) mice with microPET imaging. Based on these results, [^{18}F]FBTA appears to be a suitable candidate tracer for amyloid imaging in humans.

Keywords: Alzheimer's disease; PET; [^{18}F] FBTA; amyloid; [^{11}C] BTA

Introduction

Amyloid β (Aβ) plaques and neurofibrillary tangles (NFTs) detected in human brain *postmortem* are the pathological features of Alzheimer's disease (AD).[1–3] Aβ plaques are mainly composed of aggregated Aβ peptide fibrils, while NFTs consist primarily of filaments of hyperphosphorylated tau proteins. Clinical guidelines for AD diagnosis currently lack sensitivity and specificity.[4] Thus, the gold standard for definite diagnosis of AD is the *postmortem* neuropathological examination of the brain.[5] *In vivo* imaging of Aβ plaques would greatly improve diagnosis and therapy monitoring of AD. Based on the structure of the histological amyloid staining reagents Congo Red and Thioflavin T, several radiolabelled Aβ-specific imaging agents have been developed.[6] [^{18}F]labelled benzothiazole,[7] thiophene,[8] stilbene,[9] imidazopyridine[10,11] and naphtylidene[12] derivatives have been evaluated for the use as PET tracers. Up to the present [^{18}F]FDDNP,[13,14] [^{11}C]SB-13,[15,16] [^{11}C]BF-227,[17] [^{18}F]AV-1/ZK[18] and [^{11}C]6-OH-BTA-1[7,19] have been evaluated as Aβ tracers in clinical trials and are shown in Figure 1.

While the short half-life of the [^{11}C]radionuclide requires radiosynthesis in house, [^{18}F]labelled compounds allow centralized production and distribution to a larger community. Therefore, [^{18}F]derivatives of [^{11}C]6-OH-BTA-1, which was the first Aβ PET tracer used in humans and is now frequently used, have been developed.[20,21] A selection of the some structurally similar [^{18}F]labelled analogs of [^{11}C]6-OH-BTA-1 are shown in Figure 2 (compounds I–IV).

Among them IV (= FBTA), which was first mentioned by Mathis *et al.*[20], has the closest structural analogy to 6-OH-BTA-1 and was chosen as our target compound[22] to be evaluated as potential PET tracer for Aβ imaging. The synthesis of the cold standard material together with its confirmation as potent Aβ radioligand in a competition assay on aggregated synthetic amyloid fibrils using *N*-[^{3}H-methyl]6-OH-BTA-1 is reported. In the radiosynthesis, [^{18}F]FBTA was prepared by direct introduction of [^{18}F]fluoride via nucleophilic substitution with [^{18}F]tetra-*n*-butyl-

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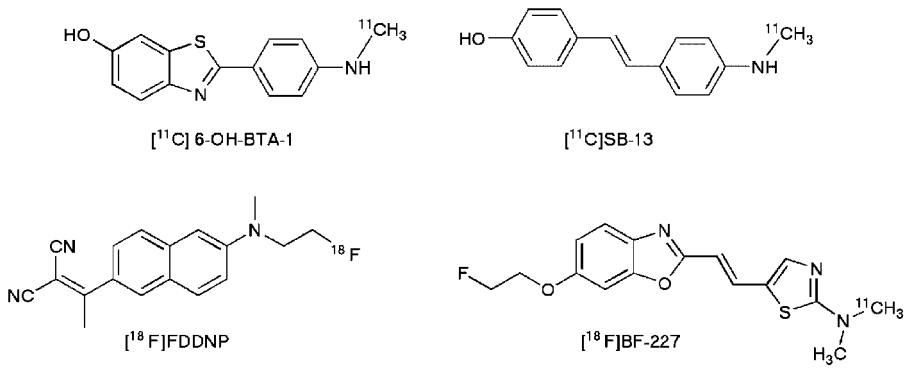


Figure 1. Clinical evaluated PET tracers for amyloid-β imaging: [^{11}C]6-OH-BTA-1: [*N*-methyl-^{11}C]2-(4′-methylaminophenyl)-6-hydroxybenzo-thiazole; [^{11}C]SB-13: [*N*-methyl-^{11}C]4-*N*-methylamino-4′-hydroxystilbene; [^{18}F]FDDNP: [^{18}F](2-(1-[6-[(2-[^{18}F]fluoroethyl)(methyl)amino]-2-naphthyl]ethylidene)); [^{11}C]BF-227: [*N*-methyl-^{11}C]2-(2-[2-dimethylaminothiazol-5-yl]ethenyl)-6-(2-[fluoro]ethoxy)benzoxazole.

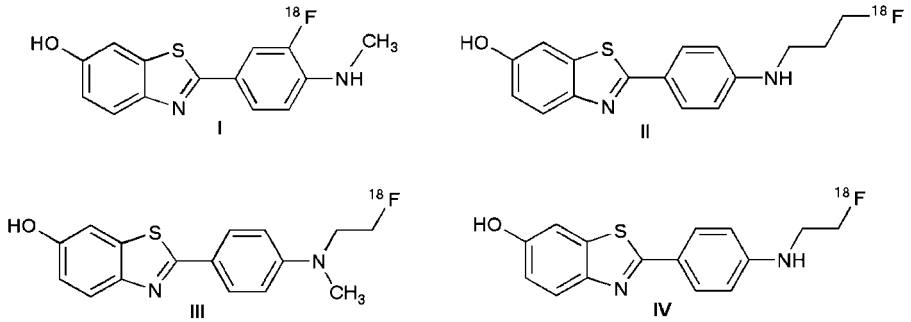


Figure 2. A selection of [^{18}F]labelled 6-OH-BTA-1 analogs: I: [^{18}F]2-(3′fluoro-4′-methylaminophenyl)-6-hydroxybenzothiazole; II: [^{18}F]6-OH-BTA-fp-1: [^{18}F]2-[4′-(3-fluoropropyl)aminophenyl]-6-hydroxybenzothiazole; III: [^{18}F]*N*-methyl-2-[4′-(2-fluoroethyl)aminophenyl]-6-hydroxybenzothiazole; IV: [^{18}F]FBTA: [^{18}F]2-[4′-(2-fluoroethyl) aminophenyl]-6-hydroxybenzothiazole.

ammonium fluoride (TBAF). The kinetics of [^{18}F]FBTA in the brain of wild-type (wt) mice were studied by means of microPET imaging and the specificity of its binding was tested with autoradiography using human AD brain slices with and without the addition of a molar excess of unlabelled 6-OH-BTA-1.

