Supporting Information

18F-Barbiturates are PET Tracers with Diagnostic Potential in Alzheimer’s Disease

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I. Chemistry and radiochemistry

General procedures. All reactions, except hydrogenations and the synthesis of sodium barbituric salt, were carried out under nitrogen atmosphere in oven-dried glassware with dry solvents. Dry N,N'-dimethylformamide (DMF) over molecular sieves, dry 1,2-dimethoxyethane (DME) over molecular sieves, dry chloroform (CHCl₃) with amylenes as stabiliser and dry pyridine were purchased from Sigma Aldrich and used without further purification. Dry acetonitrile used for the synthesis of hot tracer was purchased from Merck. Reactions were monitored by thin-layer chromatography (TLC), unless otherwise noted. TLCs were performed on Merck silica gel glass plates (60 F254). Visualisation was accomplished by irradiation with a UV lamp and/or staining with a ceric ammonium molybdate or KMnO₄ solution. Flash chromatography was performed using Silica gel (60 Å, particle size 40-63 μm) purchased from Merck. 1H NMR and 13C NMR spectra were recorded on a Varian VNMRS-400 spectrometer and calibrated using residual undeuterated solvent as internal reference. 19F NMR spectra were recorded on a Varian VNMRS-600 spectrometer and were referenced to CFCl₃. 13C NMR spectra were recorded with complete proton decoupling. Chemical shifts (δ) are reported in parts per million (ppm) and coupling constants (J) are given in Hertz (Hz). The following abbreviations are used for spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet-doublet, dt = doublet-triplet, m = multiplet, br = broad. Melting points (m.p.) are uncorrected, and recorded using a Griffin melting point apparatus. Mass Analyses (MS) were performed using Agilent 1200 HPLC system coupled to Agilent G6120 single
quadrupole detector equipped with an electrospray ionization (ESI) source in direct infusion modality. ESI-MS spectra were recorded in positive mode, unless otherwise noted. RP (reverse phase)-HPLC-MS analyses were performed with an Agilent 1200 HPLC system equipped with a DAD and an ESI-MS detector. The radiotracer purification was performed using a Gynkotek HPLC system consisting of a gradient pump (P580), column oven (STHS8S) and variable UV detector (UV340S) coupled in series with a BIOSCAN NaI detector (B-FC-3200). The purified hot tracer and the cold reference were analysed using a Shimadzu Prominence HPLC system equipped with a PDA UV detector and HERM LB500 activity detector. The dose calibrators used to measure doses were CAPINTEC CRC 15R and CAPINTEC CRC 15PET. The radioactivity of organic and aqueous phases for the determination of logP was measured using a 2 channel scintillation Detector Interface (Oakfield Instruments Ltd).

Chemistry

![Chemical Structure](image)

Diethyl 2-(6-(benzylxoy)hexyl)-2-ethylmalonate (3a). To a solution of diethyl 2-ethylmalonate (2a) (99%, d = 1.00, 1.9 mL, 10.0 mmol, 1 eq) in dry DMF (15 mL), sodium hydride (60% dispersion in mineral oil, 490 mg, 12.3 mmol, 1.2 eq) was added at 0°C. The suspension was stirred under nitrogen atmosphere at 0°C for 1.5 hours. Then ((6-bromohexyloxy)methyl)benzene (97%, d = 1.21, 2.9 mL, 12.5 mmol, 1.2 eq) was added. The solution was stirred at room temperature under nitrogen atmosphere for 3.5 hours. The reaction mixture was quenched with a saturated aqueous NH₄Cl solution and extracted with small portions of diethyl ether. The collected organic phases were dried over anhydrous Na₂SO₄, filtered and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel (CH₂Cl₂) to give 3.444 g of 3a (91%) as a yellow oil; Rₚ=0.34 (CH₂Cl₂). ¹H NMR (400 MHz, CDCl₃) δ 7.34 – 7.21 (m, 5H), 4.46 (s, 2H), 4.14 (q, J = 7.1 Hz, 4H), 3.41 (t, J = 6.6 Hz, 2H), 1.93 – 1.78 (m, 4H), 1.60 – 1.52 (m, 2H), 1.38 – 1.25 (m, 4H), 1.20 (t, J = 7.1 Hz, 6H), 1.16 – 1.06 (m, 2H), 0.77 (t, J = 7.6 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 172.2, 139.0, 128.7, 127.9, 127.8, 73.2, 70.7, 61.3, 58.3, 31.9, 30.0, 30.1, 26.3, 25.5, 24.2, 14.5, 8.8; ESI-MS m/z: calcd. for C₂₂H₃₅O₅ [M+H]⁺ 379.2, C₂₂H₃₄NaO₅ [M+Na]⁺ 401.2, found: 379.2, 401.2.
**Diethyl 2-(6-(benzyloxy)hexyl)-2-phenylmalonate (3b).** To a solution of diethyl 2-phenylmalonate (2b) (98%, d = 1.09, 1.9 mL, 8.6 mmol, 1 eq) in dry DMF (15 mL), sodium hydride (60% dispersion in mineral oil, 420 mg, 10.5 mmol, 1.2 eq) was added at 0°C. The suspension was stirred under nitrogen atmosphere at 0°C for 1 hour. Then ((6-bromohexyloxy)methyl)benzene (97%, d = 1.21, 2.5 mL, 10.8 mmol, 1.2 eq) was added. The solution was stirred at room temperature under nitrogen atmosphere for 48 hours. The reaction mixture was quenched with a saturated aqueous NH₄Cl solution and extracted with small portions of diethyl ether. The collected organic phases were dried over anhydrous Na₂SO₄, filtered and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel (n-Hex/EtOAc 9:1) to give 1.471 g of 3b (40%) as a colourless oil; Rf=0.30 (n-Hex/EtOAc 9:1). ¹H NMR (400 MHz, CDCl₃) δ 7.50 – 7.02 (m, 10H), 4.40 (s, 2H), 4.20 – 4.06 (m, 4H), 3.35 (t, J = 6.6 Hz, 2H), 2.26 – 2.16 (m, 2H), 1.54 – 1.45 (m, 2H), 1.33 – 1.22 (m, 4H), 1.22 – 1.07 (m, 8H); ¹³C NMR (100 MHz, CDCl₃) δ 171.0, 138.9, 137.4, 128.6, 128.3, 127.8, 127.7, 127.6, 73.1, 70.6, 62.8, 61.6, 35.9, 30.0, 29.9, 26.1, 24.8, 14.2; ESI MS m/z: calcd. for C₂₆H₃₅O₅ [M+H]⁺ 427.2, C₂₆H₃₄NaO₅[M+Na]⁺ 449.2, found: 427.3, 449.3.

