

## Supporting information to

# Targeting cyclic nucleotide phosphodiesterase 5 (PDE5) in brain: Toward the development of a PET radioligand labeled with fluorine-18

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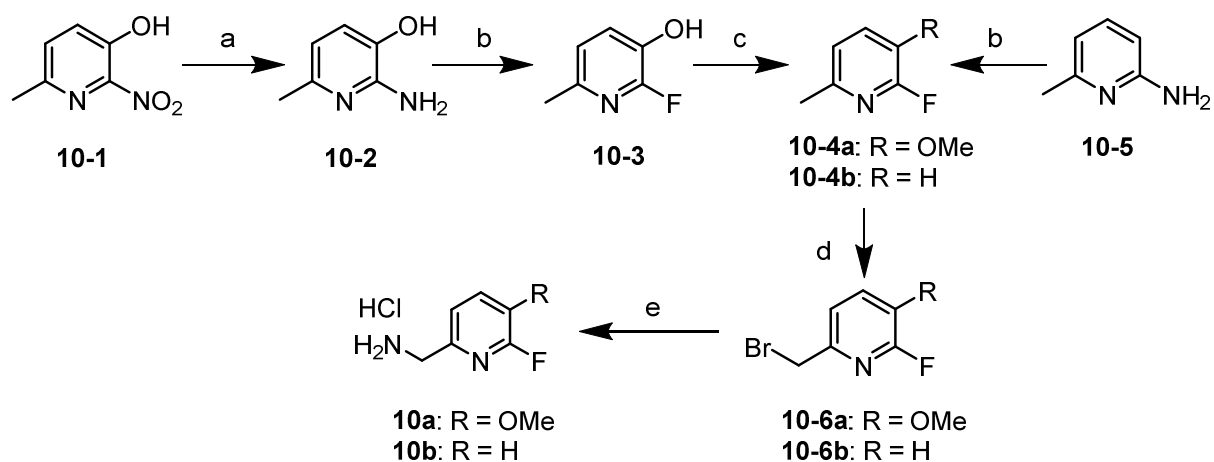
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## 1. Synthesis of the pyridine intermediates 10a and 10b



### Scheme S1. Synthesis of 10a and 10b

Reagents and conditions: (a) H<sub>2</sub>, Pd/C, EtOH, rt, 24 h; (b) NaNO<sub>2</sub>, HBF<sub>4</sub>, 0 °C, 5 h; (c) MeI, K<sub>2</sub>CO<sub>3</sub>, DMF, rt, 1 h; (d) NBS, AIBN, CCl<sub>4</sub>, 60 °C, 20 h for **10-6a** and 22 h for **10-6b**; (e) 1) HMTA, CHCl<sub>3</sub>, 50 °C, 1 h; 2) HCl<sub>conc.</sub>, EtOH, 50 °C, 2 h, then 12 h at rt.

#### 2-Amino-6-methylpyridin-3-ol (**10-2**)[1]:

A mixture of 6-methyl-2-nitropyridine-3-ol (5.0 g, 32.4 mmol, 1.0 eq.) and 10% palladium on carbon (500 mg) in ethanol (30 mL) was vigorously stirred under a hydrogen atmosphere at room temperature for one day. The mixture was filtered on Celite® 545 and washed with ethanol (100 mL). The filtrate was concentrated under reduced pressure and the residue was washed with diethyl ether (50 mL) to give the desired product (3.7 g, 29.8 mmol, 92%) as a yellow solid. Mp: 152 ± 1 °C (Literature: 150-153 °C [2]); IR (cm<sup>-1</sup>) ν 3487 (ν<sub>NH<sub>2</sub></sub>), 3371 (ν<sub>NH<sub>2</sub></sub>), 1632 (δ<sub>NH</sub>), 1488 (ν<sub>C=C</sub>), 1255 (ν<sub>as C-O</sub>), 1181 (ν<sub>s C-O</sub>); <sup>1</sup>H NMR (400 MHz, DMSO-d<sub>6</sub>) δ 9.13 (m, 1H, OH), 6.71 (d, 1H, <sup>3</sup>J<sub>H<sub>5</sub>-H<sub>4</sub></sub> = 7.6 Hz, H<sub>5</sub>), 6.21 (d, 1H, <sup>3</sup>J<sub>H<sub>4</sub>-H<sub>5</sub></sub> = 7.6 Hz, <sup>4</sup>J<sub>H<sub>4</sub>-OH</sub> = 0.4 Hz, H<sub>4</sub>), 5.31 (s, 2H, NH<sub>2</sub>), 2.14 (s, 3H, H<sub>a</sub>); ESI-MS *m/z* calculated for C<sub>6</sub>H<sub>9</sub>N<sub>2</sub>O [M+H]<sup>+</sup> 125.06, found 125.06.