Results and discussion

Chemistry

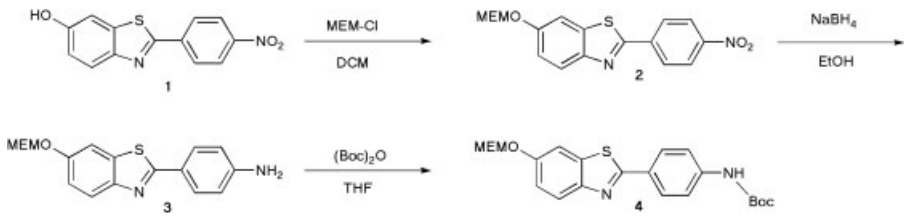
Based on protocols from the literature,[7] 2-(4-nitrophenyl)-6-hydroxybenzothiazole **1** was synthesized in four steps starting from 4-nitrobenzoic acid and *p*-anisidine. The phenolic moiety was protected as the methoxyethoxymethyl (MEM) ether[23] to give **2**. Reduction of the nitro group[7] with $NaBH_4$ in ethanol lead to aniline **3**, which was protected with a Boc group under standard conditions[24,25] to give compound **4** (Scheme 1), in order to prevent dialkylation in the labelling step. Compound **4** is the key intermediate for the synthesis of cold reference material and for the radiosynthesis of [^{18}F]FBTA.

The alkylation of **4** was achieved in dry dimethylfomamide (DMF) with NaH as base at 60°C using fluoroethyltosylate[26] as alkylating agent giving compound **5** as stable and storable material in high yield (Scheme 2).

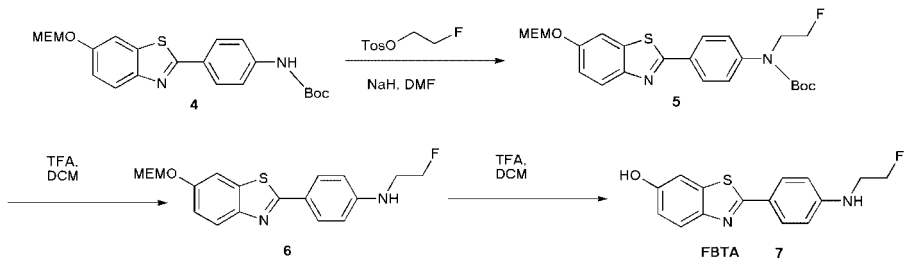
The lower stability of the Boc group compared with the MEM group allowed selective cleavage with 5% trifluoroacetic acid (TFA) in dichloromethane (DCM) at room temperature (rt) yielding fluoroethylamine **6**. Compound **6** was needed as reference material for the detection of incomplete cleavage of the MEM group in the HPLC-based monitoring of the one-pot deprotection in the planned radiosynthesis. In general, the deprotection of **5** was accomplished in a one-pot procedure with TFA/DCM at reflux temperature to give **7** (= FBTA) in high and reproducible yield.

In addition to the synthesis of FBTA, we intended to synthesize the structurally closest derivative of 6-OH-BTA-1, the *N*-fluoromethyl derivative **10**. In this approach, we succeeded in the analogous alkylation of carbamate **4** with fluoromethyl tosylate[27] giving compound **8**. Unfortunately, compound **8** turned out to be unstable and degraded to hydroxymethyl derivative **9** upon purification on silica gel and under storage in the refrigerator. Attempted acidic deprotection to compound **10** was unsuccessful as well (see Scheme 3).

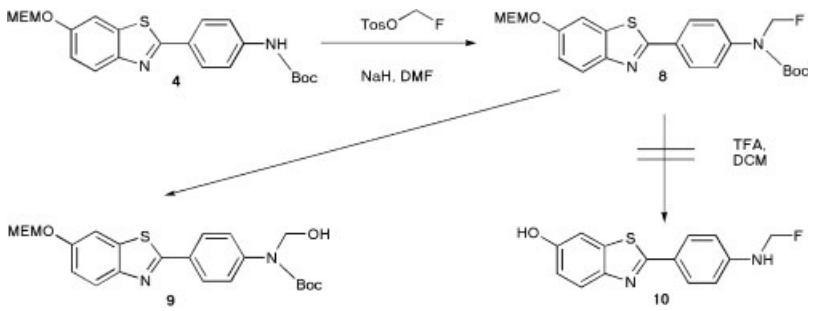
These results were not completely unexpected since there is no satisfying literature precedence for the synthesis of a secondary fluoromethylaniline, neither in cold nor hot synthesis although several patents[28,29] quote structures of cold fluoromethylated anilines - unfortunately not including any experimental procedures or analytical data. Zheng and Berridge,[30] have reported comprehensively on the alkylation of diphenylamine with [^{18}F]fluoroiodomethane, among other tertiary fluoromethyldialkylamines. Examples of *N*-fluoromethylated nitrogen containing heterocycles that have been used as PET tracers in other regards are described elsewhere.[31,32] Encouraged by the results of Zheng and Berridge,[30] we attempted alkylation of the unprotected aniline **3** with fluoromethyl tosylate in various solvents like chloroform, DMF or acetone with different bases, which all remained unsuccessful.



Scheme 1



Scheme 2



Scheme 3

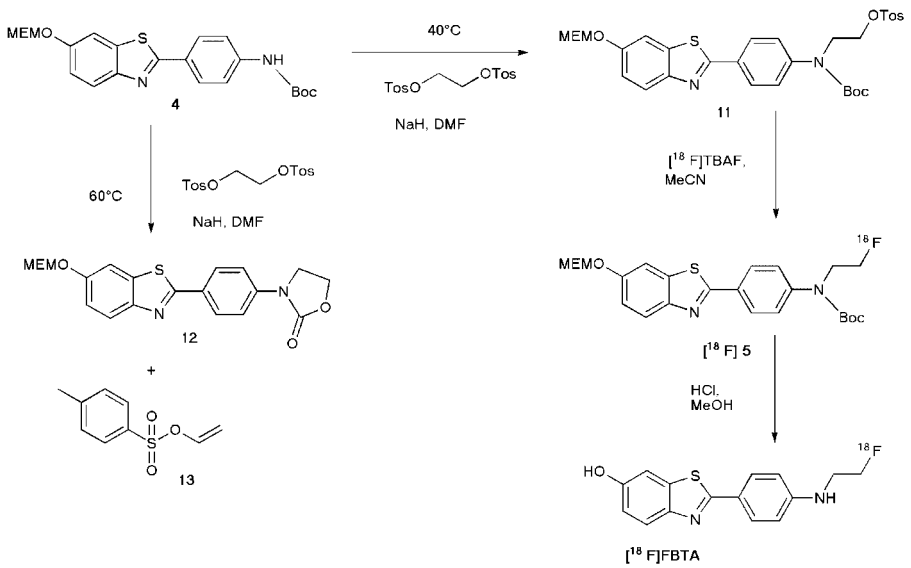
Radiosynthesis

Although there is literature[33] covering the [^{18}F]fluoroethylation of other substrates with [^{18}F]fluoroethyl tosylate we decided to synthesize precursor **11** carrying a tosylethyl group, thus allowing nucleophilic introduction of [^{18}F]fluoride. In this way, we circumvent the laborious two-step radiosynthesis using [^{18}F]fluoroethyl tosylate as an alkylating agent (see Scheme 4).