**Diethyl 2-benzyl-2-(6-(benzyloxy)hexyl)malonate (3c).** To a solution of diethyl 2-benzylmalonate (2c) (97%, d = 1.06, 1.9 mL, 7.8 mmol, 1 eq) in dry DMF (15 mL), sodium hydride (60% dispersion in mineral oil, 387 mg, 9.7 mmol, 1.2 eq) was added at 0°C. The suspension was stirred under nitrogen atmosphere at 0°C for 45 minutes. Then ((6-bromohexyloxy)methyl)benzene (97%, d = 1.21, 2.0 mL, 8.6 mmol, 1.1 eq) was added. The solution was stirred at room temperature under nitrogen atmosphere for 24 hours. The reaction mixture was quenched with a saturated aqueous NH₄Cl solution and extracted with small portions of diethyl ether. The collected organic phases were dried over anhydrous Na₂SO₄, filtered and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel (n-Hex/EtOAc 9:1) to give 3.165 g of 3c (92%) as a pale yellow oil; Rf=0.31 (n-Hex/EtOAc 9:1). ¹H NMR
(400 MHz, CDCl$_3$) $\delta$ 7.30 – 7.08 (m, 8H), 7.01 – 6.96 (m, 2H), 4.42 (s, 2H), 4.14 – 4.04 (m, 4H), 3.37 (t, $J$ = 6.6 Hz, 2H), 3.15 (s, 2H), 1.73 – 1.64 (m, 2H), 1.55 – 1.48 (m, 2H), 1.34 – 1.19 (m, 6H), 1.15 (t, $J$ = 7.1 Hz, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 171.6, 138.9, 136.6, 130.1, 128.6, 128.5, 127.9, 127.8, 127.1, 73.2, 70.6, 61.4, 59.1, 38.3, 32.0, 30.0, 29.9, 26.3, 24.4, 14.4 (s); ESI MS $m/z$: calcd. for C$_{27}$H$_{37}$O$_5$ [M+H]$^+$ 441.3, C$_{27}$H$_{36}$NaO$_5$ [M+Na]$^+$ 463.2, found: 441.2, 463.2.

5-(6-(Benzyloxy)hexyl)-5-ethylpyrimidine-2,4,6(1H,3H,5H)-trione (4a). To a suspension of urea (99%, 2.481 g, 40.9 mmol, 10 eq) in dry DMF (12.5 mL), sodium hydride (60% dispersion in mineral oil, 665 mg, 16.6 mmol, 4 eq) was added at 0°C. The mixture was stirred under nitrogen atmosphere at 0°C for 1 hour. Then a solution of 3a (1.567 g, 4.1 mmol, 1 eq) in dry DMF (2 mL) was added. The reaction mixture was stirred under nitrogen atmosphere at room temperature for 2 hours. The reaction was quenched with a cold saturated aqueous NH$_4$Cl solution. The aqueous layer was extracted with small portions of diethyl ether. The collected organic phases were dried over anhydrous Na$_2$SO$_4$, filtered and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel (n-Hex/EtOAc 1:1) to give 1.240 g of 4a (86%) as a white solid; $R_f$:0.75 (n-Hex/EtOAc 1:1). M.p. 65-67°C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 9.76 (s, 2H), 7.39–7.19 (m, 5H), 4.50 (s, 2H), 3.43 (t, $J$ = 6.6 Hz, 2H), 2.11–1.91 (m, 4H), 1.64–1.51 (m, 2H), 1.38–1.14 (m, 6H), 0.86 (t, $J$ = 7.4 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 173.2, 149.6, 138.8, 128.7, 128.0, 127.8, 73.1, 70.5, 57.7, 39.0, 32.8, 29.8, 29.6, 26.1, 25.4, 9.8; ESI MS $m/z$: calcd. for C$_{19}$H$_{27}$N$_2$O$_4$ [M+H]$^+$ 347.2, C$_{19}$H$_{26}$N$_2$NaO$_4$[M+Na]$^+$ 369.2, found: 347.2, 369.1.

5-(6-(Benzyloxy)hexyl)-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione (4b). To a suspension of urea (99%, 217 mg, 3.6 mmol, 10 eq) in dry DMF (1 mL), sodium hydride (60% dispersion in mineral oil, 98 mg, 2.4 mmol, 7 eq) was added at 0°C. The mixture was stirred under nitrogen atmosphere at 0°C for 2 hours. Then a solution of 3b (148 mg, 0.35 mmol, 1 eq) in dry DMF (0.5 mL) was added. The reaction
mixture was stirred under nitrogen atmosphere at room temperature for 2.5 hours. The reaction was quenched with a saturated aqueous NH₄Cl solution. The aqueous layer was extracted with small portions of diethyl ether. The collected organic phases were dried over anhydrous Na₂SO₄, filtered and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel (n-Hex/EtOAc 7:3) to give 48 mg of 4b (35%) as a white solid; Rᵣ=0.36 (n-Hex/EtOAc 7:3). M.p. 103-105°C; ¹H NMR (400 MHz, CDCl₃) δ 8.98 (s, 2H), 7.63 – 6.92 (m, 10H), 4.50 (s, 2H), 3.45 (t, J = 6.6 Hz, 2H), 2.48 – 2.33 (m, 2H), 1.67 – 1.54 (m, 2H), 1.42 – 1.25 (m, 6H); ¹³C NMR (100 MHz, CDCl₃) δ 171.3, 149.5, 138.8, 137.4, 129.5, 129.0, 128.6, 128.0, 127.8, 126.5, 73.1, 70.5, 61.0, 36.8, 29.8, 29.7, 26.0, 25.9; ESI MS m/z: calcd. for C₂₃H₂₇N₂O₄ [M+H]+ 395.2, C₂₃H₂₆N₂NaO₄ [M+Na]+ 417.2, found: 395.2, 417.2.

5-Benzyl-5-(6-(benzyloxy)hexyl)pyrimidine-2,4,6(1H,3H,5H)-trione (4c). To a suspension of urea (99%, 1.106 g, 18.2 mmol, 10 eq) in dry DMF (5 mL), sodium hydride (60% dispersion in mineral oil, 289 mg, 7.2 mmol, 4 eq) was added at 0°C. The mixture was stirred under nitrogen atmosphere at 0°C for 1 hour. Then a solution of 3c (803 mg, 1.8 mmol, 1 eq) in dry DMF (3 mL) was added. The reaction mixture was stirred under nitrogen atmosphere at room temperature for 5 hours. The reaction was quenched with a saturated aqueous NH₄Cl solution. The aqueous layer was extracted with small portions of diethyl ether. The collected organic phases were dried over anhydrous Na₂SO₄, filtered and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel (n-Hex/EtOAc 7:3) to give 589 mg of 4c (80%) as a white solid; Rᵣ=0.35 (n-Hex/EtOAc 7:3). M.p. 140-141°C; ¹H NMR (400 MHz, CDCl₃) δ 8.33 (br s, 2H), 7.34 – 6.97 (m, 10H), 4.42 (s, 2H), 3.37 (t, J = 6.5 Hz, 2H), 3.18 (s, 2H), 2.10 – 2.02 (m, 2H), 1.54 – 1.46 (m, 2H), 1.32 – 1.20 (m, 4H), 1.18 – 1.08 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 172.4, 148.4, 138.9, 134.8, 129.7, 129.1, 128.7, 128.2, 128.0, 127.9, 73.2, 70.5, 59.1, 45.3, 39.2, 29.8, 29.6, 26.1, 25.4; ESI MS m/z: calcd. for C₂₄H₂₉N₂O₄ [M+H]+ 409.2, C₂₄H₂₈N₂NaO₄ [M+Na]+ 431.2, found: 409.2, 431.2.
5-Ethyl-5-(6-hydroxyhexyl)pyrimidine-2,4,6(1H,3H,5H)-trione (5a). A mixture of 4a (1.611 g, 4.6 mmol) and 20% Pd(OH)$_2$/C (0.393 g) in MeOH (40 mL) was stirred at room temperature under H$_2$ atmosphere. After 17 hours the mixture was filtered through Celite and the bed was washed with MeOH. The solvent was removed in vacuo to give 1.183 g of 5a (>98%) as a white solid; $R_f=0.30$ (CH$_2$Cl$_2$/EtOAc 1:1). The product was used without further purification. M.p. 90-92°C; $^1$H NMR (400 MHz, CDCl$_3$) δ 9.09 (s, 2H), 3.63 (t, $J=6.5$ Hz, 2H), 2.12 – 1.87 (m, 4H), 1.61 – 1.44 (m, 2H), 1.39 – 1.12 (m, 7H), 0.88 (t, $J=7.4$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 173.3, 149.6, 63.0, 57.7, 38.9, 32.9, 32.6, 29.4, 25.6, 25.4, 9.8; ESI MS m/z: calcd. for C$_{12}$H$_{21}$N$_2$O$_4$ [M+H]$^+$ 257.1, C$_{12}$H$_{20}$N$_2$NaO$_4$[M+Na]$^+$ 279.1, found: 257.1, 279.1.