#### 2-Fluoro-6-methylpyridin-3-ol (**10-3**)[3]:

To a solution of **10-2** (7.7 g, 62.1 mmol, 1.0 eq.) dissolved in a 49.5-50.5% tetrafluoroboric acid solution (80 mL), sodium nitrite (10.85 g, 0.16 mol, 2.5 eq.) was portion-wise added at 0 °C under stirring over 3 hours. The resulting mixture was stirred at 0-5 °C for further 2 hours. Afterwards, aqueous 1M sodium hydroxide solution (550 mL) was added slowly to adjust pH 6-7 while keeping the temperature below 10 °C. The resulting mixture was extracted with ethyl acetate (4 × 300 mL). The combined organic layers were washed with brine (200 mL), dried over anhydrous magnesium sulfate, filtered and then concentrated under reduced pressure. The crude product was purified by column chromatography (SiO<sub>2</sub>, ethyl acetate/cyclohexane, 3/7, v/v) to give the desired product (3.3 g, 26.0 mmol, 42%) as a light yellow solid. R<sub>f</sub> (SiO<sub>2</sub>, cyclohexane/ethyl acetate, 7/3, v/v): 0.44; Mp: 134 ± 1 °C (Literature: 133-135 °C [3]); IR (cm<sup>-1</sup>) ν 1495 (ν<sub>C=C</sub>), 1268 (ν<sub>as C-O</sub>), 1119 (ν<sub>s C-O</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.27 (dd, 1H, <sup>3</sup>J<sub>H<sub>4</sub>-H<sub>5</sub></sub> = 7.9 Hz, <sup>4</sup>J<sub>H<sub>4</sub>-F</sub> = 10.7 Hz, H<sub>4</sub>), 6.92 (d, 1H, <sup>3</sup>J<sub>H<sub>5</sub>-H<sub>4</sub></sub> = 7.9 Hz, H<sub>5</sub>), 2.41 (s, 3H, H<sub>a</sub>); ESI-MS *m/z* calculated for C<sub>6</sub>H<sub>7</sub>FNO [M+H]<sup>+</sup>: 128.04, found 128.00.

**2-Fluoro-3-methoxy-6-methylpyridine (10-4a) [4]:**

To a mixture of **10-3** (1.5 g, 11.8 mmol, 1.0 eq.) and potassium carbonate (3.26 g, 23.6 mmol, 2.0 eq.) in anhydrous *N,N*-dimethylformamide (DMF, 20 mL), iodomethane (3.35 g, 23.6 mmol, 2.0 eq.) was added dropwise under stirring. The resulting mixture was stirred at rt for 1 hour before addition of water (100 mL). Afterwards, the solution was extracted with ethyl acetate (3 × 80 mL). The combined organic layers were washed with brine (2 × 100 mL), dried over anhydrous magnesium sulfate, filtered and then concentrated under reduced pressure. The crude product was purified by column chromatography (SiO<sub>2</sub>, gradient, cyclohexane/ethyl acetate, 100/0 to 30/70, v/v) to give the desired product (1.4 g, 9.9 mmol, 84%) as colorless oil. *R*<sub>f</sub> (SiO<sub>2</sub>, cyclohexane/ethyl acetate, 7/3, v/v): 0.44; IR (cm<sup>-1</sup>) ν 1577 (ν<sub>C=C</sub>), 1474 (δ<sub>CH<sub>3</sub></sub>), 1265 (ν<sub>as C-O-C</sub>), 1127 (ν<sub>s C-O-C</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.16 (dd, 1H, <sup>3</sup>J<sub>H<sub>4</sub>-H<sub>5</sub></sub> = 7.9 Hz, <sup>4</sup>J<sub>H<sub>4</sub>-F</sub> = 10.4 Hz, H<sub>4</sub>), 6.92 (d, 1H, <sup>3</sup>J<sub>H<sub>5</sub>-H<sub>4</sub></sub> = 7.9 Hz, H<sub>5</sub>), 3.85 (s, 3H, H<sub>a</sub>), 2.39 (s, 3H, H<sub>b</sub>); ESI-MS *m/z* calculated for C<sub>7</sub>H<sub>9</sub>FNO [M+H]<sup>+</sup>: 142.06; found 141.98.

