Short Note

Preparation of a novel radiotracer targeting the EphB4 receptor via radiofluorination using spiro azetidinium salts as precursor

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Abstract. The visualization of Eph receptors, which are overexpressed in various tumor entities, using selective small molecule Eph inhibitors by means of PET is a promising approach for tumor imaging. *N*-(Pyrimidinyl)indazolamines represent a class of compounds, which are known to have high affinity especially for the EphB4 receptor. Radiofluorination of these compounds could provide a highly specific imaging agent and was investigated using a classical nucleophilic introduction of [^{18}F]fluoride as well as a less common nucleophilic ring opening reaction of azetidinium salts. In the past, radiofluorinations using azetidinium precursors were demonstrated to result in high radiochemical yields in short periods of time. For this purpose, an azetidinium precursor based on the *N*-(pyrimidinyl)indazolamine lead compound was developed and radiofluorination was successfully accomplished. The respective [^{18}F]radiotracer was quickly prepared with high radiochemical purity >97% and in a radiochemical yield of 34%.

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Introduction.

Aliphatic radiofluorinations commonly involve the nucleophilic introduction of [^{18}F]fluoride into alkyl compounds containing suitable leaving groups. To this end, there are two main, well-established methods for the synthesis of the resulting tertiary [^{18}F]fluoroethylamines and [^{18}F]fluoropropylamines as illustrated in Figure 1. The first method consists of preparing a [^{18}F]fluoropropyl or ethyl moiety (e.g. 1-bromo-3-[^{18}F]fluoropropane or 2-[^{18}F]fluoroethyl tosylate)[1] and subsequent introduction of this building block into secondary amine precursors using a nucleophilic displacement reaction. This two-step reaction results in overall RCYs ranging from 10 to 51%.[2,3,4,5,6,7] In the second strategy, a tertiary amine with a propyl residue, furnished with a good leaving group at the terminal carbon atom, is directly reacted with [^{18}F]fluoride. The use of tosylate or mesylate as leaving groups is preferred, leading to RCYs ranging from 33–70%.[2,8,9,10,11,12,13,14] A RCY of 88% was reported when using bromine as the leaving group.[15]

A third approach, rarely used in the past, consists of the application of aziridinium (three membered rings) and azetidinium (four membered rings) salts as precursors (Figure 1). The reactions of nucleophiles with aziridinium ions caused by ring opening of substituted heterocycles is reported to be facile[16] especially for the introduction of fluorine-18.[17,18,19] However, only little is known about ring opening reactions using azetidinium salts. It is known that these reactions lead to unbranched propyl chains when the heterocycle is unsubstituted[20,21,22] with yields of 57–68%.

The introduction of fluorine-18 via azetidinium mesylates was first investigated by Kiesewetter and Eckelman in 2004 with RCYs for the ^{18}F-containing compounds ranging from 56–75% using n.c.a [^{18}F]fluoride.[23] Furthermore, azetidinium salts were used to successfully label molecules, which are part of pyrido[2,3-*d*]pyrimidine-scaffold, with fluorine-18 in high RCYs (81-90%)[24] As Pyrido[2,3-*d*]pyrimidines are known as cyclin-dependent kinase (CDK) inhibitors. Finally, two radiofluorinated bioorthogonal building blocks 1-(2-azidoethyl)-4-(2-[^{18}F]fluoroethyl)piperazine ([^{18}F]AFP) and 1-(but-3-yn-1-yl)-4-(2-[^{18}F]fluoroethyl)piperazine ([^{18}F]BFP) were prepared (RCYs 29 and 31%, d.c.)[25] for mild radiolabeling via the Cu-catalyzed and strain-promoted click reaction as well as both variants of the Staudinger Ligation in the case of [^{18}F]AFP.

The advantages of azetidinium salts as precursor are a convenient preparation from the open-chained compound, milder reaction conditions and a fast reaction. The strained four-membered ring, two sites for the nucleophilic attack of the [^{18}F]fluoride and the ammonium residue as good leaving group enable a quick reaction time.