The synthesis of the precursor **11** was accomplished by the alkylation of intermediate **4** with ethylene bis-tosylate.[34] The alkylation was carried out under similar conditions as the alkylation of compound **4** with fluoromethyl and fluoroethyl tosylate, but had to be performed at lower temperature of 40°C to avoid the formation of by-products **12** and **13**.[35] At higher temperatures, cyclization of precursor **11** under the loss of the *tert*-butyl group yielding cyclic carbamate **12** was observed. The same cyclization was observed when the precursor was stored at rt. By-product **13** was formed from ethylene bis-tosylate at elevated temperature.

For the radiosynthesis, [^{18}F]fluoride was generated from H_2[^{18}O]-enriched water via proton bombardment. [^{18}F]fluoride was adsorbed on an anion exchange cartridge as described in the literature[36] and eluted with tetra-*n*-butyl-ammonium hydrogen carbonate to give [^{18}F]TBAF. Precursor **11** was transformed into the [^{18}F]fluoroethyl compound [^{18}F]**5** via nucleophilic substitution with [^{18}F]TBAF[37] in dry acetonitrile at elevated temperature. A crude sample of the reaction mixture was analysed by analytical HPLC. Comparison with cold standard material **5** proved successful [^{18}F]fluorination of the precursor. Cleavage of the Boc group and the MEM group was carried out in a one-pot procedure with HCl/MeOH. Before injection onto the semipreparative HPLC, the reaction mixture was neutralized with NH_4OH and diluted with acetonitrile to redissolve precipitated material, which is crucial to achieve high isolated yield. The high lipophilicity of the target compound did not allow the use of a physiologic eluent in the HPLC purification, which was complicating the work up procedure; [^{18}F]FBTA was eluted with acetonitrile/TEAP-buffer (Figure 3, trace A).

The product peak was collected and diluted with water. This solution was adsorbed on a C18-cartridge and subsequently washed with water. The product was eluted with ethanol and transferred into an injectable solution. Radiochemical purity was greater than 98% at the end of synthesis (EOS) and was still greater than 95% after 6 h as measured by analytical HPLC (Figure 3, trace B). The chemical identity was confirmed by co-injection with the cold standard compound (Figure 3, trace C). (Retention time of FBTA was 10.0 min versus 9.9 min of [^{18}F]FBTA because the sample passed the radioactivity detector first and UV detector second in the used HPLC system.) The product contained no detectable amount of precursor and 0.7 nmol (mean) FBTA per mCi at EOS. The radiosynthesis of [^{18}F]FBTA was performed in a PC-controlled synthesizer module that had been adapted to perform all steps leading to [^{18}F]FBTA automatically. The title compound was obtained in a radio-



Scheme 4

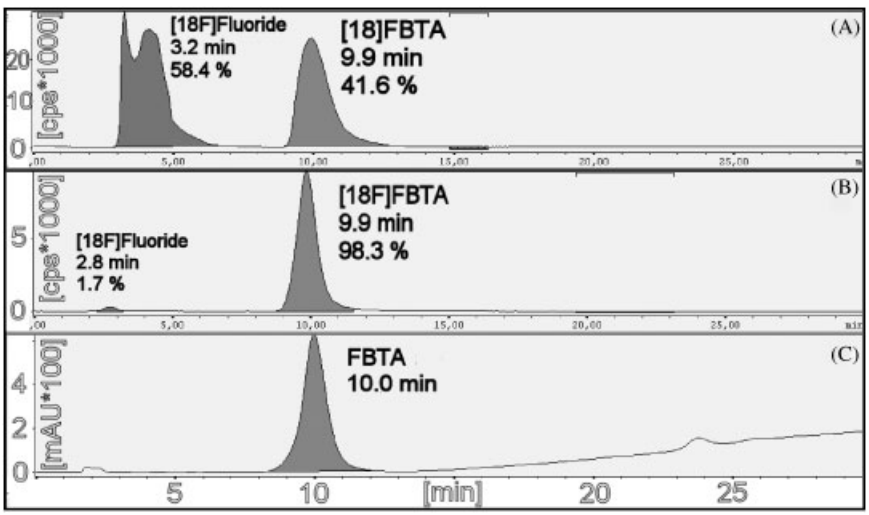


Figure 3. Isolation and analysis of [^{18}F]FBTA: trace A: semipreparative HPLC of the reaction mixture: radioactivity detector; [^{18}F]fluoride retention time 3.2 min (58.4%), [^{18}F]FBTA retention time 9.9 min (41.6%). Trace B: analytical HPLC of the end product: radioactivity detector; [^{18}F]fluoride retention time 2.8 min (1.7%); [^{18}F]FBTA retention time 9.9 min (98.3%). Trace C: analytical HPLC of FBTA standard: UV detector; retention time 10.0 min.

chemical yield of 21 ± 5% (n = 13) with a mean specific activity of 2.5 ± 1.2 mCi/nmol (n = 7) at EOS. [^{18}F]FBTA was shown to be stable *in vitro* and is available for routine synthesis.

In vitro and *in vivo* evaluation

The kinetics of [^{18}F]FBTA in brains of wt mice were studied by means of microPET imaging. Following i.v. administration of [^{18}F]FBTA, peak radioactivity uptake in mouse brain was in the order of about 0.1% ID kg/g. We analysed frontal cortex as a region where plaque load would be expected in human AD patients and cerebellum as a region which has been shown to be devoid of plaque load. In Figure 4, dose-normalized [^{18}F]FBTA time–activity curves for the frontal cortex and the cerebellum are shown. The ratio of the radioactivity concentration in frontal cortex at 2 min to that at 30 min after tracer injection was 3.5. This is less compared with [^{11}C]6-OH-BTA-1[7] but is still in a useful range for use as a PET tracer since [^{18}F]FBTA allows a longer time of measurement.