**6-(Bromomethyl)-2-fluoro-3-methoxypyridine (10-6a):**

Under stirring, *N*-bromosuccinimide (278 mg, 1.56 mmol, 1.1 eq.) and α,α'-azobisisobutyronitrile (AIBN, 47 mg, 0.29 mmol, 0.2 eq.) were added to a solution of **10-4a** (200 mg, 1.42 mmol, 1.0 eq.) in carbon tetrachloride (10 mL). The mixture was heated at 60 °C for 20 hours. After cooling to rt, water (10 mL) was added and the mixture was extracted with dichloromethane (2 × 30 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous magnesium sulfate, filtered and then concentrated under reduced pressure. The crude product was purified by column chromatography (SiO<sub>2</sub>, ethyl acetate/cyclohexane, 2/8, v/v) to give, by order of elution: the desired product (200 mg, 0.91 mmol, 64%) as a white solid and the by-product 6-(dibromomethyl)-2-fluoro-3-methoxypyridine (25 mg, 0.08 mmol, 6%) as yellow oil. *R*<sub>f</sub> (Al<sub>2</sub>O<sub>3</sub>, cyclohexane/ethyl acetate, 7/3, v/v): 0.60; Mp: 60 ± 1 °C; IR (cm<sup>-1</sup>) ν 1577 (ν<sub>C=C</sub>), 1475 (δ<sub>CH<sub>3</sub></sub>), 1258 (ν<sub>C-O-C</sub>), 1172 (ν<sub>C-O-C</sub>); <sup>1</sup>H NMR (400 MHz, MeOD-d<sub>4</sub>) δ 7.50 (dd, 1H, <sup>3</sup>J<sub>H<sub>4</sub>-H<sub>5</sub></sub> = 8.1 Hz, <sup>4</sup>J<sub>H<sub>4</sub>-F</sub> = 10.2 Hz, H<sub>4</sub>), 7.35 (dd, 1H, <sup>3</sup>J<sub>H<sub>5</sub>-H<sub>4</sub></sub> = 8.1 Hz, <sup>5</sup>J<sub>H<sub>5</sub>-F</sub> = 0.7 Hz, H<sub>5</sub>), 4.48 (s, 2H, H<sub>b</sub>), 3.91 (s, 3H, H<sub>a</sub>); <sup>13</sup>C NMR (100 MHz, MeOD-d<sub>4</sub>) δ 153.8 (d, <sup>1</sup>J<sub>C<sub>2</sub>-F</sub> = 240 Hz, C<sub>2</sub>), 145.7 (d, <sup>3</sup>J<sub>C<sub>6</sub>-F</sub> = 11 Hz, C<sub>6</sub>), 144.1 (d, <sup>2</sup>J<sub>C<sub>3</sub>-F</sub> = 25 Hz, C<sub>3</sub>), 123.8 (d, <sup>3</sup>J<sub>C<sub>4</sub>-F</sub> = 4 Hz, C<sub>4</sub> or C<sub>5</sub>), 123.1 (d, <sup>4</sup>J<sub>C<sub>5</sub>-F</sub> = 4 Hz, C<sub>4</sub> or C<sub>5</sub>), 56.9 (C<sub>a</sub>), 33.0 (C<sub>b</sub>); HRMS (ESI) *m/z* calculated for C<sub>7</sub>H<sub>8</sub><sup>79</sup>BrFNO [M+H]<sup>+</sup> 219.9773, found 219.9757 (100%); C<sub>7</sub>H<sub>8</sub><sup>81</sup>BrFNO [M+H]<sup>+</sup>: 221.8744 (98%).

