11C and 18F Radiolabeling of Tetra- and Pentathiophenes as PET-Ligands for Amyloid Protein Aggregates

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Supporting information

Table of contents

General Information.............................................................................................................S-2
Chemistry.............................................................................................................................S-2
Radiochemistry....................................................................................................................S-12
Biology.................................................................................................................................S-13
NMR Spectra.......................................................................................................................S-17
References.........................................................................................................................S-23
**General Information**

Organic extracts were dried over anhydrous magnesium sulfate, filtered and concentrated *in vacuo* at 40 °C. NMR-spectra were recorded on a Varian instrument (\(^1\)H 300 MHz, \(^{13}\)C 75.4 MHz). Chemical shifts were assigned with the solvent residual peak as the reference. TLC was carried out on Merck precoated 60 F254 plates using UV-light (\(\lambda = 254\) nm and 366 nm) and charring with EtOH/H\(_2\)SO\(_4\)/p-anisaldehyde/HOAc 90:3:2:1 for visualization. Flash column chromatography (FC) was performed using silica gel 60 (0.040-0.063 mm, Merck). Gradient HPLC-MS was performed on a Gilson system (Column: Waters X-Bridge C-18 or C-8 5 µ, 250 x 15 mm and Waters X-Bridge C-18 or C-8 2.5 µ, 150 x 4.6 mm for semi-preparative and analytical runs respectively; Pump: Gilson gradient pump 322; UV/VIS-detector: Gilson 155; MS detector: Thermo Finnigan Surveyor MSQ; Gilson Fraction Collector FC204) gradient system. Eluent A: MeCN:H\(_2\)O 90:10 with 0.05% NH\(_4\)OAc. Eluent B: MeCN. Eluent C: MeCN with 0.05% Et\(_3\)N. Eluent D: MeCN:H\(_2\)O 5:95 with 0.05% NH\(_4\)OAc. Eluent E: H\(_2\)O with 0.05% Et\(_3\)N. \(^{[1]}\)C]CN and \(^{[18]}\)F]F was produced at the Uppsala University Hospital using a Scanditronix MC-17 cyclotron. Semi-preparative radio-HPLC was performed using a VWR LaPrep HPLC system (P110, P311) with a Beckman CoutlerTM UltrapshereTM ODS column (10×250 mm). Analytical radio-HPLC was performed using a VWR LaChrom ELITE system (L-2130, L-2200, L-2400) with a Merck Chromolith Performance RP-18e column (4.6×100 mm). Both systems were set to detect UV at 254 nm and were equipped with a Bioscan Flow-Count PMT radioactivity detector. The precursors and non-radioactive standards were >95% pure as deducted by LC-MS and NMR.

**4.2 Chemistry**

**4.2.1 Compound 1**

Synthesis and analytical data in accordance with literature procedure.\(^1\)
4.2.2. Compound 2

A mixture of 1 (1.01 g, 2.14 mmol), 5-carboxythiophene-2-boronic acid (0.461 g, 2.68 mmol), and K$_2$CO$_3$ (0.740 g, 5.36 mmol) in MeOH/toluene 1:1 (40 mL) was heated at 75 °C for 1 min. PEPPSI™-IPr catalyst (0.025 mg, 0.037 mmol) was added and the mixture was heated at 75 °C for an additional 30 min. HOAc (conc.) and EtOAc were added and the solution was washed with brine and water. The organic phase was dried, filtered and concentrated. Crystallization from MeOH gave a yellow solid that was suspended in dry dioxane (10 mL) and BF$_3$•Et$_2$O (0.081 mL, 0.639 mmol) and tert-butyl 2,2,2-trichloroacetimidate (0.767 mL, 4.29 mmol) were added. Stirring for 2 h, concentration, and FC (toluene, toluene/EtOAc 60:1 → 9:1) gave 2 (1.030 g, 84%) as a yellow solid over two-steps.$^1$H NMR (300 MHz, CDCl$_3$) δ 1.57 (s, 9H), 3.72 (s, 3H), 3.74 (s, 3H), 3.77 (s, 2H), 3.78 (s, 2H), 7.05 (d, $J = 5.3$ Hz, 1H), 7.10 (d, $J = 4.0$ Hz, 1H), 7.15 (d, $J = 3.8$ Hz, 1H), 7.17 (d, $J = 3.8$ Hz, 1H), 7.20 (s, 1H), 7.24 (s, 1H), 7.60 (d, $J = 3.8$ Hz, 1H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 28.4, 34.8, 34.9, 52.3, 52.5, 82.1, 124.1, 125.0, 127.6, 127.7, 128.1, 130.6, 130.8, 131.3, 132.8, 133.4, 133.6, 134.3, 135.1, 135.4, 136.4, 142.7, 161.4, 171.1, 171.4; ESI-MS m/z 575 [(M+H)$^+$ calcd for C$_{27}$H$_{27}$O$_6$S$_4$ + 575].