Binding affinities of FBTA for aggregated synthetic amyloid fibrils ($A\beta_{1-40}$ and $A\beta_{1-42}$) were determined in a radioligand competition assay using *N*-[^{3}H-methyl]6-OH-BTA-1 according to literature.[38] At a concentration of 100 nM, FBTA inhibited [^{3}H]6-OH-BTA-1 binding with 93 ± 1 and 83 ± 1% efficiency for $A\beta_{1-40}$ and $A\beta_{1-42}$, respectively. In comparison, cold 6-OH-BTA-1 tested in an identical manner, resulted in 72 ± 2 and 70 ± 3% inhibition of binding for $A\beta_{1-40}$ and $A\beta_{1-42}$, respectively.

Further, binding of [^{18}F]FBTA to $A\beta$ plaques was also proven by autoradiography of human congophilic AD brain slices. Incubation[39] and washing[40] of the slices were done according to literature with minor modifications. Intense cortical binding was observed (Figure 5(a)). In a displacement experiment done in

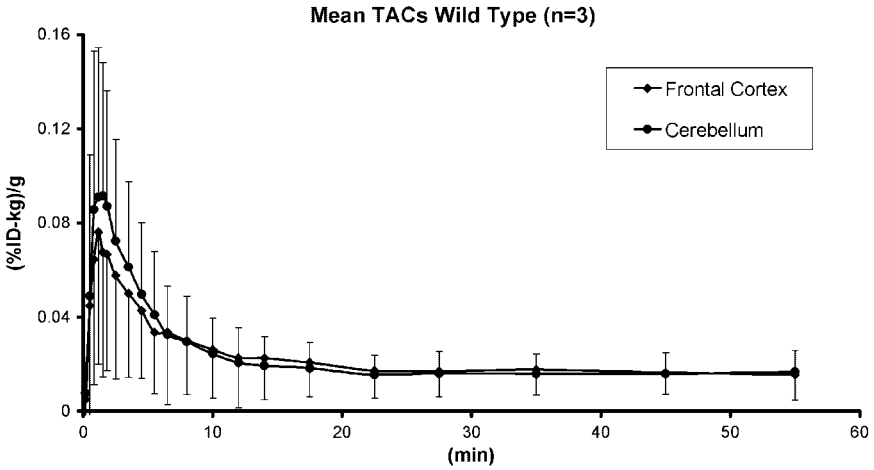


Figure 4. Mean TACs of three wt mice in the frontal cortex and in the cerebellum.

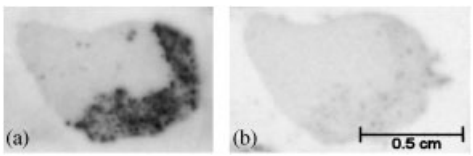


Figure 5. Specific Binding (a: ~1 nM [^{18}F]FBTA) and selective displacement (b: ~1 nM [^{18}F]FBTA+1 μM 6-OH-BTA-1) of [^{18}F]FBTA to Aβ plaques shown by autoradiography using postmortem human brain tissue sections.

parallel selective displacement of [^{18}F]FBTA by unlabelled 6-OH-BTA-1 can clearly be seen (Figure 5(b)).

Conclusion

Radiosynthesis of a [^{18}F]labelled analog of [^{11}C]6-OH-BTA-1 was successfully accomplished. The [^{11}C]methyl group was formally replaced by a [^{18}F]fluoroethyl group. Cold reference material FBTA was obtained by high-yielding alkylation of Boc aniline **4** with fluoroethyltosylate and subsequent deprotection. In the hot synthesis the lower yielding alkylation with ethylene bistosylate giving tosylethyl precursor **11** was accepted to allow one-step nucleophilic introduction of [^{18}F]fluoride with [^{18}F]TBAF followed by one-pot deprotection giving [^{18}F]FBTA. A detailed discussion of the problems encountered in the attempted synthesis of fluoromethyl derivative **10** is included in this report. Acceptable peak brain uptake at 2 min p.i. and 2 min/30 min washout ratios in brains of wt mice indicate that [^{18}F]FBTA might be a useful Aβ tracer in humans. Binding affinity of FBTA for Aβ_{1-40} and Aβ_{1-42} fibrils, as determined by an inhibition assay using *N*-[^{3}H-methyl]6-OH-BTA-1, was shown to be higher than that of 6-OH-BTA-1. Autoradiography of human AD brain slices showed that [^{18}F]FBTA binds specific and in a displaceable manner to the same binding sites as 6-OH-BTA-1.

Experimental

All starting materials and reagents were purchased from commercial sources and used without further purification. Analytical HPLC and semipreparative HPLC were performed with an Agilent ChemStation 1100 System, which was equipped with a quaternary gradient pump and was operated with the designated software. In analytical HPLC a multi-wavelength UV detector and a Gabi Star scintillation detector model 2″ was used. The semipreparative HPLC was equipped with a WellChrom Filter-Photometer K-2001 from Knaur and a Geiger–Müller tube as a radioactivity detector. UV detection was done at 254 nm. The radiosynthesis was performed in a PC-controlled TRACERlab FX-FDG Synthesiser Module from GE Healthcare, which had been adapted for the synthesis of [^{18}F]FBTA. The system was operated with the GINA Star^{TM} 4.0 software.

Melting points were measured on a Leica Galen III instrument and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker-Spectrospin 200 (200 and 50 MHz, respectively) spectrometer or a Varian Unity spectrometer (500 and 125 MHz, respectively). HRMS were obtained with a Finnigan MAT 900 (ESI) or a Finnigan MAT 8230 (EI). Elemental analysis was performed on a 2400 CHN Elemental Analyzer from Perkin Elmer.

Synthetic chemistry

2-(4′-Nitrophenyl)-6-(methoxyethoxymethoxy)benzothiazole (**2**): 2-(4′-Nitrophenyl)-6-hydroxybenzothiazole **1** (2.68 g, 9.8 mmol) was dissolved in DCM (100 ml) before *N*-ethyl-di-isopropylamine (Huenig's base) (4.8 ml, 41.8 mmol) and MEM-Cl (7.3 ml, 34.3 mmol) were added successively. The solution was stirred at rt for 49 h. The reaction mixture was washed with 0.05 N HCl and 0.05 N NaOH, dried over Na_2SO_4 and evaporated. The crude product was purified by flash column chromatography (SiO_2; LP:EtOAc = 4:1) to give 2.39 g (68%) of the pure product as yellow solid.