5-(6-Hydroxyhexyl)-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione (5b). A mixture of 4b (58 mg, 0.15 mmol) and 20% wt of 20% Pd(OH)$_2$/C (12 mg) in MeOH (3.5 mL) was stirred at room temperature under H$_2$ atmosphere. After 15 hours the mixture was filtered through Celite with MeOH. The solvent was removed in vacuo and the residue was purified by flash chromatography on silica gel (n-Hex/EtOAc 3:7) to afford 42 mg of 5b as a white solid (93%). $R_f=0.45$ (n-Hex/EtOAc 3:7). M.p. 158-160°C; $^1$H NMR (400 MHz, CD$_3$OD) δ 7.46 – 7.24 (m, 5H), 3.54 (t, $J=6.6$ Hz, 2H), 2.42 – 2.31 (m, 2H), 1.57 – 1.47 (m, 2H), 1.42 – 1.28 (m, 6H), $^1$H NMR data are in agreement with those previously reported in literature;[1] $^{13}$C NMR (100 MHz, CD$_3$OD) δ 174.2, 152.1, 140.6, 130.9, 130.3, 128.1, 63.7, 62.3, 38.0, 34.3, 31.4, 27.4, 27.3; ESI MS m/z: calcd. for C$_{16}$H$_{21}$N$_2$O$_4$ [M+H]$^+$ 305.1, C$_{16}$H$_{20}$N$_2$NaO$_4$[M+Na]$^+$ 327.1, found: 305.2, 327.1.
5-Benzyl-5-(6-hydroxyhexyl)pyrimidine-2,4,6(1H,3H,5H)-trione (5c). A mixture of 4c (450 mg, 1.1 mmol) and 20% wt of 20% Pd(OH)₂/C (114 mg) in MeOH (20 mL) was stirred at room temperature under H₂ atmosphere. After 17 hours the mixture was filtered through Celite and the bed was washed with MeOH and then with EtOAc. The solvent was removed in vacuo and the residue was purified by flash chromatography on silica gel (gradient: from n-Hex/EtOAc 2:3 to n-Hex/EtOAc 3:7) to afford 163 mg of 5c as a white solid (46%). Rᵢ=0.55 (n-Hex/EtOAc 3:7). M.p. 162-164°C; ¹H NMR (400 MHz, CD₃OD) δ 7.31–6.80 (m, 5H), 3.43 (t, J = 6.6 Hz, 2H), 3.08 (s, 2H), 2.04–1.93 (m, 2H), 1.45–1.35 (m, 2H), 1.28–1.18 (m, 4H), 1.17–1.07 (m, 2H); ¹³C NMR (100 MHz, CD₃OD) δ 175.5, 151.7, 137.3, 131.4, 130.4, 129.4, 63.7, 60.2, 47.2, 40.4, 34.2, 31.3, 27.3, 27.0; ESI MS m/z: calcd. for C₁₇H₂₃N₂O₄ [M+H]⁺ 319.2, C₁₇H₂₂N₂NaO₄[M+Na]⁺ 341.1, found: 319.1, 341.1.

5-Ethyl-5-(6-fluorohexyl)pyrimidine-2,4,6(1H,3H,5H)-trione (1a). To a solution of 5a (250 mg, 1.0 mmol, 1 eq) in dry DME (8 mL) DAST (99%, 1.23, 260 µL, 2.0 mmol, 2 eq) was added at -78 °C. The reaction mixture was stirred at this temperature for 15 minutes under nitrogen atmosphere and then it was allowed to warm to room temperature. After 4.5 hours water was added and the mixture was extracted with diethyl ether. The combined organic extracts were dried over anhydrous Na₂SO₄, filtered and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel (gradient: from n-Hex/EtOAc 4:1 to n-Hex/EtOAc 3:1) to afford 181 mg of 1a as a white solid (72%); Rᵢ=0.14 (n-Hex/EtOAc 4:1). M.p. 131-132°C; analytical HPLC [(Phenomenex Luna C18, 5 μm, 100 Å, 250 x 4.6 (L x ID) inj. volume 20 µL, flow rate 1 mL/min, solvent A: H₂O+0.1%TFA, solvent B: CH₃CN+0.1%TFA, method: 45%B for 3 min, linear gradient to 100%B in 15 min], 6.8 min, > 99% λ = 214 nm, 254 nm, 220 nm; ¹H NMR (400 MHz, CDCl₃) δ 9.22 (s, 2H), 4.39 (dt, J = 47.3, 6.1 Hz, 2H), 4.39 (dt, J = 47.3, 6.1 Hz, 2H), 4.39 (dt, J = 47.3, 6.1 Hz, 2H), 4.39 (dt, J = 47.3, 6.1 Hz, 2H), 4.39 (dt, J = 47.3, 6.1 Hz, 2H), 4.39 (dt, J = 47.3, 6.1 Hz, 2H), 4.39 (dt, J = 47.3, 6.1 Hz, 2H), 4.39 (dt, J = 47.3, 6.1 Hz, 2H), 4.39 (dt, J = 47.3, 6.1 Hz, 2H).
5-(6-Fluorohexyl)-5-phenylpyrimidine-2,4,6(1H,3H,5H)-trione (1b). To a -78°C (dry ice-acetone bath) cooled solution of 5b (91 mg, 0.30 mmol, 1 eq) in dry DME (3 mL) DAST (99%, d = 1.23, 80 µL, 97 mg, 0.6 mmol, 2 eq) was added. The reaction mixture was stirred under nitrogen atmosphere at -78 °C for 10 minutes and then warmed to room temperature. The reaction was stirred at room temperature for another 2.5 hours, cooled again to -78°C and a second portion of DAST (99%, 40 µL, 49 mg, 0.30 mmol, 1 eq) was added. Reaction mixture was stirred at this temperature for 10 minutes and then it was warmed to room temperature and stirred for 1.5 hours. Water was added and the mixture was extracted with diethyl ether. The combined organic extracts were dried over anhydrous Na₂SO₄, filtered and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel (n-Hex/EtOAc 7:3) to afford 64 mg of 1b as a white solid (70%); Rf=0.32 (n-Hex/EtOAc 7:3). M.p. 135-137°C; analytical HPLC [(Phenomenex Luna C18, 5 μm, 100 Å, 250 x 4.6 (L x ID) inj. volume 20 μL, flow rate 1 mL/min, solvent A: H₂O+0.1%TFA, solvent B: CH₃CN+0.1%TFA, method: 45%B for 3 min, linear gradient to 100%B in 15 min], 9.4 min, >99% λ = 214 nm, 254 nm, 220 nm; ¹H NMR (400 MHz, CD₃OD) δ 7.45 – 7.31 (m, 5H), 4.42 (dt, J = 47.5, 6.1 Hz, 2H), 2.45 – 2.32 (m, 2H), 1.77 – 1.60 (m, 2H), 1.49 – 1.29 (m, 6H); ¹³C NMR (100 MHz, CD₃OD) δ 174.2, 152.1, 140.6, 130.9, 130.3, 128.1, 85.6 (d, J = 163.7 Hz), 62.3, 37.9, 32.2 (d, J = 19.5 Hz), 31.2, 27.3, 26.7 (d, J = 5.4 Hz); ¹⁹F NMR (564 MHz, CD₃OD) δ -219.77 – -220.08 (m); ESI MS m/z: calcd. for C₁₆H₂₀FN₂O₃ [M+H]⁺ 307.1, C₁₆H₁₉FN₂NaO₃ [M+Na]⁺ 329.1, found: 307.1, 329.1.
5-Benzyl-5-(6-fluorohexyl)pyrimidine-2,4,6(1H,3H,5H)-trione (1c). To a -78°C (dry ice-acetone bath) cooled solution of 5c (120 mg, 0.38 mmol, 1 eq) in dry DME (3 mL) DAST (99%, d = 1.23, 100 µL, 122 mg, 0.75 mmol, 2 eq) was added. The reaction mixture was stirred under nitrogen atmosphere at -78 °C for 10 minutes and then warmed to room temperature. The reaction was stirred at room temperature for another 2.5 hours, cooled again to -78°C and a second portion of DAST (99%, 50 µL, 61 mg, 0.38 mmol, 1 eq) was added. Reaction mixture was stirred at this temperature for 10 minutes and then it was warmed to room temperature and stirred for 1.5 hours. Water was added and the mixture was extracted with diethyl ether. The combined organic extracts were dried over anhydrous Na₂SO₄, filtered and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel (n-Hex/EtOAc 7:3) to afford 73 mg of 1c as a white solid (60%); Rᵢ=0.46 (n-Hex/EtOAc 7:3). M.p. 149-151°C; analytical HPLC [(Phenomenex Luna C18, 5 µm, 100 Å, 250 x 4.6 (L x ID) inj volume 20 µL, flow rate 1 mL/min, solvent A: H₂O+0.1%TFA, solvent B: CH₃CN+0.1%TFA, method: 45%B for 3 min, linear gradient to 100%B in 15 min], 9.9 min, > 99% λ = 214 nm, 254 nm, 220 nm; ¹H NMR (400 MHz, CDCl₃) δ 8.48 (s, 2H), 7.25 – 7.15 (m, 3H), 7.13 – 7.03 (m, 2H), 4.42 (dt, J = 47.3, 6.1 Hz, 2H), 3.27 (s, 2H), 2.23 – 2.08 (m, 2H), 1.73 – 1.57 (m, 2H), 1.46 – 1.30 (m, 4H), 1.29 – 1.18 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 172.5, 148.5, 134.7, 129.7, 129.1, 128.2, 84.2 (d, J = 164.5 Hz), 59.1, 45.4, 39.0, 30.5 (d, J = 19.6 Hz), 29.3, 25.4, 25.1 (d, J = 5.3 Hz); ¹⁹F NMR (564 MHz, CDCl₃) δ -218.05 – -218.56 (m); ESI MS m/z: calcd. for C₁₇H₂₂FN₂O₃ [M+H]⁺ 321.2, C₁₇H₂₁FN₂NaO₃ [M+Na]⁺ 343.3, found: 321.2, 343.1.