Our aim consisted of the development of a new radiolabeling procedure for a fluorine-18-containing radiotracer using azetidinium salts based on inhibitor molecules for the EphB4 receptor, because EphB4 with its preferential ligand, ephrinB2, also from a radiopharmacologist's point of view is one of the most important Eph receptors.[26] Pro-tumorigenic effects of EphB4 activation have been described for prostate cancer[27] and breast cancer, whereas EphB4 knockdown resulted in reduced tumor growth in mice.[28] Inconsistent outcome of EphB4/ephrinB2 signalling is also described for tumorigenesis. Anti-tumorigenic effects of activated EphB4 in mouse melanoma and breast cancer cells were observed, and reduction or loss of EphB4 expression in human colorectal tumors and invasive breast carcinoma.[29,30,31]

Several potent Eph kinase inhibitors were reported either based on high molecular weight compounds like peptides,[32,33] which block the extracellular domain of the appropriate

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receptor or based on small organic molecules[34,35,36] which bind to the intramolecular ATP binding pocket. To date, mainly peptide-based radiotracers containing Cu-64, In-111 or F-18 are known for multimodal imaging purposes using PET or SPECT which are specific for Eph receptors.[37,38,39]

Due to their favorable chemical and biological properties, small organic inhibitors (e.g. **I** or **III**) published by Bardelle *et al.* are the basis of our research.[40,41,42,43] Recently, the novel fluorine-18-containing radiotracer **II** based on the benzodioxolylpyrimidine structural motif was developed and analyzed via *in vitro* and *in vivo* studies.[44] Unfortunately, despite the high affinity of the original inhibitor **I** to the EphB4 receptor (IC_{50} = 90 nM, recombinant EphB4), the tracer **II** showed only a moderate uptake in tumor cells (A375) and no uptake *in vivo* in the respective tumor (A375) bearing mice. This can partially be explained due to its unfavorable (bio)chemical properties like a high lipophilicity (logP = 3.77). To overcome these problems, a novel lead structure was chosen based on a *N*-(pyrimidinyl)indazolamine core containing inhibitor **III** with an excellent affinity to the Eph receptor (IC_{50} = 1.3 nM)[42] and practical physicochemical properties like a logP = 2.98.

For this purpose, we have choosen compound **III** as basis published by Bardelle *et al.* in 2010.[42] Previous *in silico* studies were accomplished to find the best labeling positions (structures **IV**, **V**, and **VI** in Figure 2).[45] Based on these findings, we tried to prepare an ^{18}F-labeled compound **V** by replacing the methyl group of the original compound **III**, but without success. According to the *in silico* studies, compound **VI** should have comparable affinity. Thus, we aimed to prepare the radiotracer according this structure and to use azetidinium salts as precursor.

Results and Discussion.

Synthesis. The original compound, the resulting ^{18}F-tracer and its precursor consists of two molecules parts as shown in Figure 3. The first part of the inhibitor (part A) was synthesized as previously described.[45] The second structure (part B) was synthesized from 4-(3-iodo-5-nitrophenyl)morpholine (**1**) which was reacted with *N*-Boc-piperazine in a Buchwald-Hartwig reaction with xantphos and Pd_2dba_3. The cross-coupled product **2** was obtained in 71% yield. Afterwards, the protecting group was removed quantitatively, leading to **3** which was then reacted with either 1-fluoro-3-iodopropane leading to **4** (80% yield) or 3-bromopropanol leading to **5** (69% yield). Next, the nitro groups in **2**, **4**, and **5** were reduced yielding anilines **6-8** (95%, 78% and 100% yields, respectively). The complete pathway to anilines **6-8** is shown in Scheme 1.

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Finally, both parts A and B were connected. Thus, anilines **7** and **8** were reacted with **9** to give the EOE-protected reference compound **10** (62% yield) and the hydroxyl compound **11** (29% yield). The EOE group of **10** was cleaved under acidic conditions using 1 M HCl to give the final reference compound **14**.
To prepare the precursor, compound **11** was first treated with *p*-TsCl but no suitable product was isolable. Then, compound **11** was treated with MsCl to give the open chained compound **12**. After purification, **12** was dissolved in different solvents (dichloromethane, chloroform, acetonitrile) and warmed to prepare the final azetidinium precursor **13**. The progress of the reaction was monitored by ^{1}H NMR. For example, the signal for the mesyl group in **12** was found at δ = 3.01 ppm whereas the same signal for the mesylate of azetidinium salt **13** was found at δ = 2.76 ppm. The whole synthesis pathway is pointed out in Scheme 2.

Radiolabeling. Both precursors **12** and **13** were used for the radiolabeling. However under the used labeling conditions, **12** converted into **13**; no differences between **12** and **13** were found. Previously published conditions were used: K[^{18}F]F, K_2CO_3, K_{222}, anhydrous acetonitrile to give [^{18}F]**10** (t_R = 30.7 min).[45] For the removal of the EOE group, [^{18}F]**10** was treated with 2 M HCl to yield [^{18}F]**14** (t_R = 11.9 min) (Scheme 3/Figure 3). After deprotection, a spot at R_f = 0.37 (solvent: dichloromethane/methanol = 9/1) belonging to the final ^{18}F-tracer [^{18}F]**14** was determined by comparison with the non-radioactive compound **13** using radio-TLC analyses (Figure 4). Purification was done using semi-preparative HPLC yielding [^{18}F]**14** in 34% RCY.

logP determination: The lipophilicities of the original inhibitor **III** and the fluorine-containing compound **14** were determined according to the shake flask method reported elsewhere.[46] A logP value of 2.98 was determined for the original inhibitor **III** and a value of 2.55 for compound **14**. Both compounds seemed to be more hydrophilic compared to the previous prepared radiotracers. The results are summarized in Table 1.