**2-(Bromomethyl)-6-fluoropyridine (10-6b)[5]:**

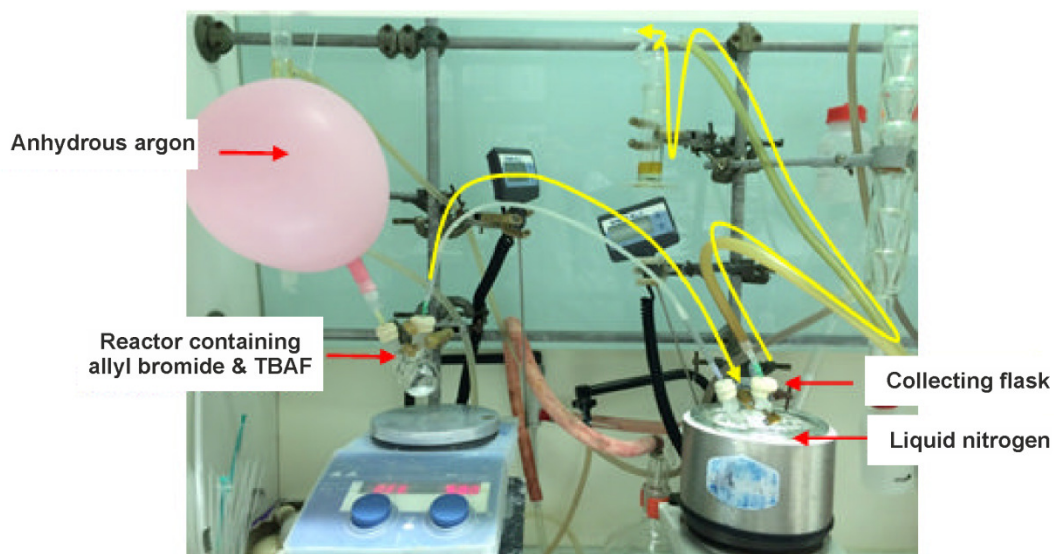
Under stirring, *N*-bromosuccinimide (1.65 g, 9.27 mmol, 1.0 eq.) and α,α'-azobisisobutyronitrile (152 mg, 0.93 mmol, 0.1 eq.) were added to a solution of **10-4b** (1.0 g, 9.00 mmol, 1.0 eq.) in carbon tetrachloride (30 mL). The mixture was heated at 60 °C for 22 hours. After cooling to rt, water (60 mL) was added and the aqueous layer was extracted with dichloromethane (2 × 50 mL). The combined organic layers were washed with brine (100 mL), dried over anhydrous magnesium sulfate, filtered and then concentrated under reduced pressure. The crude residue was purified by column chromatography (SiO<sub>2</sub>, dichloromethane/cyclohexane, 1/1, v/v) to give by order of elution: 2-dibromomethyl-6-fluoropyridine (0.31 g, 1.17 mmol, 13%) and the desired product (0.60 g, 3.16 mmol, 35%) as colorless oils. *R*<sub>f</sub> (SiO<sub>2</sub>, dichloromethane/cyclohexane, 1/1, v/v): 0.33; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.70 (m, 1H, H<sub>4</sub>), 7.22 (m, 1H, H<sub>5</sub>), 6.76 (m, 1H, H<sub>3</sub>), 4.36 (s, 2H, H<sub>a</sub>).

**General procedure for 10a and 10b:**

Hexamethylenetetramine (159 mg, 1.13 mmol, 1.0 eq.) was added under stirring to a solution of **10-6a** or **10-6b** (1.0 eq.) in chloroform (15 mL). The resulting mixture was stirred at 50 °C for 1 hour. After

cooling to rt, the solvent was removed under reduced pressure. The residue was dissolved with ethanol (10 mL) and concentrated hydrochloric acid (0.5 mL) was added. This mixture was stirred at 50 °C for 2 hours and at room temperature overnight. The solvent was then evaporated under reduced pressure to give the desired crude products **10a** (white solid) or **10b** (yellow solid) which were used in the next step without further purification.