4.2.3. Compound 3

Compound 2 (0.166 g, 0.289 mmol) was dissolved in DMF (3 mL) and the solution was cooled to 0 °C. NIS (0.072 g, 0.320 mmol) was added portion wise during one min. The solution was stirred at 0 °C for 15 min and then at RT for 1 h. TFA (0.023 ml, 0.301 mmol was added and the reaction mixture was stirred for an additional 4 h. Subsequent (without workup) purification by HPLC (C-18, 80-100% eluent B in eluent D in a linear gradient over 10 min followed by isocratic 100% eluent B for 15 min) gave 3 (0.115 g, 57%) as an orange solid. $^1$H NMR (300 MHz, acetone-d$_6$) δ 1.58 (s, 9H), 3.70 (s, 3H), 3.71 (s, 3H), 3.82 (s, 2H), 3.85 (s, 2H), 7.23 (d, $J = 3.9$ Hz, 1H), 7.29 (d, $J = 3.9$ Hz, 1H), 7.30 (d, $J = 3.9$ Hz, 1H), 7.32 (s, 1H), 7.41 (s, 1H),
7.64 (d, J = 3.9 Hz, 1H); $^{13}$C NMR (75 MHz, acetone-$d_6$) δ 28.4, 34.5, 35.1, 52.4, 52.5, 74.0, 82.4, 125.3, 128.5, 128.7, 130.0, 133.3, 133.5, 134.2, 134.6, 135.0, 135.4, 135.4, 138.9, 141.8, 143.0, 161.4, 171.2; ESI-MS m/z 701 [(M+H)$^+$ calcd for C$_{27}$H$_{26}$IO$_6$S$_4$$^+$ 701].

4.2.4. Compound 4

Compound 2 (0.965 g, 1.68 mmol) was dissolved in DMF (10 mL) and the solution was cooled to -15 °C. NBS (0.210 g, 1.18 mmol) was added portion wise during one min. The solution was allowed to attain RT during 2 h, again cooled to -15 °C, more NBS (0.095 g, 0.534 mmol) was added portion wise during one min, and the solution was allowed to attain RT. Dilution with EtOAc, washing with brine, drying, filtering, concentration, and FC (toluene, toluene/EtOAc 60:1 → 9:1) gave 4 (1.020 g, 93%) as a yellow solid. $^1$H NMR (300 MHz, CDCl$_3$) δ 1.59 (s, 9H), 3.72 (s, 2H), 3.74 (s, 3H), 3.75 (s, 3H), 3.77 (s, 2H), 7.04 (s, 1H), 7.11 (d, J = 3.8 Hz, 1H), 7.12 (d, J = 3.8 Hz, 1H), 7.18 (d, J = 3.8 Hz, 1H), 7.22 (s, 1H), 7.61 (d, J = 3.8 Hz, 1H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 28.4, 34.6, 34.9, 52.5, 82.1, 111.9, 124.2, 127.7, 128.1, 128.2, 131.3, 131.5, 133.1, 133.3, 133.6, 134.3, 134.4, 134.9, 135.6, 135.7, 142.6, 161.4, 170.9, 171.0; ESI-MS m/z 653 [(M+H)$^+$ calcd for C$_{27}$H$_{26}$BrO$_6$S$_4$$^+$ 653].

4.2.5. Compound 5

Compound 3 (0.080 g, 0.114 mmol) and CuCN (0.100 g, 1.12 mmol) were transferred to a microwave vial and DMF (2 mL) was added. Microwave irradiation at 170 °C for 15 min was followed by addition of EtOAc and washing with saturated NaHCO$_3$ (aq) and water. Purification by HPLC (C-18, 80-100% eluent B in eluent D in a linear gradient over 10 min followed by isocratic 100% eluent B for 15 min) gave 5 (0.023 g, 34%) as an orange solid. $^1$H NMR (300 MHz, CDCl$_3$) δ 1.58 (s, 9H), 3.75 (s, 6H), 3.76 (s, 2H), 3.78 (s, 2H), 7.12 (d, J = 4.0 Hz, 1H), 7.22 (s, 1H), 7.18 (d, J = 3.8 Hz, 1H), 7.22 (s, 1H), 7.58 (s, 1H), 7.61 (d, J = 4.0 Hz, 1H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 28.3, 34.3, 34.8, 52.4, 52.5, 77.5, 108.2, 113.7, 124.2, 127.8, 128.1,
129.2, 130.9, 131.9, 132.2, 133.1, 133.5, 134.5, 135.9, 137.3, 139.9, 140.3, 142.2, 161.2, 170.2, 170.7; ESI-MS m/z 600 [(M+H)+ calcd for C\textsubscript{28}H\textsubscript{26}NO\textsubscript{6}S\textsubscript{4}+ 600].