Conclusion. In this short note, we have demonstrated the successful radiolabeling of a radiotracer based on the *N*-(pyrimidinyl)indazolamine core structure suitable for the visualization of the EphB4 receptor. [^{18}F]**14** was prepared via a nucleophilic ring opening reaction of the appropriate azetidinium salt precursor **13** in a RCY of 34% after cleavage of the EOE protecting group. The precursor **13** was formed from the respective open-chained mesyl compound **12**. Further analyses were accomplished showing an improved lipophilicity with a logP of 2.55 compared to the recently published tracer **II**. Biological and radiobiological studies are in progress and will be reported separately.

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Experimental

Materials and Methods

All reagents were purchased from commercial suppliers and used without further purification. Compounds **1** and **9** were prepared according to the literature. Analytical TLC was performed on pre-coated Silica Gel 60 F_{254} plates (Merck) and the results visualized under UV-light (λ = 254 nm). ^{1}H NMR, ^{13}C NMR and ^{19}F NMR spectra were recorded on a Varian Inova-400 or Agilent DD2-400 (OneNMR Probe) spectrometer at 400, 101, and 376 MHz, respectively. Chemical shifts are reported in ppm with tetramethylsilane (^{1}H, ^{13}C) and trichlorofluoromethane (^{19}F) as internal standard, respectively. MS spectra were obtained on a Xevo TQ-S (Waters) by electron spray (ESI) as the ionization method. Melting points were determined using a Cambridge Instruments Galen III apparatus and are uncorrected. Analytical HPLC was performed on a VWR/Hitachi Elite La Chrome HPLC system, equipped with a reverse phase column (Nucleosil 100-5C18 Nautilus), a UV-diode array detector (254 nm) and a scintillation radiodetector (Raytest, Gabi Star) at a flow rate of 1 mL/min (eluent: acetonitrile/water, 20:80 + 0.1% TFA, isocratic). The radioactive compound was identified with analytical radio-HPLC by comparison of the retention time of the reference compound. Decay-corrected RCYs were quantified by integration of radioactive peaks on a radio-TLC using a radio-TLC scanner (Fuji, BAS2000). [^{18}F]Fluoride was produced by the ^{18}O(p,n)^{18}F nuclear reaction utilizing the PET cyclotron Cyclone 18/9 (IBA, Belgium); [^{18}O]H_2O was irradiated.

tert-Butyl 4-(3-morpholino-5-nitrophenyl)piperazine-1-carboxylate **2**

Compound **1** (600 mg, 1.80 mmol) was dissolved in anhydrous toluene (15 mL) in a Schlenk tube under argon atmosphere. 1-Boc-piperazine (672 mg, 3.60 mmol) and Cs_2CO_3 (2.93 g, 8.99 mmol) were added. Pd_2dba_3 and xantphos were added in catalytic amounts. The mixture was stirred at 90 °C for 24 h. After cooling to rt, the reaction was quenched with H_2O (15 mL) and extracted with EtOAc (3 x 15 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure and the crude product was purified by flash column chromatography (PE–EtOAc = 2:1 → 1:1) to yield compound **2** (634 mg, 90%) as an orange solid. Mp. 202°C; R_f = 0.66 (PE–EtOAc = 1:1); ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 1.49 (9H, s, CH_3), 3.18–3.24 (8H, m, CH_2N), 3.59 (4H, t, ^{3}J = 5.1, CH_2N), 3.87 (4H, t, ^{3}J = 4.9, CH_2O), 6.64 (1H, t, ^{4}J = 2.1, 2-H), 7.26 (2H, d, ^{4}J = 2.1 Hz, 4/6-H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 28.6 (CH_3), 49.1 (CH_2N), 66.8 (CH_2O), 80.3 (C_q), 102.2, 102.7 (C-4/6), 108.6 (C-2), 150.3 (C-1), 152.7, 152.8 (C-3/5), 154.8 (C=O); MS (ESI$^+$): *m/z* 415 (49) [M$^+$+Na], 393 (100) [M$^+$+H]; elemental analysis calcd. for $C_{19}H_{28}N_4O_5$ (392.45): C, 58.15, H, 7.19, N, 14.28; found: C, 58.44, H, 7.50, N, 14.42%.