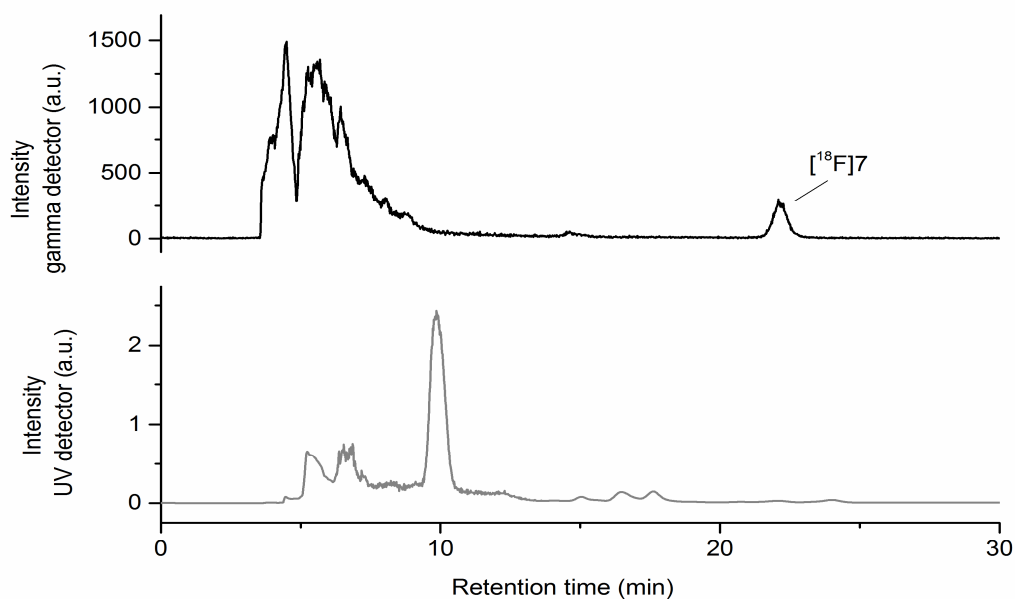
## 2. Production of allyl fluoride for the synthesis of 19:



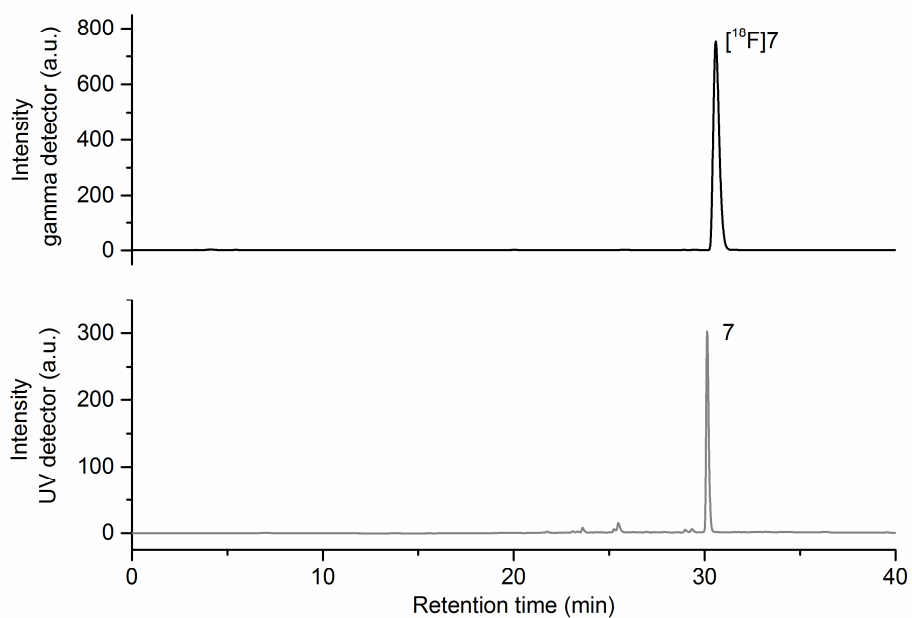
**Figure S1.** Installation for production of allyl fluoride

### 3. Radiosynthesis data

Chromatograms of the radiosynthesis of [<sup>18</sup>F]7 based on the tosylate precursor