4.2.6. q-FTAA-CN

To a solution of 5 (0.023 g, 0.038 mmol) in DCM (2 mL) was added TFA (0.5 mL) and the solution was stirred for 2 h and co-concentrated with toluene. To the residual in dioxane (3 mL) at 0 °C was added 1M NaOH (aq) (0.100 mL, 0.100 mmol) and water (1 mL). The reaction mixture was stirred at 0 °C for 30 min and for an additional 4 h at RT. Subsequent (without workup) purification by HPLC (C-8, 0-70% eluent C in eluent E in a linear gradient over 15 min followed by isocratic 100% eluent C for 10 min) gave q-FTAA-CN (0.011 g, 56%) as an orange solid as a TEA-salt. To the solid was added 1M NaOH (aq) (0.070 mL, 0.070 mmol) and water until everything went into solution. Concentration gave q-FTAA-CN as an orange sodium salt. \textsuperscript{1}H NMR (300 MHz, CD\textsubscript{3}OD:D\textsubscript{2}O 1:1) δ 3.70 (s, 2H), 3.71 (s, 2H), 7.19 (d, \(J = 3.8\) Hz, 1H), 7.27 (s, 1H), 7.31 (d, \(J = 3.8\) Hz, 1H), 7.38 (d, \(J = 3.8\) Hz, 1H), 7.48 (d, \(J = 3.8\) Hz, 1H), 7.71 (s, 1H); \textsuperscript{13}C NMR (75 MHz, CD\textsubscript{3}OD:D\textsubscript{2}O 1:1) δ 38.0, 38.1, 107.0, 115.2, 125.2, 128.5, 129.4, 130.2, 131.9, 132.4, 134.3, 136.0, 136.1, 136.8, 138.7, 140.7, 141.3, 142.1, 143.3, 169.3, 177.1; HRMS m/z 513.9548 [((M-H)- calcd for C\textsubscript{22}H\textsubscript{12}NO\textsubscript{6}S\textsubscript{4} 513.9547].

4.2.7. q-FTAA-Br

To a solution of 4 (0.126 g, 0.206 mmol) in DCM (3 mL) was added trifluoroacetic acid (1 mL) and the solution was stirred for 2 h and co-concentrated with toluene. To the residual in dioxane (3 mL) was added 1M NaOH aq. (0.927 mL, 0.927 mmol) and water (1 mL). After 4 h AcOH (conc.) and EtOAc were added and the solution was washed with brine and water. The organic phase was dried, filtered and concentrated to give the protonated tricarboxylic acid of q-FTAA-Br (0.110 g, 94%) as a yellow solid. To the solid was added 1M NaOH aq. (0.637 mL, 0.637 mmol) and water until everything went into solution. Concentration gave q-FTAA-Br as a
yellow sodium salt. $^1$H NMR (300 MHz, D$_2$O) δ 3.60 (s, 2H), 3.62 (s, 2H), 6.89 (d, $J = 3.9$ Hz, overlapped, 1H), 6.90 (s, 1H), 6.94 (d, $J = 3.9$ Hz, 1H), 6.99 (d, $J = 3.9$ Hz, 1H), 7.10 (s, 1H), 7.43 (d, $J = 3.9$ Hz, 1H); $^{13}$C NMR (75 MHz, D$_2$O) δ 38.2, 38.4, 110.3, 124.4, 126.4, 126.5, 129.1, 131.9, 132.1, 133.7, 134.0, 134.4, 134.5, 134.6, 135.4, 139.6, 140.9, 169.8, 179.2, 179.3; HRMS m/z 566.8694 [(M-H)$^-$ calcd for C$_{21}$H$_{12}$O$_6$S$_4$- 566.8700].

4.2.8. Compound 6

A mixture of 4 (0.047 g, 0.072 mmol), 5-cyanothiophene-2-boronic acid pinacol ester (0.026 g, 0.111 mmol), and K$_2$CO$_3$ (0.025 g, 0.181 mmol) in MeOH/toluene 1:1 (3 mL) was heated at 75 °C for 1 min. PEPPSI™-IPr catalyst (0.004 g, 0.006 mmol) was added and the mixture was heated at 75 °C for an additional 30 min. HOAc (conc.) and EtOAc were added and the solution was washed with brine and water. The organic phase was dried, filtered and concentrated. Purification by HPLC (C-18, 80-100% eluent B in eluent D in a linear gradient over 10 min followed by isocratic 100% eluent B for 15 min) gave 6 (0.031 g, 63%) as an orange solid. $^1$H NMR (300 MHz, CDCl$_3$) δ 1.59 (s, 9H), 3.76 (s, 3H), 3.77 (s, 3H), 3.78 (s, 2H), 3.80 (s, 2H), 7.13 (apparently t, $J = 4.1$ Hz, 2H), 7.21 (s, overlapped, 2H), 7.22 (d, $J = 3.2$ Hz, 1H), 7.26 (d, $J = 3.2$ Hz, 1H), 7.53 (d, $J = 3.9$ Hz, 1H), 7.62 (d, $J = 3.9$ Hz, 1H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 28.4, 34.8, 34.9, 52.5, 52.6, 82.2, 108.1, 114.2, 123.8, 124.2, 127.9, 128.2, 128.2, 129.2, 131.6, 131.7, 132.9, 133.6, 134.4, 134.5, 135.0, 135.7, 136.1, 138.4, 142.5, 143.9, 161.3, 170.9, 171.0; ESI-MS m/z 682 [(M+H)$^+$ calcd for C$_{32}$H$_{28}$NO$_6$S$_5^+$ 682].