^{1}H-NMR (200 MHz, $CDCl_3$) δ = 8.35 (d, J = 9.0 Hz, 2H), 8.22 (d, J = 8.8 Hz, 2H), 8.01 (d, J = 9.0 Hz, 1H), 7.65 (d, J = 2.4 Hz, 1H), 7.25 (dd, J = 2.7, J = 8.6, 1H), 5.37 (s, 2H), 3.90–3.86 (m, 2H), 3.61–3.57 (m, 3H), 3.39 (s, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 162.9 (C), 156.0 (C), 149.3 (C), 148.7 (C), 139.2 (C), 136.8 (C), 127.8 (CH), 124.4 (CH), 124.2 (CH), 117.9 (CH), 107.4 (CH), 93.9 (CH_2), 71.5 (CH_2), 67.9 (CH_2), 59.0 (CH_3). Calculated for $C_{17}H_{16}N_2O_5S$: C 55.66%; H 4.48%; N 7.77%; S 8.90%; found: C 56.32%; H 4.34%; N 7.79%; S 8.71%. Melting point: 144–146°C (analytical sample was recrystallized from EtOAc).

2-(4′-Aminophenyl)-6-(methoxyethoxymethoxy)benzothiazole (**3**): To a mixture of 2-(4′-nitrophenyl)-6-(methoxyethoxymethoxy)-benzothiazole **2** (4.83 g, 13.40 mmol) and $Cu(OAc)_2$ (2.43 mg, 13.40 mmol) in anhydrous ethanol (160 ml), $NaBH_4$ (17.74 g,

469.0 mmol) was added portionwise and the reaction mixture was stirred at rt for 20 h. The solvent was evaporated and the residue was taken up in water and extracted with EtOAc. The extracts were combined, dried over Na_2SO_4 and evaporated. The crude product was purified by flash column chromatography (SiO_2; LP:EtOAc = 1:2) to give 2.76 g (62%) of the pure product as yellow solid.

^{1}H-NMR (200 MHz, $CDCl_3$) δ = 7.88 (d, J = 6.1 Hz, 1H), 7.84 (d, J = 5.9 Hz, 2H), 7.55 (d, J = 2.4 Hz, 1H), 7.14 (dd, J = 2.5 Hz, J = 8.8 Hz, 1H), 6.71 (d, J = 8.5 Hz, 2H), 5.32 (s, 2H), 3.89–3.84 (m, 2H), 3.60–3.55 (m, 2H), 3.38 (s, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 166.8 (C), 154.8 (C), 149.3 (C), 149.0 (C), 135.5 (C), 128.9 (CH), 123.8 (C), 122.8 (CH), 116.7 (CH), 114.8 (CH), 107.7 (C), 94.0 (CH_2), 71.6 (CH_2), 67.7 (CH_2), 59.0 (CH_3). Calculated for $C_{17}H_{18}N_2O_3S$: C 61.80%, H 5.49%. N 8.48%, S 9.70%; found: C 61.55%; H 5.58; N 8.48%; S 9.39%. Melting point: 145–147°C (analytical sample was recrystallized from MeOH).

N-Boc-2-(4′-aminophenyl)-6-(methoxyethoxymethoxy)benzothiazole (**4**): A mixture of **3** (2.00 g, 6.05 mmol) and di-*tert*-butyl dicarbonate (3.02 g, 13.84 mmol) in THF (10 ml) was heated to reflux for 42 h. The mixture was poured on water and extracted with DCM. The combined organic layers were washed with water, dried over Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography (SiO_2; LP:Et_2O = 1:2) to give 2.53 g (97%) of the pure product as yellow solid.

^{1}H-NMR (500 MHz, $CDCl_3$) δ = 7.97 (d, J = 8.5 Hz, 2H), 7.92 (d, J = 9.1 Hz, 1H), 7.58 (d, J = 2.2 Hz, 1H), 7.48 (d, J = 8.5 Hz, 2H), 7.17 (dd, J = 2.4 Hz, J = 9.0 Hz, 1H), 6.71 (s, 1 H, NH), 5.33 (s, 2H), 3.89–3.86 (m, 2H), 3.59–3.57 (m, 2H), 3.38 (s, 3H), 1.53 (s, 9H). ^{13}C-NMR (125 MHz, $CDCl_3$) δ = 166.0 (C), 155.1 (C), 152.3 (C), 149.3 (C), 140. 73 (C), 135.9 (C), 128.2 (CH), 123.3 (CH), 118.3 (CH), 116.9 (CH) 107.6 (CH), 94.0 (CH_2), 81.1 (C), 71.6 (CH_2), 67.8 (CH_2) 59.0 (CH_3), 28.3 (CH_3). Calculated for $C_{22}H_{26}N_2O_5S$: C 61.38%; H 6.09%; N 6.515; S 7.45%; found: C 61.05%; H 6.22%; N 6.54%; S 7.17%. Melting point: 127–129°C (analytical sample was recrystallized from MeOH).

N-Boc-2-[4′-(2-fluoroethyl)aminophenyl]-6-(methoxyethoxymethoxy)benzothiazole (**5**): NaH (27 mg, 1.28 mmol) was dissolved in dry DMF (3 ml) with external ice/salt cooling. Boc-amine **4** (243 mg, 0.56 mmol) and fluoroethyl tosylate (247 mg, 1.13 mmol) were added and the reaction mixture was stirred at 60°C for 3 h. It was cooled to rt, diluted with MTBE, quenched with water and extracted with MTBE. The combined organic layers were washed with water, dried over Na_2SO_4 and evaporated to give the crude product, which was purified by flash column chromatography (SiO_2; LP:Et_2O = 1:1) to give 247 mg (92%) of the pure product as yellow solid.

^{1}H-NMR (200 MHz, $CDCl_3$) δ = 8.01 (d, J = 8.6 Hz, 2H), 7.94 (d, J = 9.0 Hz, 1H), 7.60 (d, J = 2.4 Hz, 1H), 7.36 (d, J = 8.6 Hz, 2H), 7.19 (dd, J = 2.5 Hz, J = 9.0 Hz, 1H), 5.34 (s, 2H), 4.62 (dt, J = 47.4 Hz, J = 4.9 Hz, 2H), 4.01 (t, J = 4.99 Hz, 1H), 3.91–3.85 (m, 3H), 3.60–3.56 (m, 2H), 3.38 (s, 3H), 1.45 (s, 9H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 165.5 (C), 155.3 (C), 154.1 (C), 149.4 (C), 144.8, 136.2 (C), 131.3 (C), 127.7 (CH), 127.3 (CH), 123.6 (CH), (CH), 117.1 (CH), 107.6 (CH), 94.0 (CH_2), 81.6 (d, J = 169.8 Hz, CH_2), 81.2 (C), 76.4 (CH_2), 71.6 (CH_2), 59.0 (CH_3), 50.6 (d, J = 20.6 Hz, CH_2), 28.3 (CH_3). Calculated for $C_{24}H_{29}FN_2O_5S$: C 60.49%, H 6.13%, N 5.88%, S 6.73%; found: C 6.34%, H 6.28%, N 5.91%, S 6.55%. Melting point: 70–72°C (analytical sample was recrystallized from Et_2O).