6-(5-Ethyl-2,4,6-trioxohexahydropyrimidin-5-yl)hexyl 4-methylbenzenesulfonate (6a). To a suspension of 5a (507 mg, 2.0 mmol, 1 eq) in dry CHCl₃ (15 mL) dry pyridine (d = 0.98, 320 µL, 4.0 mmol, 2 eq) was added. The mixture was cooled in an ice bath and p-toluenesulfonyl chloride (98%,
1.163 g, 6.0 mmol, 3 eq) was added. The reaction mixture was stirred at room temperature under nitrogen atmosphere for 4 days. Diethyl ether and water were added. The layers were separated and the aqueous phase was extracted with diethyl ether. The combined organic extracts were washed with 2 N HCl, 5% NaHCO₃, H₂O and then dried over anhydrous Na₂SO₄. The solvent was removed in vacuo and the residue was purified by flash chromatography on silica gel (n-Hex/EtOAc 3:2) to afford 691 mg of 6a as a white solid (85%). \( R_f = 0.28 \) (n-Hex/EtOAc 3:2); M.p. 89-90°C; analytical HPLC [(Phenomenex Luna C18, 5 μm, 100 Å, 250 x 4.6 (L x ID) inj volume 20 µL, flow rate 1 mL/min, solvent A: H₂O+0.1%TFA, solvent B: CH₃CN+0.1%TFA, method: 45%B for 3 min, linear gradient to 100%B in 15 min], 11.1 min, > 99% \( \lambda = 214 \) nm, 254 nm, 220 nm; \(^1\)H NMR (400 MHz, CDCl₃) \( \delta \) 8.57 (s, 2H), 7.77 (d, \( J = 8.3 \) Hz, 2H), 7.34 (d, \( J = 8.5 \) Hz, 2H), 3.98 (t, \( J = 6.4 \) Hz, 2H), 2.45 (s, 3H), 2.02 (q, \( J = 7.4 \) Hz, 2H), 1.97 – 1.88 (m, 2H), 1.64 – 1.54 (m, 2H), 1.31 – 1.11 (m, 6H), 0.88 (t, \( J = 7.4 \) Hz, 3H); \(^13\)C NMR (100 MHz, CDCl₃) \( \delta \) 173.2, 149.7, 145.0, 133.3, 130.1, 128.1, 70.6, 57.5, 38.5, 32.9, 29.0, 28.9, 25.2, 21.9, 9.7; ESI MS \( m/z \): calcd. for C₁₉H₂₇N₂O₆S⁺ \( [M+H]^+ \) 411.2, \( [M+Na]^+ \) C₁₉H₂₆N₂NaO₆S⁺ 433.1, found: 411.1, 433.1.

6-(2,4,6-Trioxo-5-phenylhexahydropyrimidin-5-yl)hexyl 4-methylbenzenesulfonate (6b). To a suspension of 5b (27 mg, 0.09 mmol, 1.0 eq) in dry CHCl₃ (1.5 mL) dry pyridine (\( d = 0.98 \), 18 µL, 0.22 mmol, 2.4 eq) was added. The mixture was cooled in an ice bath and \( p \)-toluenesulfonyl chloride (98%, 31 mg, 0.16 mmol, 1.8 eq) was added. This suspension was stirred at 0 °C under nitrogen atmosphere for 1 hour. Then the reaction mixture was refluxed at 60 °C. After 24 hours under stirring at 60 °C, another portion of \( p \)-toluenesulfonyl chloride (33 mg, 0.17 mmol, 1.9 eq) was added and the reaction mixture was stirred again for 24 hours at reflux. Then cooled to room temperature and diethyl ether and water were added. The layers were separated and the aqueous phase was extracted with diethyl ether. The combined organic extracts were dried over anhydrous Na₂SO₄. The solvent was removed in vacuo and the residue was purified by flash chromatography on silica gel (n-Hex/EtOAc 3:2) to afford 27 mg of 6b as a white solid (65%). \( R_f = 0.27 \) (n-Hex/EtOAc 3:2). M.p. 52-54°C; analytical HPLC [(Phenomenex Luna C18, 5 \( \mu m \), 100 Å, 250 x 4.6 (L x ID) inj volume 20 µL, flow rate 1 mL/min, solvent A: H₂O+0.1%TFA, solvent B: CH₃CN+0.1%TFA, method: 45%B for 3 min, linear gradient to 100%B in 15 min], 12.6 min, > 99% \( \lambda = 214 \) nm, 254 nm, 220 nm; \(^1\)H NMR (400 MHz,CDCl₃) \( \delta \) 8.85 (s, 2H), 7.85 – 7.70 (d, 2H), 7.45 – 7.27 (m, 7H), 3.99 (t, \( J = 6.4 \) Hz, 2H), 2.43 (s, 3H), 2.39 – 2.29 (m, 2H), 1.66 – 1.56 (m, 2H), 1.34 – 1.23 (m,
$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.33 (br s, 2H), 7.78 (d, $J$ = 8.3 Hz, 2H), 7.35 (d, $J$ = 8.0 Hz, 2H), 7.24 – 7.17 (m, 3H), 7.11 – 7.02 (m, 2H), 3.99 (t, $J$ = 6.4 Hz, 2H), 3.24 (s, 2H), 2.45 (s, 3H), 2.17 – 2.03 (m, 2H), 1.66 – 1.55 (m, 2H), 1.31 – 1.13 (m, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 172.3, 148.3, 145.0, 134.6, 133.4, 130.2, 129.7, 129.1, 128.2, 70.6, 59.0, 45.5, 38.9, 29.1, 29.0, 25.3, 22.0; ESI MS $m/z$: calcd. for C$_{24}$H$_{29}$N$_2$O$_6$S [M+H]$^+$ 473.2, C$_{24}$H$_{28}$N$_2$NaO$_6$S [M+Na]$^+$ 495.2, found: 473.2, 495.1.