4.2.9. p-FTAA-CN

To a solution of 6 (0.020 g, 0.029 mmol) in DCM (2 mL) was added trifluoroacetic acid (0.5 mL) and the solution was stirred for 2 h and co-concentrated with toluene. To the residual in dioxane (3 mL) at 0 °C was added 1M NaOH (aq) (0.132 mL, 0.132 mmol) and water (1 mL). The reaction mixture was stirred at 0 °C for 30 min and for an additional 4 h at RT. Subsequent
(without workup) purification by HPLC (C-8, 0-70% eluent C in eluent E in a linear gradient over 15 min followed by isocratic 100% eluent C for 10 min) gave p-FTAA-CN (0.010 g, 58%) as an orange solid. To the solid was added 1M NaOH (aq) (0.055 mL, 0.055 mmol) and water until everything went into solution. Concentration gave p-FTAA-CN as a red sodium salt. ¹H NMR (300 MHz, CD₃OD:D₂O 1:1) δ 3.61 (s, 4H), 7.10 (d, J = 3.9 Hz, 1H), 7.11-7.17 (m, 4H), 7.23 (s, 1H), 7.43 (d, J = 3.9 Hz, 1H), 7.58 (d, J = 3.9 Hz, 1H); ¹³C NMR (75 MHz, CD₃OD:D₂O 1:1) δ 38.9, 106.6, 115.6, 124.7, 125.0, 127.7, 128.2, 129.5, 131.3, 132.2, 132.4, 133.1, 134.3, 135.7, 135.9, 136.9, 140.8, 141.4, 141.5, 145.7, 170.0, 178.7; HRMS m/z 595.9430 [(M-H)⁻ calcd for C₂₆H₁₅NO₆S₅⁻ 595.9425].

4.2.10. Compound 7

A mixture of 4 (0.126 g, 0.193 mmol), 2-thienylboronic acid (0.037 g, 0.289 mmol), and K₂CO₃ (0.067 g, 0.485 mmol) in MeOH/toluene 1:1 (6 mL) was heated at 75 °C for 1 min. PEPPSI™-IPr catalyst (0.008 g, 0.012 mmol) was added and the mixture was heated at 75 °C for an additional 30 min. HOAc (conc.) and EtOAc were added and the solution was washed with brine and water. The organic phase was dried, filtered and concentrated. Purification by FC (toluene, toluene/EtOAc 18:1) gave compound 7 (0.108 g, 85%) as an orange solid. ¹H NMR (300 MHz, CDCl₃) δ 1.58 (s, 9H), 3.75 (s, 3H), 3.76 (s, 3H), 3.77 (s, 2H), 3.78 (s, 2H), 7.02 (dd, J = 3.6, 5.1 Hz, 1H), 7.11 (d, J = 3.9 Hz, 1H), 7.12 (s, 1H), 7.17 (dd, J = 1.1, 3.6 Hz, 1H) 7.18 (s, 2H), 7.22 (s, 1H), 7.23 (dd, J = 1.1, 5.1 Hz, 1H), 7.61 (d, J = 3.9 Hz, 1H); ¹³C NMR (75 MHz, CDCl₃) δ 28.4, 34.9, 35.0, 52.4, 52.5, 82.1, 124.1, 124.2, 125.1, 126.9, 127.5, 127.8, 128.0, 128.2, 131.2, 131.3, 131.5, 133.3, 133.6, 134.3, 135.1, 135.4, 136.0, 136.5, 136.7, 142.6, 161.3, 171.0, 171.1; ESI-MS m/z 657 [(M+H)⁺ calcd for C₃₁H₂₉O₆S₅⁺ 657].

4.2.11. Compound 8
Compound 7 (0.683 g, 1.039 mmol) was dissolved in DMF (7 mL) and the solution was cooled to -15 °C. NBS (0.130 g, 0.730 mmol) was added portion wise during one min. The solution was allowed to attain RT during 2 h, again cooled to -15 °C, more NBS (0.055 g, 0.309 mmol) was added portion wise during one min, and the solution was allowed to attain RT. Dilution with EtOAc, washing with brine, drying, filtering, concentration, and FC (toluene → toluene/EtOAc 18:1) gave 8 (0.609 g, 80%) as a yellow solid. \(^1\)H NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 1.54 (s, 9H), 3.67 (s, 3H), 3.68 (s, 3H), 3.86 (s, 2H), 3.88 (s, 2H), 7.20 (d, \(J = 3.9\) Hz, 1H), 7.25 (d, \(J = 3.9\) Hz, 1H), 7.30 (d, \(J = 3.9\) Hz, 1H), 7.30 (s, 1H), 7.32 (d, \(J = 3.9\) Hz, 1H) 7.39 (d, \(J = 3.9\) Hz, 1H), 7.48 (s, 1H), 7.66 (d, \(J = 4.0\) Hz, 1H); \(^{13}\)C NMR (75 MHz, DMSO-\(d_6\)) \(\delta\) 28.2, 34.5, 52.5, 82.3, 111.3, 125.4, 125.7, 128.1, 128.3, 128.9,130.3, 131.3, 132.3, 132.6, 132.8, 133.5, 133.9, 134.1, 134.7, 134.8, 135.1, 137.6,142.1, 160.7, 170.8, 170.9; ESI-MS m/z 735 [(M+H\(^+\)) calcd for C\(_{31}\)H\(_{28}\)BrO\(_6\)S\(_5\)\(^+\)]