2-[4′-(2-Fluoroethyl)aminophenyl]-6-(methoxyethoxymethoxy)-benzothiazole (**6**): TFA (2.5 ml) was added to a solution of **5** (150 mg, 0.32 mmol) in DCM (47.5 ml). The solution was stirred at rt for 1 h. The solvent was evaporated. The residue was dissolved in DCM, washed with water and 2N NaOH, dried over Na_2SO_4 and evaporated. The crude product was purified by flash column chromatography (SiO_2; LP:MTBE = 3:1) to give 73 mg (62%) of the pure product as yellow solid.

^{1}H-NMR (200 MHz, MeOD) δ = 7.81 (d, J = 2.7 Hz, 1H), 7.76 (d, J = 2.9 Hz, 2H), 7.60 (d, J = 2.4 Hz, 1H), 7.17 (dd, J = 2.5 Hz, J = 8.8 Hz), 6.73 (d, J = 8.8 Hz, 2H), 5.32 (s, 2H), 4.59 (dt, J = 47.6 Hz, J = 5.0 Hz, 2H), 3.86–3.82 (m, 2H), 3.60–3.53 (m, 3H), 3.43 (t, J = 5.1 Hz, 1H), 3.33 (s, 3H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 156.4 (C), 152.3 (C), 150.3 (C), 136.4 (C), 129.8 (CH), 123.1 (CH), 122.7 (C), 118.0 (CH), 113.3 (CH), 109.1 (CH), 95.2 (CH_2), 83.4 (d, J = 167.6 Hz, CH_2), 72.8 (CH_2), 68.9 (CH), 59.1 (CH_3), 44.5 (N–CH_2–CH_2–F, d, J = 21.2). Calculated for $C_{19}H_{21}FN_2O_3S$: C 60.62%, H 5.62%, N 7.44%, S 8.52%, F 5.05%; found: C 60.53%, H 5.65%, N 7.45%, S 8.34%, F 5.31%. Melting point: 84–85°C (analytical sample was recrystallized from MeOH).

2-[4′-(2-Fluoroethyl)aminophenyl]-6-hydroxybenzothiazole (**7**): TFA (5 ml) was added to a solution of **5** (851 mg, 1.79 mmol) in DCM (30 ml) and the solution was stirred at reflux for 1 h. The reaction mixture was allowed to cool to rt and a saturated solution of $NaHCO_3$ was added until the aqueous layer reached a pH of 8. The product was extracted with MTBE. The combined organic layers were dried over Na_2SO_4 and evaporated. The crude product was purified by flash column chromatography (SiO_2; LP:MTBE = 1:1) to give 287 mg (56%) of the pure product as yellow solid.

^{1}H-NMR (500 MHz, CD_3CN) δ = 7.80 (d, J = 8.8 Hz), 7.71 (d, J = 8.8 Hz, 1H), 7.32 (d, J = 2.1 Hz, 1H), 6.94 (dd, J = 2.5 Hz, J = 8.5 Hz, 1H), 6.72 (d, J = 8.8 Hz, 2H), 4.58 (dt, d, J = 47.4 Hz, J = 5.0 Hz, 2H), 3.47 (dt, d, J = 26.9 Hz, J = 5.0 Hz, 2H). ^{13}C-NMR (125 MHz, CD_3CN) δ = 166.3 (C-2), 155.5 (C-6), 151.7 (C-4′), 149.2 (C-3a), 136.7 (C-7a), 129.4 (C-2′, C-6′), 123.6 (C-4), 123.2 (C-1′), 116.3 (C-5), 113.3 (C-3′, C-5′), 107.7 (C-7), 83.6 (d, J = 165.4 Hz, CH_2F), 44.2 (d, J = 19.4 Hz, CH_2N). HRMS (EI) *m/z* calculated for $C_{15}H_{13}FN_2OS$ 288.0733; found: $[M]^+$ 288.0736. Melting point: 194–196°C (analytical sample was recrystallized from LP/MTBE).

N-Boc-2-[4′-(fluoromethylamino)phenyl]-6-(methoxyethoxymethoxy)benzothiazole (**8**): NaH (19 mg, 0.79 mmol) was dissolved in dry DMF (2 ml) with external ice/salt cooling. Boc-amine **4** (171 mg, 0.40 mmol) and fluoromethyl tosylate (16.2 mg, 0.79 mmol) were added and the reaction mixture was stirred at 60°C for 3 h. It was cooled to rt, diluted with MTBE, quenched with water and extracted with MTBE. The combined organic layers were washed with water, dried over Na_2SO_4 and evaporated to give the crude product, which was purified by flash column chromatography (SiO_2; LP:Et_2O = 1:1) to give 38 mg (21%) of the pure target compound as yellow oil.

^{1}H-NMR (200 MHz, $CDCl_3$) δ = 8.03 (d, J = 8.5 Hz, 2H), 7.95 (d, J = 9.0 Hz, 1H), 7.60 (d, J = 2.4 Hz, 1H), 7.42 (d, J = 8.6 Hz, 2H), 7.19 (dd, J = 2.5 Hz, J = 8.9 Hz), 5.68 (d, J = 54.8 Hz, 2H), 5.34 (s, 2H), 3.89–3.85 (m, 2H), 3.60–3.55 (m, 2H), 3.38 (s, 3H), 1.49 (s, 9H). ^{13}C-NMR (50 MHz, $CDCl_3$) δ = 165.2 (C), 155.3 (C), 153.3 (C), 149.4 (C), 143.3/143.2 (C), 136.3 (C), 132.1 (C), 127.7 (CH), 126.63/126.60 (CH), 123.7 (C), 117.1 (C), 107.6 (C), 93.9 (CH_2), 88.5 (d, J = 199.1 Hz, CH_2), 82.6 (C), 71.6 (CH_2), 67.8 (CH_2), 59.0 (CH_3), 28.1 (CH_3).