5-(6-Azidohexyl)-5-ethylpyrimidine-2,4,6(1H,3H,5H)-trione (7). To a solution of 6a (206 mg, 0.5 mmol, 1 eq) in dry DMF sodium azide (67 mg, 1.0 mmol, 2 eq) was added. This suspension was stirred...
under nitrogen atmosphere at room temperature for 2 hours and then for other 2 hours at 50 °C. The resulting solution was cooled in an ice bath and cold water (12 mL) was added, and extracted with small portions of diethyl ether. The collected organic phases were dried over anhydrous Na₂SO₄, filtered and the solvent was removed in vacuo. The residue was purified by flash chromatography on silica gel (n-Hex/EtOAc 3:2) to afford 127 mg of 7 as a white solid (90%). \( R_f = 0.53 \) (n-Hex/EtOAc 3:2). M.p. 84-85°C; \(^1\)H NMR (400 MHz, CDCl₃) \( \delta \) 8.13 (s, 2H), 3.24 (t, \( J = 6.9 \) Hz, 2H), 2.09 – 1.94 (m, 4H), 1.59 – 1.50 (m, 2H), 1.38 – 1.17 (m, 6H), 0.89 (t, \( J = 7.5 \) Hz, 3H), \(^1\)C NMR (100 MHz, CDCl₃) \( \delta \) 173.2, 149.6, 57.7, 51.6, 38.7, 33.0, 29.2, 28.9, 26.6, 25.3, 9.7; ESI MS (+) \( m/z \): calcd. for C₁₂H₂₀N₃O₃ [M+H-N₂]+ 254.1, C₁₂H₁₉N₅NaO₃ [M+Na]+ 304.1, found: 254.2, 304.1; ESI MS (-) \( m/z \): calcd. for C₁₂H₁₈N₅O₃ [M-H]- 280.1, found: 280.1.

5-(6-Aminoethyl)-5-ethylpyrimidine-2,4,6(1\( H \),3\( H \),5\( H \))-trione hydrochloride (8). To a solution of 7 (126 mg, 0.45 mmol) in THF (1.5 mL) was added the catalyst 20% Pd(OH)₂/C (33 mg). Reaction mixture was stirred at room temperature under H₂ atmosphere. After 6 hours the precipitate was filtered on buckner funnel with filter paper. The residue on the filter was washed with MeOH and then dissolved with 1N HCl. The acidic solution was evaporated to afford 104 mg of the hydrochloride salt 8 as a white solid (79%). The product was used without further purification. M.p. 53-55°C; \(^1\)H NMR (400 MHz, D₂O) \( \delta \) 2.97 (t, \( J = 7.6 \) Hz, 2H), 2.05 – 1.91 (m, 4H), 1.68 – 1.56 (m, 2H), 1.40 – 1.16 (m, 6H), 0.86 (t, \( J = 7.5 \) Hz, 3H); \(^1\)C NMR (100 MHz, D₂O) \( \delta \) 175.5, 150.7, 57.0, 39.3, 37.6, 32.3, 27.9, 26.3, 24.9, 23.9, 8.3; ESI MS \( m/z \): calcd. for C₁₂H₂₂N₃O₃ [M+H-HCl]+ 256.3, C₁₂H₂₁N₃NaO₃, found: 256.2.

5-(3-(6-(5-Ethyl-2,4,6-trioxohexahydropyrimidin-5-yl)hexyl)thioureido)-2-(6-hydroxy-3-oxo-3\( H \)-xanthen-9-yl)benzoic acid (9). To a solution of 8 (51.7 mg, 0.18 mmol, 1 eq) in dry DMF (2 mL) DIPEA (\( d = 0.74 \), 62 µL, 0.36 mmol, 2 eq) and FITC isomer I (77.1 mg, 0.20 mmol, 1.1 eq) were added. Reaction
mixture was stirred at room temperature under nitrogen atmosphere for 5.5 hours until completion of the reaction. The reaction was monitored by analytical HPLC [(Phenomenex Luna C18, 5 μm, 100 Å, 250 x 4.6 (L x ID) inj. volume 20 μL, flow rate 1 mL/min, solvent A: H2O+0.1%TFA, solvent B: CH3CN+0.1%TFA, method: 10%B for 3 min, linear gradient from 10 to 100%B in 20 min], Rf (FITC) = 19.4 min, Rf (8) = 9.9 min, Rf (9) = 16.2 min. The reaction mixture was concentrate in vacuo and the residue was purified by flash chromatography on silica gel (DCM/EtOH 8.7:1.3) to afford 102 mg of 9 as an orange solid (88%). Rf = 0.29 (DCM/EtOH 8.7:1.3). M.p. 210-212°C; analytical HPLC (conditions see above) Rf: 16.4 min, > 94% λ = 214 nm, 254 nm; 1H NMR (400 MHz, CD3OD) δ 8.12 (s, 1H), 7.75 (d, J = 8.2 Hz, 1H), 6.71 – 6.59 (m, 4H), 6.54 (dd, J = 8.7, 2.3 Hz, 2H), 3.57 (br s, 2H), 2.01 – 1.83 (m 4H), 1.68 – 1.55 (m, 2H), 1.41 – 1.17 (m, 6H), 0.84 (t, J = 7.4 Hz, 3H); 13C NMR (100 MHz, CD3OD) δ 191.8, 183.6, 175.9, 172.0, 171.9, 162.2, 155.0, 152.2, 150.5, 143.3, 132.8, 131.2, 131.1, 129.8, 126.5, 120.7, 114.5, 112.3, 112.2, 104.4, 58.7, 46.4, 40.4, 34.4, 31.1, 30.5, 28.3, 26.9, 10.4; ESI MS m/z: calcd. for C33H33N4O8S [M+H]+ 645.2, found: 645.2.