4.2.12. p-FTAA-Br

To a solution of 8 (0.047 g, 0.064 mmol) in DCM (3 mL) was added TFA (1 mL) and the solution was stirred for 2 h and co-concentrated with toluene. To the residual in dioxane (1 mL) was added 1M NaOH (aq) (0.288 mL, 0.288 mmol) and water (0.5 mL). After 4 h HOAc (conc.) and EtOAc were added and the solution was washed with brine and water. The organic phase was dried, filtered and concentrated to give the protonated tricarboxylic acid of p-FTAA-Br (0.040 g, 96%) as an orange solid. To the solid was added 1M NaOH (aq) (0.190 mL, 0.190 mmol) and water until everything went into solution. Concentration gave p-FTAA-Br as a red sodium salt. Compound was characterized as the protonated tricarboxylic acid: \(^1\)H NMR (300 MHz, DMSO-\(d_6\)) \(\delta\) 3.74 (s, 2H), 3.77 (s, 2H), 7.17 (d, \(J = 3.9\) Hz, 1H), 7.22 (d, \(J = 3.9\) Hz, 1H), 7.26 (s, 1H), 7.29 (d, \(J = 3.9\) Hz, 1H), 7.31 (d, \(J = 3.9\) Hz, 1H), 7.37 (d, \(J = 3.9\) Hz, 1H), 7.44 (s, 1H), 7.67 (d, \(J = 3.9\) Hz, 1H); \(^{13}\)C NMR (75 MHz, DMSO-\(d_6\)) \(\delta\) 34.6, 34.8, 110.8, 125.0,
125.1, 127.5, 127.7, 128.6, 130.0, 130.7, 131.8, 132.0, 133.0, 133.1, 133.5, 133.5, 133.9, 134.4, 134.4, 134.8, 137.3, 141.8, 162.6, 171.5, 171.6; HRMS m/z 648.8574 [(M-H)⁻ calcd for C_{25}H_{15}BrO_{6}S_5⁻ 648.8577].

4.2.13. Compound 9

A mixture of 4 (0.990 g, 1.51 mmol), 5-carboxythiophene-2-boronic acid (0.450 g, 2.62 mmol), and K_{2}CO_{3} (0.600 g, 4.34 mmol) in MeOH/toluene 1:1 (30 mL) was heated at 75 °C for 1 min. PEPPSI™-IPr catalyst (0.025 g, 0.037 mmol) was added and the mixture was heated at 75 °C for an additional 30 min. HOAc (conc.) and EtOAc were added and the solution was washed with brine and water. The organic phase was dried, filtered and concentrated. Crystallization from MeOH gave compound 9 (0.810 g, 77%) as an orange solid. \(^{1}\)H NMR (300 MHz, DMSO-d_6) δ 1.54 (s, 9H), 3.67 (s, 6H), 3.88 (s, 4H), 7.33 (s, 2H), 7.39 (d, J = 4.1 Hz, 1H), 7.39 (d, J = 3.9 Hz, 1H) 7.47 (s, 1H), 7.48 (s, 1H) 7.66 (d, J = 3.9 Hz, 1H), 7.68 (d, J = 4.1 Hz, 1H); \(^{13}\)C NMR (75 MHz, DMSO-d6) δ 27.8, 34.1, 52.0, 81.7, 124.8, 125.0, 127.7, 129.6, 129.7, 132.1, 132.2, 133.0, 133.2, 133.6, 133.7, 134.1, 134.2, 134.5, 134.5, 141.6, 141.7, 160.3, 162.5, 170.4; ESI-MS m/z 701 [(M+H)⁺ calcd for C_{32}H_{29}O_{8}S_5⁺ 701].

4.2.14. Compound 10

To a solution of 9 (0.101 g, 0.144 mmol), 2-(2-(2-aminoethoxy)ethoxy)ethoxy)-ethanol (0.060 g, 0.31 mmol), and DIPEA (0.101 mL, 0.580 mmol) in DMF (3 mL) was added O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluoro-phosphate (HATU) (0.085 g, 0.22 mmol). After 2 h EtOAc and brine were added and the organic phase was separated and washed with saturated NH_{4}Cl (aq), saturated NaHCO_{3} (aq) and water, dried, filtered and concentrated. Purification by gradient HPLC (C-18, 60-100% eluent A in eluent D in a linear gradient over 15 min followed by isocratic 100% eluent A for 10 min) gave compound 10 (0.091 g, 72%) as an orange solid. \(^{1}\)H NMR (300 MHz, CDCl_{3}) δ 1.57 (s, 9H), 3.61-3.73 (m,
16H), 3.74 (s, 6H), 3.77 (s, 4H), 7.09 (d, J = 3.9 Hz, 1H), 7.10 (d, J = 4.0 Hz, 1H), 7.18 (s, 3H),
7.20 (s, 1H), 7.50 (d, J = 4.0 Hz, 1H), 7.59 (d, J = 3.9 Hz, 1H), 7.61 – 7.67 (b, 1H); $^{13}$C NMR
(75 MHz, CDCl$_3$) δ 28.3, 34.8, 34.8, 39.9, 52.4, 61.6, 70.0, 70.2, 70.5, 70.6, 72.6, 82.1, 124.1,
127.7, 127.9, 128.1, 128.8, 131.4, 132.7, 133.1, 133.6, 134.2, 135.4, 135.6, 135.7, 138.5, 140.8, 142.5, 161.3, 161.9, 171.0; ESI-MS m/z 876 [(M+H)$^+$ calcd for C$_{40}$H$_{46}$NO$_{11}$S$_5^+$ 876].