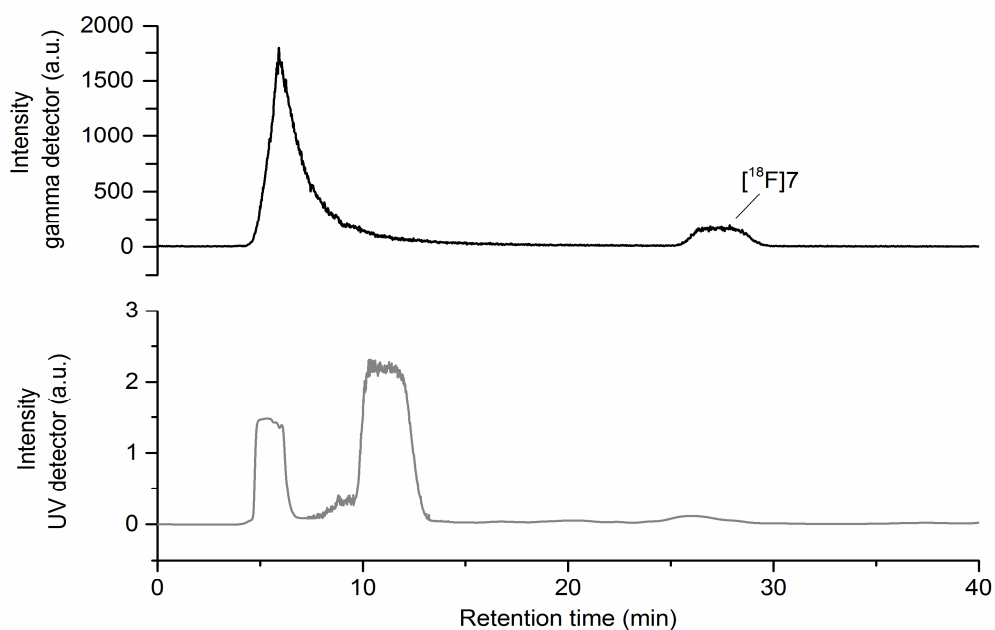


**Figure S2:** Exemplary semi-preparative HPLC chromatogram for separation of [<sup>18</sup>F]7 after labeling of the tosylate precursor (conditions: Reprisil-Pur AQ 250 × 10 mm, 55% ACN/40 mM NH<sub>4</sub>OAc<sub>aq</sub>, Flow 3.5 mL/min)

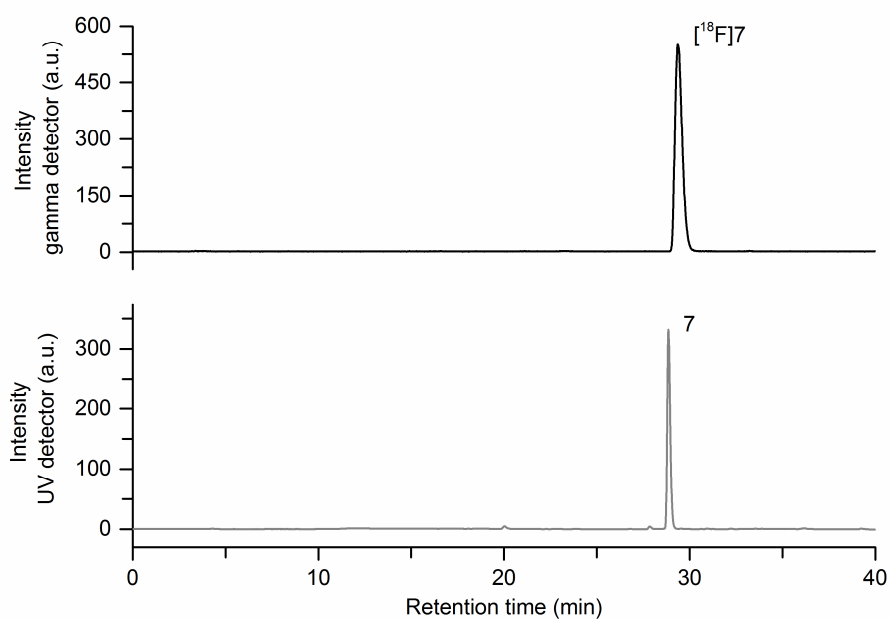


**Figure S3:** Radio- and UV-HPLC chromatogram of [<sup>18</sup>F]7 spiked with the nonlabeled reference compound **7**, (conditions: Reprisil-Pur AQ 250 × 4.6 mm, gradient run with ACN/20 mM NH<sub>4</sub>OAc<sub>aq</sub> system, Flow 1.0 mL/min).

Chromatograms of the radiosynthesis of [<sup>18</sup>F]7 based on the nosylate precursor



**Figure S4:** Exemplary semi-preparative HPLC chromatogram for separation of [<sup>18</sup>F]7 after labeling of the nosylate precursor (conditions: Reprisil-Pur AQ 250 × 10 mm, 55% ACN/40 mM NH<sub>4</sub>OAc<sub>aq.</sub>, Flow 2.8 mL/min)



**Figure S5:** Radio- and UV-HPLC chromatogram of [<sup>18</sup>F]7 spiked with the nonlabeled reference compound 7, (conditions: Reprisil-Pur AQ 250 × 4.6 mm, gradient run with ACN/20 mM NH<sub>4</sub>OAc<sub>aq.</sub> system, Flow 1.0 mL/min).

## References

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