Sodium 5-ethyl-5-(6-fluorohexyl)-2,4,6-trioxotetrahydro-1H-pyrimidin-3-ide. The fluorinated barbiturate 1a (70.8 mg, 0.27 mmol, 1 eq) was dissolved in 700 µL of THF at room temperature and a solution of 1.35N NaOH in absolute ethanol (220 µL, 11.9 mg, 0.30 mmol, 1.1 eq) was added. Immediately the solution became milky and after few minutes a precipitate was detected. Reaction mixture was stirred at room temperature for 2 hours. Then the white solid was filtered and dried at 55 °C in oven for one night to constant weight of 33.6 mg (44%). M.p. 230-232°C; ESI MS (+) m/z: calcd. for C12H19FN2NaO3 [M+H]+, C12H20FN2O3 [M+2H-Na]+ 281.1, 259.1, found: 259.1, 281.1; ESI MS (-) m/z: calcd. for C12H18FN2O3 [M-H]- 257.1, found: 257.1.

Sodium 5-(6-fluorohexyl)-2,4,6-trioxo-5-phenyltetrahydro-1H-pyrimidin-3-ide. The fluorinated barbiturate 1b (56.8 mg, 0.18 mmol, 1 eq) was dissolved in 300 µL of THF at room temperature and a
solution of 1 N NaOH in absolute ethanol (200 µL, 8.0 mg, 0.20 mmol, 1.1 eq) was added. After few hours under stirring at room temperature, precipitation of solid was not observed. The solution was evaporated in vacuo and a white solid was obtained. In order to separate the sodium salt from the unreacted barbiturate 1b, the solid was solubilised in water and then filtered on 0.22 µm filter. The aqueous solution was evaporated in vacuo and the desired salt was obtained as a white solid (59.3 mg, 98%). *ESI MS* m/z: calcd. for C_{16}H_{19}FN_{2}NaO_{3} [M+H]^+ 329.1, found: 329.1.

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Sodium 5-benzyl-5-(6-fluorohexyl)-2,4,6-trioxotetrahydro-1H-pyrimidin-3-ide. The fluorinated barbiturate 1c (39.0 mg, 0.12 mmol, 1 eq) was dissolved in 300 µL of THF at room temperature and a solution of 0.55 N NaOH in absolute ethanol (240 µL, 5.2 mg, 0.13 mmol, 1.1 eq) was added. After few hours under stirring at room temperature, precipitation of solid was not observed. The solution was evaporated in vacuo and a white solid was obtained. In order to separate the sodium salt from the unreacted barbiturate 1c, the solid was solubilised in water and then filtered on 0.22 µm filter. The aqueous solution was evaporated in vacuo and the desired salt was obtained as a white solid (25.2 mg, 60%). *ESI MS* m/z: calcd. for C_{17}H_{21}FN_{2}NaO_{3} [M+H]^+, C_{17}H_{22}FN_{2}O_{3} [M+2H-Na]^+ 343.1, 321.2, found: 343.1, 321.1.
Radiochemistry

5-Ethyl-5-(6-[18F]fluorohexyl)pyrimidine-2,4,6(1H,3H,5H)-trione ([18F]1a). [18F]Fluoride (20±4 GBq, n=5) was produced by CTI RDS-111 cyclotron (CTI/Siemens) via the 18O(p,n)18F nuclear reaction by bombardment of isotopically enriched [18O]H2O target and passed through a Chromafix(R) 30-PS-HCO3 cartridge (Macherey Nagel) as an aqueous solution in 18O-enriched water. [18F]F- was eluted from the cartridge with 0.5 mL of K2CO3 solution in water (6 mg/mL) into a reaction vessel in the hot cell. Then a solution of Kryptofix® 222 (15 mg) in dry CH3CN (1 mL) was added. Water was removed by azeotropic distillation with acetonitrile under stream of helium at 85 °C. A solution of tosyl precursor 6a (5.5 mg) in dry CH3CN (1 mL) was added to the reaction vessel containing the dried [K222][18F]F complex (15±3 GBq, n=5). After heating at 100 °C for 15 min the reaction was cooled to room temperature. Then a solution of 1N HCl (150 µL) in dry CH3CN (350 µL) was added, and the mixture was passed through two preconditionated Sep-Pak® Light Alumina N cartridges (Waters) in order to remove the unreacted fluoride. The cartridges were preconditionated with 10% 1N HCl in CH3CN and dried. The product was purified by semipreparative HPLC [Phenomenex Luna C18, 5 µm, 100 Å, 250 x 10.0 (L x ID), volume inj. 400 µL, flow rate 4 mL/min, solvent A: H2O, solvent B: CH3CN, gradient: 40%-95% B in 15 min]. A simultaneous detection by UV and radioactivity monitors was performed to isolate the desired product. Retention time of [18F]1a was 8.3 min. The solvent was removed in vacuo and the residue was dissolved in 0.5 mL of saline. The saline solution of [18F]1a was passed through a sterile 0.22 µm filter. The activity of the final formulation was 900±100 MBq/mL (n=5). The radiosynthesis of [18F]1a was accomplished in 90±10min (n=7), with a decay corrected radiochemical yield of 17±3% (n=7) considering the activity produced, and of 20±3% (n=7) considering the activity transferred into the reaction vessel. The radiochemical purity of the final formulated product was ≥ 99%.

The identity of the radiolabelled tracer was confirmed via HPLC by comparison with the non-radioactive 1a (6.56 min, λ=215 nm) [Phenomenex Luna C18, 5 µm, 100 Å, 250 x 4.6 (L x ID), inj. volume 20 µL, flow rate 1 mL/min, solvent A: H2O, solvent B: CH3CN, gradient: 40%-95% B in 15 min] (figure S1).
Figure S1: HPLC profiles of “cold” reference 1a (top) and $[^{18}\text{F}]$1a (bottom). HPLC conditions: Phenomenex Luna C18 column, 5 μm, 100 Å, 250 x 4.6 (L x ID), inj. volume 20 μL, flow rate 1 mL/min, solvent A: H$_2$O, solvent B: CH$_3$CN, gradient: 40%-95% B in 15 min], Rt (UV) = 6.56 min, λ=215 nm, Rt ($\gamma$) = 6.64 min.
Metabolite analyses. Cardiac blood samples were drawn at the end of the scans (ca. 1h post injection) from two WT, two APP/PS1 and two PLB2 mice. Blood samples were centrifuged at 13500 rpm for 5 min in order to separate plasma. Plasma samples were denaturated by mixing equal volumes of ice-cold acetonitrile and centrifuged (3 min, 13500 rpm). Then the supernatant was analysed by injection into analytical HPLC.

**Figure S2.** Representative HPLC chromatograms of blood samples drawn at 60 min after administration of $[^{18}\text{F}]\text{1a}$ in wild type mouse (A), APP/PS1 mouse (B) and PLB2-Tau mouse (C).

HPLC conditions: Phenomenex Luna C18 column, 5 μm, 100 Å, 250 x 4.6 (L x ID), inj. volume 20 μL, flow rate 1 mL/min, solvent A: H$_2$O, solvent B: CH$_3$CN, gradient: 40%-95% B in 15 min.
II. LogP determination

LogP determination via HPLC of compounds 1a-c.

Method. LogP values were determined according an HPLC-based method reported in the Official Journal of the European Communities.[3] All samples were analysed using an analytical column [Phenomenex Luna C18, 5 μm, 100 Å, 250 x 4.6 (L x ID) inj volume 20 μL, flow rate 1 mL/min] and an isocratic mobile phase of 40% CH₃CN and 60% sodium acetate buffer (100 mM, pH=4.7). UV absorption was detected at 214 nm. A set of seven reference compounds (aprobarbital, phenol, butalbital, pentobarbital, secobarbital, toluene, naphthalene), whose logP is known in literature, was coinjected with thiourea and compounds 1a-1c (figure S3 A). In order to increase the confidence in the measurements, quintuplicate coinjections were made.