4.2.15. Compound 12

Compound 11 (0.232 g, 1.05 mmol) was dissolved in MeOH (3 mL). PPh$_3$ (0.550 g, 2.10
mmol) and H$_2$O (0.1 mL) were added and the mixture was stirred at 50 °C for 2 h and
concentrated. Purification by FC (EtOAc/MeOH 9:1 + 1% MeOH sat. with NH$_3$ and
EtOAc/MeOH 4:1 + 1% MeOH sat. with NH$_3$) gave 12 (0.175 g, 85%) as a colorless oil. $^1$H
NMR (300 MHz, CD$_3$OD) δ 2.78-2.90 (m, 2H), 3.55 (t, J = 5.3 Hz, 2H), 3.56-3.72 (m, 9H),
3.75-3.80 (m, 1H), 4.50-4.64 (m, 2H); $^{13}$C NMR (75 MHz, CD$_3$OD) δ 41.9, 71.2, 71.4, 71.5,
71.5, 71.6, 71.7, 84.1 (d, $J_{CF} = 167.5$ Hz).

4.2.16. Compound 13

To a stirred solution of 9 (0.054 g, 0.077 mmol), 12 (0.030 g, 0.154 mmol), and DIPEA (0.050
mL, 0.287 mmol) in DMF (3 mL) was added $O$-(7-azabenzotriazol-1-yl)-$N,N',N'$-tetramethyluronium hexafluoro-phosphate (HATU) (0.045 g, 0.118 mmol). After 2 h EtOAc and
brine were added and the organic phase was separated and washed with saturated NH$_4$Cl (aq),
saturated NaHCO$_3$ (aq) and water, dried, filtered and concentrated. Purification by gradient
HPLC (C-18, 70-100% eluent A in eluent D in a linear gradient over 15 min followed by
isocratic 100% eluent A for 10 min) gave 13 (0.042 g, 62%) as an orange solid. $^1$H NMR (300
MHz, CDCl$_3$) δ 1.58 (s, 9H), 3.59-3.71 (m, 13H), 3.75 (s, 6H), 3.76-3.80 (m, 1H), 3.78 (s,
overlapped, 4H), 4.49-4.59 (m, 2H), 6.59-6.67 (b, 1H), 7.11 (d, J = 3.9 Hz, 2H), 7.19 (s, 3H),
7.22 (s, 1H), 7.42 (d, J = 3.9 Hz, 1H), 7.61 (d, J = 3.9 Hz, 1H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 28.4, 34.9, 39.9, 52.5, 69.9, 70.4, 70.7, 70.8, 71.0, 83.2 (d, \(J_{CF} = 169.3\) Hz), 124.2, 124.2, 127.8, 128.1, 128.2, 128.8, 131.5, 132.9, 133.1, 133.6, 134.3.

4.2.17. p-FTAA-TEG-F

**Protetected p-FTAA-TEG-F** (0.008 g, 0.009 mmol) was dissolved in DCM (1 mL) and trifluoroacetic acid (0.333 mL) was added. The reaction mixture was stirred for 2 h and co-concentrated with toluene. To the residual in dioxane (1 mL) at 0 °C was added 1M NaOH (aq) (0.045 mL, 0.045 mmol) and water (0.333 mL). The reaction mixture was stirred at 0 °C for 30 min and for an additional 4 h at RT. Subsequent (without workup) purification by HPLC (C-8, 0-70% eluent C in eluent E in a linear gradient over 15 min followed by isocratic 100% eluent C with 0.05% Et\(_3\)N for 10 min) gave the protonated tricarboxylic acid of **p-FTAA-TEG-F** (0.005 g, 70%) as a red solid. To the solid was added 1M NaOH (aq) (0.025 mL, 0.025 mmol) and water until everything went into solution. Concentration gave compound **p-FTAA-TEG-F** as a red sodium salt. \(^1\)H NMR (300 MHz, D\(_2\)O) \(\delta\) 3.38 (t, \(J = 5.7\) Hz, 2H), 3.58 (t, \(J = 5.7\) Hz, 2H), 3.61-3.68 (m, 8H), 3.66 (s, overlapped, 4H), 3.68-3.73 (m, 1H), 3.78-3.84 (m, 1H), 4.52-4.57 (m, 1H), 4.67-4.73 (m, 1H), 6.90-6.97 (m, 3H), 7.06 (d, \(J = 3.8\) Hz, 1H), 7.10 (s, 1H), 7.12 (s, 1H), 7.35 (d, \(J = 4.0\) Hz, 1H), 7.47 (d, \(J = 3.8\) Hz, 1H); \(^{13}\)C NMR (75 MHz, D\(_2\)O) \(\delta\) 38.4, 39.3, 68.6, 69.4, 69.5, 69.7, 69.8, 70.0, 83.3 (d, \(J_{CF} = 163.3\) Hz), 124.1, 126.0, 126.2, 129.0, 129.4, 130.4, 131.7, 131.9, 132.4, 133.1, 133.8, 134.1, 134.6, 134.9, 135.1, 139.5, 140.8, 141.8, 163.4, 169.3, 178.7, 178.8; HRMS m/z 794.0703 [(M+H)\(^+\) calcd for C\(_{34}\)H\(_{32}\)FNO\(_{10}\)S\(_5\)\(^+\) 794.0692].