N-Boc-2-[4′-(hydroxymethylamino)phenyl]-6-(methoxyethoxymethoxy)benzothiazole (**9**): *Obtained as a degradation product from* **8**: ^{1}H-NMR (500 MHz, DMSO) $\delta = 8.01$ (d, $J = 8.5$ Hz, 2H), 7.96 (d, $J = 9.1$ Hz, 1H), 7.80 (d, $J = 2.2$ Hz, 1H), 7.49 (d, $J = 8.5$ Hz, 2H), 7.22 (dd, $J = 2.4$ Hz, $J = 9.0$ Hz, 1H), 6.21 (t, $J = 7.0$ Hz, 1H), 5.35 (s, 2H), 5.00 (d, $J = 7.0$ Hz, 2H), 3.77–3.75 (m, 2H), 3.49–3.47 (m, 2H), 3.22 (s, 3H), 1.43 (s, 9H). ^{13}C-NMR (125 MHz, DMSO) $\delta = 164.8$ (C-2), 154.8 (C-6), 152.6 (COON), 148.8 (C-3a) 144.5 (C-4′), 135.7 (C-7a), 130.0 (C-1′), 127.1 (C-2′, C-4′), 126.5 (C-3′, C-5′), 123.4 (C-4), 117.2 (C-5), 108.2 (C-7), 93.4 (O–CH_2–O), 80.4 (t-Bu), 72.5 (N–CH_2–O), 71.0 (O–CH_2–CH_2–O), 67.5 (O–CH_2–CH_2–O), 58.0 (OCH_3), 27.9 (t-Bu-CH_3).

N-Boc-2-[4′-(2-tosylethyl)aminophenyl]-6-(methoxyethoxymethoxy)benzothiazole (**11**): NaH (7 mg, 0.29 mmol) was dissolved in dry DMF (0.8 ml) and Boc-amine **4** (124 mg, 0.26 mmol) was added at 0°C. This solution was added dropwise to a solution of ethylene bis-tosylate (213 mg, 58 mmol) in dry DMF (0.8 ml) at 40°C. Upon complete addition, the reaction mixture was stirred at 40°C for another 10 min before it was cooled to rt, diluted with MTBE (3 ml), quenched with water (5 ml) and finally extracted with MTBE. The combined organic layers were washed with water and brine, dried over Na_2SO_4 and evaporated. The crude product was purified by flash column chromatography (SiO_2; LP:MTBE = 1:1) to give 64 mg (35%) of pure target compound as white solid.

^{1}H-NMR (500 MHz, $CDCl_3$) $\delta = 7.96$–7.94 (m, 3H), 7.72 (d, $J = 8.2$ Hz, 2H), 7.62 (d, $J = 2.1$ Hz, 1H), 7.30 (d, $J = 8.2$ Hz, 2H), 7.23 (d, $J = 8.5$ Hz, 2H), 7.20 (dd, $J = 2.2$ Hz, $J = 8.8$ Hz, 1H), 5.35 (s, 2H), 4.24 (t, $J = 5.4$ Hz, 2H), 3.91 (t, $J = 5.4$ Hz, 2H), 3.89–3.87 (m, 2H), 3.60–3.58 (m, 2H), 3.39 (s, 3H), 1.41 (s, 9H). ^{13}C-NMR (125 MHz, $CDCl_3$) $\delta = 165.3$ (C), 155.3 (C), 153.9 (C), 144.9 (C), 144.3 (C), 136.2 (C), 131.4 (C), 129.9 (CH), 127.9 (CH), 127.7 (CH), 127.1 (CH), 123.7 (CH), 117.1 (CH), 107.6 (CH), 94.0 (CH_2), 81.4 (C), 71.6 (CH_2), 67.8 (CH_2), 67.3 (CH_2), 59.0 (CH_3), 49.1 (CH_2) 28.2 (CH_3), 21.6 (CH_3) HRMS (ESI) *m/z* calculated for $C_{31}H_{37}N_2O_8S_2$ 629.1991; found 629.2003 ($[M+H]^+$).

2-[4′-(2-Oxo-3-oxazolidinyl)phenyl]-6-(methoxyethoxymethoxy)-benzothiazole (**12**): *Isolated as a by-product in the synthesis of* **11**: ^{1}H-NMR (500 MHz, $CDCl_3$) $\delta = 8.04$ (d, $J = 8.7$ Hz, 2H), 7.96 (d, $J = 8.8$ Hz, 1H), 7.66 (d, $J = 8.8$ Hz, 2H), 7.57 (d, $J = 1.9$ Hz, 1H), 7.19 (dd, $J = 2.4$ Hz, $J = 9.0$ Hz), 5.33 (s, 2H), 4.51 (t, $J = 7.7$ Hz, 2H), 4.09 (t, $J = 8.0$ Hz, 2H), 3.87–3.86 (m, 2H), 3.59–3.57 (m, 2H), 3.38 (s, 3H). ^{13}C-NMR (125 MHz, $CDCl_3$) $\delta = 165.8$ (C-2), 155.4 (C-6), 154.8 (COON), 148.3 (C-3a), 140.5 (C-1′, C-4′), 135.6 (C-7a), 128.2 (C-2′, C-6′), 123.2 (C-4), 118.0 (C-3′, C-5′), 117.3 (C-5), 107.5 (C-7), 93.9 (O–CH_2–O), 71.5 (O–CH_2–CH_2–O), 67.8 (O–CH_2–CH_2–O), 61.3 (O–CH_2), 59.0 (OCH_3), 44.9 (N–CH_2).

Vinyl 4-methylbenzenesulphonate (**13**). *Isolated as a by-product in the synthesis of* **11**: ^{1}H-NMR (200 MHz, $CDCl_3$) $\delta = 7.79$ (d, $J = 8.2$ Hz, 2H), 7.36 (d, $J = 7.5$ Hz, 2H), 6.60 (dd, $J = 5.8$ Hz, $J = 13.5$ Hz, 1H), 4.89 (dd, $J = 2.4$ Hz, $J = 13.5$ Hz, 1H), 4.68 (dd, $J = 2.4$ Hz, $J = 5.8$ Hz), 2.45 (s, 1H). ^{13}C-NMR (50 MHz, $CDCl_3$) $\delta = 145.3$ (C), 141.6 (CH), 132.5 (C), 129.9 (CH), 128.0 (CH), 102.7 (CH_2), 21.7 (CH_3).

Radiochemistry

*[^{18}F]N-Boc-2-[4′-(2-fluoroethyl)aminophenyl]-6-(methoxyethoxymethoxy)benzothiazole ([^{18}F]***5***)*: [^{18}F]Fluoride was separated from the proton-irradiated H_2 [^{18}O]-enriched water on an anion exchange cartridge (SEP-PAK light, Waters Accell Plus QMA, USA). Elution of the activity was achieved with 0.5 ml of tetra-*n*-butyl-ammonium hydrogen carbonate (38 μmol, pH ~ 8) in water. Azeotropic drying was repeatedly performed with acetonitrile in a glassy carbon (Sigradur®G) reaction vessel. The precursor **11** (5 mg, 16 μmol) was dissolved in dry acetonitrile (1 ml), added to the dry n.c.a. [^{18}F]TBAF and the mixture was heated to 90°C for 5 min to achieve nucleophilic substitution of the tosyl group.