Preparation of sample injected in column. For each solid compound a solution with the mobile phase was prepared (1 mg/mL). Toluene was dissolved in CH₃CN (1 mg/mL). Then, from each solution 100 μL were drawn (with the exceptions of thiourea and toluene, 50 μL and 200 μL, respectively) and collected together to form the final sample that was analysed.

Calibration graph. The retention time of the first peak (thiourea) was used as t₀. For each reference compounds, the corresponding capacity factors (k), were calculated according to the equation: \( k = \frac{t_r - t_0}{t_0} \). Then the corresponding logarithms of the capacity factors (logk), were calculated and plotted as function of partition coefficients (logP) that are known in literature. The best-fit curve was reported in figure S3 B.

Determination of logP. The logarithms to base 10 of the capacity factors (logk) of compounds 1a-c were calculated from the corresponding retention times (tᵣ). Then, from the calibration graph, the logP values were interpolated.

The logP values of compounds 1a-c calculated according this procedure are: logP(1a)=1.84; logP(1b)=2.44; logP(1c)=2.70.
Figure S3. (A) Representative HPLC chromatogram. (B) Calibration graph.

Reference compounds and corresponding logP: 1=apobarbital, logP=1.15[^4]; 2=phenol, logP=1.5[^3]; 3=butalbital logP=1.65[^4]; 4=pentobarbital, logP=1.95[^4]; 5=secobarbital, logP=2.15[^4]; 6=toluene logP=2.7[^3]; 7=naphthalene logP=3.6[^3].

Octanol-buffer LogP determination of [18F]1a. The experimental determination of partition coefficient of radioactive tracer [18F]1a was performed in n-octanol and phosphate buffer (PBS pH 7.4) as previously reported by Wilson et al[^2] with slight modifications. In their work they demonstrated that hydrophilic impurities affect significantly the logP determination. The error can be reduced carrying out a pre-wash of the organic phase containing the radiotracer with the buffer solution. The tracer was diluted with PBS in order to obtain a solution with an activity of 0.5 MBq/mL. 200 µL of this solution were transferred in 4 eppendorf vials. Then 200 µL of n-octanol were added to each vials. The eppendorf vials were shaken (10 min, 2000 rpm) and then centrifuged (5 min, 13500 rpm). From each vials 210 µL were drawn from the bottom and discarded. To the remaining organic phases were added 190 µL of fresh PBS. These solutions were shaken (10 min, 13500 rpm) and centrifuged (5 min, 13500 rpm) again. Then 100 µL were drawn from each layer and the activity (counts per 10 secs) was measured with a γ counter. The ratio of the (no decay corrected) activity average of organic phase to that of the aqueous buffer solution was determined for four partitioning cycles. The base-10 logarithm of the activity ratios was calculated for each cycle, and finally, the average was calculated providing the value of logP. The logP of [18F]1a determined according this procedure is 1.12±0.01 (n=4).
III. In Vivo validation using microPET/CT

A total of 16 PET/CT scans were performed; 13 using a dynamic brain protocol and 3 using a dynamic whole body protocol. Details of the animals used are shown in Table 1. All animal handling was performed under the University’s Code of Practice on the Use of Animals in Research as well as the legal requirements of the Animals Act 1986 and Home Office Code of Practice guidance.

Data were acquired using a SEDECAL ARGUS PET/CT scanner (SEDECAL, Madrid, Spain). The scanner has two 11.8 cm diameter rings of phoswich detectors coupled to position sensitive photomultiplier tubes, giving a 4.8 cm axial field of view. A complete performance evaluation of the scanner has been done by Wang et al.[5]

Animals were anaesthetised using 2% isofluorane, a cannula was inserted in a tail vein and the animal was placed on the imaging couch in the scanner. The tracer was injected (through the cannula) and PET imaging started at the same time as the injection. After the PET acquisition was completed, a CT scan was performed. For the Dynamic Brain protocol, PET data were acquired in list mode for one hour. These data were then re-framed into 17 time frames 4x15s, 4x30s, 2x60s, 3x300s, 4x600s). For the Dynamic Whole Body protocol data were acquired for a total of one hour using two (repeated) bed positions with 5 minutes per frame for each bed position, giving a total of 6 interleaved time frames for each bed position. In all cases data were acquired in 3-Dimensional (3D) mode. These data were converted into 2-Dimensional (2D) sinograms by Fourier rebinning. Images were then reconstructed using a 2D-OSEM (two-dimensional ordered subset expectation maximization) reconstruction algorithm (provided by the manufacturer). Corrections for attenuation, randoms and photon scatter were applied (using the manufacturer's software).

Following the CT scan three of the mice (one of each genotype) were scanned using the Dynamic Brain protocol (scan numbers 4, 9 and 10) and all three of the mice scanned using the Dynamic Whole Body protocol were sacrificed via cardiac puncture and all of the blood was removed for metabolite analysis.

Registered PET and CT images were transferred from the scanner to a processing workstation and all subsequent analysis was carried out using Pmod (Pmod Technologies, CH) version 3.2. All of the brain CT images were registered to those from the first scan using the non-linear brain normalisation algorithm in Pmod. The same transforms were then applied to the PET images so that these were also registered to the first scan. The MRI image from the Mirrione mouse brain atlas (provided with Pmod) was also registered to the CT scan from the first mouse and the same transform applied to the atlas to provide a set of brain regions of interest, which were used for analysis.
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**Table S1.** Details of the mice used for the PET/CT imaging studies.
**Figure S4.** Brain uptake and activity of $[^{18}F]1a$ in aged (>12 months) WT, Alzheimer mice (APP/PS1) and Tau mice (PLB2). Left: Mean activity (in %ID/g) during first 60 seconds; right: peak values. ns: not significant (p>0.05).

**Figure S5.** Brain area analyses after i.v. injection of $[^{18}F]1a$ (last frame, t = 55 min) revealed an overall highly significant difference between transgenic APP/PS1 cf. WT and Tau mice. Post-hoc analysis identified significant differences in all brain areas excluding the brainstem (*: p<0.05; **:p<0.01, ns: not significant).
**Figure S6.** Whole brain analysis for the PET/CT imaging studies on 13 mice (4 WT, 5 APP/PSEN1 and 4 PLB2 Tau mice).
**Figure S7.** Full body PET/CT scan n. 16 (Table S1) of a Tau mouse.
Figure S8. Brain PET-CT scans from the three different mice genotypes with $^{[18}\text{F}]\text{Ia}$, taken at various times post injection, as indicated. The images were converted to units of %ID/g and a colour scale running from 0-10%ID/g was used (bottom left).
IV. In Vitro amyloid binding assays

**Amyloid preparation.** Human amyloid \(\beta_{1-42}\) (A\(\beta\); Abcam Biochemicals, Cambridge, UK), was dissolved in Dimethyl Sulfoxide (DMSO, Sigma-Aldrich, Pool, UK) to a stock solution of \(\sim 294 \mu M\) and stored at \(-80^oC\) in 10 \(\mu l\) aliquots until the day of use. On the day of use, A\(\beta\) was diluted in sterile filtered Dulbecco’s 0.1 mM phosphate buffered saline (PBS; Sigma) to a concentration of 9 \(\mu M\).

**Barbiturate preparation.** The cold barbiturate 1a was dissolved in DMSO to a stock concentration of 90 mM and stored at \(-20^oC\) until the day of use. Stock solutions were further diluted to the desired concentrations in sterile filtered 0.1 mM PBS for the Congo Red assay.