4.2.18. p-FTAM-tBu-TEG-OTs

To a solution of 10 (0.088 g, 0.100 mmol) in dry CHCl\(_3\) (3 mL) were added \(p\)-toluenesulphonyl chloride (0.056 g, 0.294 mmol) and pyridine (1 mL). After 4 h the solution was diluted with
EtOAc and washed with HCl (1 M, aq.) and H2O. Purification by gradient HPLC (C-18, 80-100% eluent B in eluent D in a linear gradient over 15 min followed by isocratic 100% eluent B for 10 min) gave compound p-FTAM-tBu-TEG-OTs (0.079 g, 76%) as an orange solid. 1H NMR (300 MHz, CDCl3) δ 1.57 (s, 9H), 2.41 (s, 3H), 3.57-3.69 (m, 14H), 3.74 (s, 6H), 3.77 (s, 4H), 4.11-4.16 (m, 2H), 6.70-6.78 (b, 1H), 7.08 (d, J = 3.9 Hz, 1H), 7.10 (d, J = 4.0 Hz, 1H), 7.18 (s, 3H), 7.21 (s, 1H), 7.31 (d, J = 8.3 Hz, 2H), 7.40 (d, J = 3.9 Hz, 1H), 7.60 (d, J = 4.0 Hz, 1H), 7.77 (d, J = 8.3 Hz, 2H); 13C NMR (75 MHz, CDCl3) δ 21.7, 28.3, 34.8, 39.9, 52.4, 68.8, 69.3, 69.8, 70.4, 70.5, 70.7, 70.9, 82.1, 124.1, 124.2, 127.7, 127.7, 128.0, 128.0, 128.2, 128.7, 129.3, 131.4, 132.8, 133.0, 133.1, 133.6, 134.3, 135.4, 135.5, 137.9, 140.9, 142.5, 145.0, 161.3, 161.6, 170.9; HRMS m/z 1030.1761 [(M+H)+ calcd for C47H51NO13S6+ 1030.1763].

4.3. Radiochemistry

4.3.1. Palladium-mediated 11C-cyanation

To an oven dried 2 mL vial was added precursor (q-FTAA-Br (2.5 mg, 4 µmol) or p-FTAA-Br (3 mg, 4 µmol)) togheter with dichloro-[9,9-dimethyl-4,5-bis(diphenylphosphino)xanth-ene]palladium(II) (2.3 mg, 3 µmol). The vial was capped and flushed with nitrogen before 400 µL of dry DMF was added. The solution was cooled in a freezer (-20 °C) up until use. [11C]CO2 was produced by proton bombardment of nitrogen gas (AGA, Nitrogen 6.0) containing 0.05% oxygen (AGA, Oxygen 4.6) through the 14N(p,α)11C nuclear reaction. NH4[11C]CN was produced by an standard online method by reduction of [11C]CO2 using nickel catalyst and hydrogen to give [11C]CH4 followed by conversion to NH4[11C]CN by a platinum catalyzed reaction with ammonia. When all the reactivity was trapped, the reaction mixture was heated for 5 min at 130 °C. After cooling for 1 min, aq. TFA (10%, total volume 100 µL) was added. The crude mixture was diluted with 1 mL of aqueous MeCN (50%) and the pure compounds
were obtained after semi-preparative HPLC purification (isocratic gradient, 40% MeCN in H₂O (1% TFA), 6 mL/min). The compound eluted at 10.5-11.5 min \textbf{p-FTAA-[¹¹CN]} and 9.5-10.5 min \textbf{q-FTAA-[¹¹CN]}. After evaporation of solvents, the radioligand was formulated using 2 mL of hydroxypropyl β-cyclodextrin (300mg/mL) and 2 mL of phosphate buffer (pH 7.4) and filtered through a sterile 0.2 μm filter. \textbf{p-FTAA-[¹¹CN]} and \textbf{q-FTAA-[¹¹CN]} was identified by co-elution of the corresponding non-radioactive entities and by LC-MS.

4.3.2. \textbf{¹⁸F}-nucleophilic fluorination

\textbf{[¹⁸F]}Fluoride was produced by proton bombardment of enriched H₂¹⁸O and transferred to the hot lab where it was trapped on a SPE cartridge (Waters Accell Plus QMA Carbonate). The \textbf{[¹⁸F]}fluoride was eluted with a MeCN/H₂O (80/20, v/v) Kryptofix-2.2.2/K₂CO₃ solution and dried by azeotropic distillation with MeCN at 100 °C for 10 min under a stream of helium. Precursor \textbf{p-FTAM-tBu-TEG-OTs} (5 mg, 4 µmol) was dissolved in 600 µL of dry MeCN and added to the reaction vial containing \textbf{[¹⁸F]}fluoride/Kryptofix-2.2.2 and heated at 80 °C for 10 min. 100 µL aqueous NaOH (7 M) was added and the mixture was heated at 100 °C for 15 min. After cooling, aq. TFA (0.100 mL, total volume 0.200 mL) was added and the crude mixture was purified using semi-preparative radio-HPLC (30-90 %, 15 min, MeCN in H₂O (1% TFA), 6 mL/min). The pure \textbf{p-FTAA-TEG-[¹⁸F]} eluted at 11.9-12.5 min. After evaporation of solvents, the tracer was formulated in 2 mL of hydroxypropyl β-cyclodextrin (300mg/ml) and 2 mL of phosphate buffer (pH 7.4) and filtered through a sterile 0.2 µm filter. \textbf{p-FTAA-TEG-[¹⁸F]} was identified by co-elution of the non-radioactive entity and by LC-MS.