A sample of the reaction mixture was analysed by analytical HPLC to determine percent incorporation into [^{18}F]**5**. Retention time (reaction intermediate): 23.9 min. Column: Hamilton PRP-1, 10 μm 290 × 4 mm analytical column (solvent A: 0.2% triethylammonium phosphate buffer pH 7.2, solvent B: acetonitrile; gradient: 1–10 min 50% B, 10–24 min 50–100% B, 24–30 min 100% B; flow 1 ml/min).

*[^{18}F]2-[4′-(2-fluoroethyl)aminophenyl]-6-hydroxybenzothiazole ([^{18}F]FBTA = [^{18}F]***7***)*: After the nucleophilic substitution, the reaction mixture was evaporated to dryness by an inert gas stream for 5 min, maintaining the temperature at 60°C. After cooling to about 45°C a solution of MeOH/HCl (1 ml, 2/1 MeOH/concentrated HCl) was added and the mixture was heated for 5 min at 90°C. The reaction mixture was cooled down to 40°C, neutralized by adding 2 ml of NH_4OH (water/concentrated NH_4OH = 1/1) and diluted with 2 ml of acetonitrile. The product was purified by semipreparative HPLC using a Hamilton PRP-1 10 μm 250 × 8 mm semipreparative column eluted with 60% acetonitrile/40% 0.2% triethylammonium phosphate buffer pH 7.2 (flow 3 ml/min). The [^{18}F]FBTA peak eluted at about 10 min. The fraction containing [^{18}F]FBTA was diluted with 80 ml of water and adsorbed on a Waters C18 SepPak Plus cartridge. The C18 SepPak was washed with 10 ml of water and the product was eluted with 1 ml of anhydrous ethanol into a sterile vial followed by 9 ml of saline. Radiochemical and chemical purity were >98% as determined by analytical HPLC. Retention time ([^{18}F]FBTA): 9.9 min Column: Hamilton PRP-1, 10 μm 290 × 4 mm analytical column (solvent A: 0.2% triethylammonium phosphate buffer pH 7.2, solvent B: acetonitrile; gradient: 1–10 min 50% B, 10–24 min 50–75% B, 24–30 min 75% B; flow 1 ml/min). The radiochemical yield averaged 21% ± 5 @ EOS based on [^{18}F]fluoride, and the specific activity averaged 2.5 mCi/nmol (93 GBq/μmol) @ EOS.

MicroPET imaging

MicroPET imaging was performed on three female wt mice C56 black six (age: 6–8 weeks; weight: 21.5 ± 2.7 g) using a microPET Focus220 scanner (Siemens, Medical Solutions). The animals were kept under isoflurane anaesthesia (1.5%) and positioned on a temperature-regulated animal bed (37°C) in the microPET scanner. [^{18}F]FBTA was administered as an intravenous bolus injection (4–10 MBq in a volume of 100 μl of physiological saline/ethanol, ethanol content ⩽10% (v/v)) via the tail vein. At the start of radiotracer injection, dynamic microPET imaging was initiated. List mode data were acquired for 60 min with an energy window of 250–750 keV and 6 ns timing window. The dynamic microPET image data were sorted into three-dimensional sinograms as follows: six frames of 20 s, five frames of 60 s, four frames of 120 s, three frames of 300 s and three frames of 600 s. Transmission scans using a Co-57 point source were performed on each mouse for 10 min. Dynamic images were reconstructed by Fourier rebinning of the

three-dimensional sinograms followed by two-dimensional FBP (filtered backprojection) with a ramp filter. Normalization, decay- and attenuation correction was applied to the data. MicroPET data were analysed using a dedicated software package (PMOD, version 2.7.5, PMOD group, Switzerland). Frontal cortex and cerebellum were manually outlined as volumes of interest (VOIs) on multiple planes of the PET summation images (0–60 min) by using anatomical landmarks from a standard anatomical mouse atlas.[41] VOIs were transferred to the individual PET time frames and time–activity curves (TACs), expressed in units of kBq/g, were calculated for the individual VOIs. Radioactivity concentrations in individual mice were normalized to the injected radiotracer amount and corrected for individual body weights (%ID kg/g). The study was approved by the local Animal Welfare Committee and all study procedures were performed in accordance with the Austrian Animal Experiments Act.

In vitro Autoradiography

Binding of [^{18}F]FBTA to Aβ plaques was studied by *in vitro* autoradiography of human AD brain slices (16 µm) following procedures described in the literature.[39,40] Freshly frozen human AD brain slices were incubated for 1 h in a ~1 nM solution of [^{18}F]FBTA. Afterwards, the slices were washed, dried, exposed to a multisensitive phosphor screen (type: MS, Perkin Elmer Life Sciences) and measured using a Cyclone™ Storage Phosphor Scanner Model B431201 (Packard, USA) equipped with Opti-Quant 3.0 software.

Binding Affinity

Preparation of Aβ_{1-40} and Aβ_{1-42} fibrils: human Aβ_{1-40} and Aβ_{1-42} peptides (Bachem) were incubated at 0.5 mg/ml in a solution of 10 mM Na_2HPO_4, 1 mM EDTA (pH 7.4) at 37°C for 48 h. The formation of fibrils was confirmed by [^{3}H]6-OH-BTA-1 binding. Fibrils were either used immediately or aliquoted and stored at −80°C until use.

Solutions of FBTA (97% purity according to analytical HPLC) or 6-OH-BTA-1 (ABX Biochemicals, Radeberg, Germany) and *N*-[^{3}H-methyl]6-OH-BTA-1 were prepared as 1–10 mM dimethyl sulfphoxide (DMSO) stocks before dilution into assay buffer. The maximum final concentration of DMSO in the assays was 1%. All assays were performed in 10 mM Na_2HPO_4. The incubation was performed at 25°C for 180 min. The bound and free fractions were separated by vacuum filtration through GF/B glass filters (Whatman, Maidstone, UK) using a PerkinElmer harvester (PerkinElmer, 96 Micro B Filtermat) followed by 6 × 0.2 ml washes with ice cold phosphate buffer. Filters containing the bound ligand were counted with a liquid scintillation counter (Wallac Trilux, 1450 Microbeta).

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