**Congo Red Assay.** Congo Red (CR; VWR, Lutterworth, UK), was dissolved in sterile filtered 0.1 M PBS containing 5% ethanol to prevent CR micelle formation\[^5\] to a stock concentration of 3 mM. The stock solution was further diluted to a working concentration of 120 \(\mu M\) in 0.1 M PBS. The binding assay was conducted in clear 96 well plates (VWR), with 100 \(\mu l\) of prepared A\(\beta\) (final concentration: 4.5 \(\mu M\)) added to each well, with and without 50 \(\mu l\) 1a-containing solution (in 0.1 mM PBS) at the appropriate concentrations. Each well was supplemented with 50 \(\mu l\) of the 120 \(\mu M\) CR working solution (final well concentration: 30 \(\mu M\)), incubated at room temperature for 30 min, before absorbance spectra reading (300-600 nm, at 10 nm intervals), taken by a Synergy HT plate reader (NorthStar Scientific, Leeds, UK). Each experimental group was normalised to provide differential spectral analysis by subtracting respective control values, i.e. [CR + vehicle] for CR+A\(\beta\), and [CR + vehicle + 1a] for CR+A\(\beta\) + 1a. Each group was repeated in 4 wells. Measurements of inhibition were quantified at 530 nm, as the maximal differential peak absorbance determined for [CR + A\(\beta\)] vs. [CR + vehicle].\[^6\] Data was used to determine a non-linear specific binding curve, K\(d\) value provide as calculated by K\(d\)= (XB\(max\)/Y) –X, where B\(max\), is predicted maximal binding (Graphpad Prism ver 5.00).

**Immunohistochemistry.** Following the conjugation of fluorescein (FLC), FLC-barbiturate 9 and FLC alone were diluted in N,N’-Dimethylformamide (DMF; Sigma) to stock concentrations of 9 mM and stored at \(-20^oC\), until use. On the day of staining, 9 and FLC were diluted to 9 \(\mu M\) in 0.1 M PBS + 10% Tween-20 (Sigma). Staining protocols were modified from Platt et al., 2011.\[^8\] Briefly, 5 \(\mu m\) thick coronal brain section from WT and APP/PS1 mice (age:>18 months) were de-waxed in Xylene, rehydrated in sequential steps of 100%, 70%, and 0% ethanol. Potential binding sites lost by the fixation processes were unmasked by heat-assisted citric acid incubation for 20 min. Sections were then incubated in 9 (9 \(\mu M\)) or in FLC (9 \(\mu M\)) in 0.1 M PBS + 10% Tween-2 for 1 h. Each step was followed with appropriate washing steps in 0.1 mM PBS.
Confirmation of the specific labelling of Aβ plaques was obtained by co-staining of a number of sections with an antibody raised against the 1-16 sequence of Aβ peptide, 6E10 (Cambridge Bioscience, Cambridge, UK). As above, sections were de-waxed, rehydrated, incubated in citric acid for 20 min, before being blocked and permeabilized for 1 h in blocking buffer (1.5% normal goat serum: 2% bovine serum albumin, 1% milk powder and 0.001% Triton, in 0.1 mM PBS) incubated in 6E10 (1:200) containing antibody solution (1.5% normal goat serum: 2% bovine serum albumin and 0.001% Triton, in 0.1 mM PBS) and subsequently in secondary antibody (1:200, goat anti-mouse Alexa 594, Invitrogen, Paisley, UK) containing solution (1.5% normal goat serum and 2% bovine serum albumin, in 0.1 mM PBS) for 1 h, prior to incubation to 9 µM of 9 (as above). All steps were preceded with appropriate wash steps in 0.1 M PBS.

Slides were dried and mounted with Prolong Gold containing DAPI (Invitrogen). Images were taken with a digital camera (Axiocam, Carl Zeiss; Hertfordshire, UK) mounted on a Zeiss microscope (Axioskop 2 Plus).

Co-localisation of fluorescent barbiturate 9, amyloid and zinc

In a separate line of experiments, confirmation of the specific plaque and zinc labeling was obtained using triple staining of brain sections with an antibody raised against the 1-16 sequence of Aβ peptide, 6E10 (Cambridge Bioscience, Cambridge, UK), a florescent zinc probe (N-(6-Methoxy-8-Quinolyl)-p-Toluenesulfonamide, TSQ) as well as 9. As TSQ staining must be conducted in unfixed tissues (Côté et al., 2005), brains snap-frozen in liquid nitrogen were cut into 5µM thick cryosections. Sections were first stained with 9 (as above), before staining in 45µM TSQ in 0.1M PBS prepared from 1.5% (W/V) TSQ DMSO stock for 2mins. Subsequently, sections were permeabilized for 1hr in blocking buffer (1.5% normal goat serum: 2% bovine serum albumin, 1% milk powder and 0.001% Triton, in 0.1M PBS) incubated with 6E10 antibody solution (1:200, 1.5% normal goat serum: 2% bovine serum albumin and 0.001% Triton, in 0.1M PBS) followed by secondary antibody incubations (1:200, goat anti-mouse Alexa 594, Invitrogen, Paisley, UK; 1.5% normal goat serum and 2% bovine serum albumin, in 0.1M PBS) for 1hr. All steps were preceded with appropriate wash steps in 0.1 M PBS.
Figure S9. *In vitro* and *in situ* analyses of 9-binding. **A**: Transmission electron micrograph of aggregated β-amyloid 20 (βA). Example fibrils highlighted by black arrows, black bar: 0.2μm. **B**: A Congo Red βA assay (data shown as % inhibition, absorbance measured at 530nm) depict interactions of 1a with aggregated βA (concentrations between 25 nM and 100 μM); 1a inhibited absorbance in a
concentration-dependent manner. $K_D$ value from 25 a non-linear regression analysis (purple line) is also provided. (C) Example images of fluorescein control (FLC Con.) and the fluorescein conjugated 9 (FLC-Barb) binding in brain tissue from transgenic APP/PS1 mouse brain sections; individual FLC and merged images (with the nuclear dye DAPI) are also shown. (D) Triple staining of transgenic APP/PS1 mouse brain sections with the Aβ antibody 6E10, TSQ (zinc stain) and 9 (FLC-Barb). Co-localisation is evident from merged images. White bar: 50µm. The arrows highlight multiple plaque labelling in images acquired with a 10x objective.
V. References

VI. $^1$H, $^{13}$C and $^{19}$F NMR spectra
$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^1$H NMR (400 MHz, CDCl$_3$)

$^{13}$C NMR (100 MHz, CDCl$_3$)
$^1$H NMR (400 MHz, CD$_3$OD)

$^{13}$C NMR (100 MHz, CD$_3$OD)
$^1$H NMR (400 MHz, CDCl$_3$)

![Chemical structure](image)

$^1$C NMR (100 MHz, CDCl$_3$)

![Chemical structure](image)
$^{19}$F NMR (564 MHz, CDCl$_3$)

$^1$H NMR (400 MHz, CD$_3$OD)

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$^{13}$C NMR (100 MHz, CD$_3$OD)

$^{19}$F NMR (564 MHz, CD$_3$OD)
$^{19}$F NMR (564 MHz, CDCl$_3$)

$^1$H NMR (400 MHz, CDCl$_3$)
$^{13}$C NMR (100 MHz, CDCl$_3$)

$^1$H NMR (400 MHz, D$_2$O)
\[^{13}\text{C} \text{NMR (100 MHz, CD}_3\text{OD)}\]

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