4.4. Biology

4.4.1. Tissue staining using fluorescence

Archived paraffin-embedded formalin fixed sections were deparaffinized by sequential washing for 2 x 15 minutes in Xylene, 2 x 10 minutes Absolute Ethanol, 2x 10 minutes 95%
Ethanol, and 5 minutes respectively in 80% Ethanol, 60% Ethanol, 40% Ethanol. After rehydration with deionized water, the sections were incubated with PBS for 10 min and then stained for 30 min at RT with reference compounds q-FTAA-CN, p-FTAA-CN and p-FTAA-TEG-F. All probes were diluted 1:500 in PBS from a 1.5 mM stock. After rinsing with PBS three times, the sections were mounted with Dako fluorescence mounting medium (Dako Cytomation, Glostrup, Denmark). The medium was allowed to solidify for three hours before the rims were sealed with nail polish. Fluorescence images were collected with an inverted LSM 780 confocal microscope (Carl Zeiss, Oberkochen, Germany) using single excitation wavelength at 458 nm.

4.4.2. Homogenate Binding

Tissues (50-100 mg) were homogenized in ice-cold 0.32 M sucrose at a concentration of 20 mg/mL using a polytron tissue homogenizer (Polytron® PT 3000, Kinematica AG, Littay-Luzern, Switzerland). The resulting, crude homogenates were diluted 20 times with phosphate-buffered saline (PBS, pH 7.4) to a final concentration of 1 mg/mL and aliquots of the homogenates were stored at -80°C until used. Homogenates were thawed and incubated with the radioligands, at a concentration of 10 nM in the assay, for 40 min at RT in PBS, in a final incubation volume of 1 mL. Molar concentrations of the three radioligands were calculated using the specific radioactivity of each radiopharmaceutical. For experiments in which the non-specific binding was assessed, 10 µM of p-TFAA or unlabeled PIB was added. The reactions were stopped by vacuum assisted filtration using a Brandel M-48 cell harvester (Brandel, Gaithersburg, USA) with Whatman GF/C filter (presoaked with PBS) and washed four times with 3 ml PBS (RT). For incubations with radioligands alone, triplicate tubes were used, whereas duplicate tubes were used for incubations with excess of blocking substances. The filters were transferred to scintillation tubes where the trapped radioactivity was measured in a
well-type NaI(Tl) scintillation counter, applying correction for dead-time and for radioactive decay.

4.2.3. In vivo studies

4.2.3.1. In vivo monkey PET-CT

One female cynomolgus monkey (*Macaca fascicularis*), weighing 9 kg was used in the monkey PET study. The animal permission was granted by the local Research Animal Ethics Committee C 38/9. The monkey is sedated with ketamine (approximately 10 mg/kg), weighed, and maintained on a constant infusion of ketamine 15 mg/kg/h Propofol is administered until the animal is anesthetized enough to intubate. After intubation the animal is maintained on sevoflurane inhalation anesthesia and artificial ventilation. Body temperature, heart rate, ECG, pCO₂, pO₂, SaO₂ and blood pressure are monitored throughout the PET study. Ringer-Acetate (Fresenius-Kabi) is given during the study (0.5-1 mL/kg/h). The imaging was performed on a Discovery ST PET/CT camera. Three consecutive PET-CT scans were performed during the same day in the same monkey. An iv bolus injection of 184 MBq ([¹¹CN]-p-FTAA), 201 MBq ([¹¹CN]-q-FTAA) and 51.5 MBq ([¹⁸F]-p-FTAA-TEG) was given. The scans lasted 60 min per ¹¹C-labeled compound, and 90 min for the ¹⁸F-labeled compound, with 60-90 min between scans. Dynamic scans were performed 0-60 min for the brain and static whole body scans 60-75 (3x5 min). Images were reconstructed with attenuation, scatter and random corrections, and with reconstruction parameters generating a resolution of about 4 mm. The whole brain was delineated manually as one region of interest (ROI) and the time-activity curves were drawn (Figure S1). Different blood samples are taken throughout the study (Figure S2): i) A 115 μL venous sample was taken at the induction of anaesthesia and at the middle and end of experiment for estimation of blood gases, electrolytes, hematocrite and glucose. ii) Blood samples (0.2 mL) for radioactivity determination were taken at 0.5, 1, 3, 5, 10, 15, 20, 30, 45
and 60 min after each injection. iii). Three blood samples comprise 0.5 mL, which were centrifuged after radioactive measurement and the plasma was transferred to a new vial for measurement of plasma concentration of radioactivity.

**Figure S1.** Time activity SUV curves of the uptake in the brain of the three ligands in a female Cynomolgus monkey as measured using PET.

**Figure S2.** Time activity SUV curves of the uptake in the blood of the three ligands.
NMR Spectra of Precursors and Non-Radioactive Standards
References