4-(3-Nitro-5-(piperazin-1-yl)phenyl)morpholine **3**

Compound **2** (1.85 g, 4.71 mmol) was dissolved in anhydrous dichloromethane (40 mL), trifluoroacetic acid (4 mL) was added dropwise and the mixture was stirred overnight at rt. After the removal of the solvent, 50 mL of a 10% K_2CO_3 solution was added and the aqueous layer extracted with dichloromethane (3 x 15 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure to yield compound **3** (1.36 g, 99%) as an orange solid without further purification. Mp 144 °C; R_f = 0.17 ($CHCl_3$–MeOH = 9:1); ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 1.68 (1H, br. s, NH), 3.04 (4H, t, ^{3}J = 4.9, CH_2N), 3.21 (8H, t, ^{3}J = 4.9, CH_2N), 3.87 (4H, t, ^{3}J = 4.9, CH_2O), 6.65 (1H, t, ^{4}J = 2.1, 6-H), 7.23 (1H, t, ^{4}J = 2.0, H_{Ar}), 7.27 (1H, t, ^{4}J = 2.0, H_{Ar}); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 46.1, 49.2, 50.1 (3 x CH_2N), 66.8 (CH_2O), 101.7, 102.4 (C-4/6), 108.1 (C-2), 150.3 (C-1), 152.7, 153.1 (C-3/5); MS (ESI$^+$): *m/z* 293 (100) [M$^+$+H]; elemental calcd. for $C_{14}H_{20}N_4O_3$ (292.33): C, 57.52, H, 6.90, N, 19.17; found: C, 57.36, H, 6.94, N, 19.04%.

4-(3-(4-(3-Fluoropropyl)piperazin-1-yl)-5-nitrophenyl)morpholine **4**

Compound **3** (500 mg, 2.57 mmol), 1-fluoro-3-iodopropane (482 mg, 2.57 mmol) and Et_3N (346 mg, 3.42 mmol) were dissolved in anhydrous THF (20 mL) and the reaction mixture was stirred at 60 °C overnight. The

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reaction was quenched with H_2O (30 mL), extracted with dichloromethane (3 x 20 mL), the organic layer was dried over Na_2SO_4 and the solvent removed. Purification was performed by flash chromatography ($CHCl_3$–MeOH = 20:1) to yield compound **4** as an orange solid (479 mg, 80%). Mp 102 °C; R_f = 0.82 ($CHCl_3$–MeOH = 10:1); ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 1.85–2.00 (2H, m, CH_2CH$_2$F), 2.55 (2H, t, ^3J = 7.4, CH_2N), 2.62 (4H, t, ^3J = 4.8, CH_2N), 3.21 (4H, t, ^3J = 5.0, CH_2N), 3.25 (4H, t, ^3J = 5.0, CH_2N), 3.86 (4H, t, ^3J = 5.0, CH_2O), 4.54 (2H, dt, $^2J_{H,F}$ = 47.2, ^3J = 5.9, CH_2F), 6.65 (1H, t, ^4J = 2.1, 2-H), 7.23 (1H, t, ^4J = 1.9, H_{Ar}), 7.27 (1H, t, ^4J = 2.0, H_{Ar}); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 28.1 (d, $^2J_{C,F}$ = 19.4, *C*H$_2$CH$_2$F), 48.9, 49.2, 53.1 (3 x CH_2N), 54.3 (d, $^4J_{C,F}$ = 5.3, CH_2N), 66.8 (CH_2O), 82.5 (d, $^1J_{C,F}$ = 165.1, CH$_2$*C*H$_2$F), 101.7, 102.4 (C-6/4), 108.0 (C-2), 150.3 (C-5), 152.6, 152.7 (C-1/3); ^{19}F NMR (376 MHz, $CDCl_3$): δ = –220.6; MS (ESI$^+$): *m/z* 352 (100) [M$^+$+H]; elemental calcd. for $C_{17}H_{25}FN_4O_3$ (352.40): C, 57.94, H, 7.15, N, 15.90; found: C, 57.76, H, 7.08, N, 15.35.

3-(4-(3-Morpholino-5-nitrophenyl)piperazin-1-yl)propan-1-ol **5**

Compound **3** (500 mg, 1.71 mmol), 3-bromopropan-1-ol (380 mg, 2.73 mmol) and Et_3N (0.38 mL, 2.73 mmol) were dissolved in anhydrous THF (15 mL), NaI (50 mg) was added and the reaction mixture was stirred at 60 °C overnight. The mixture was treated with H_2O (20 mL), extracted with dichloromethane (3 x 20 mL), the organic layer was dried over Na_2SO_4 and the solvent removed. Purification was performed by flash chromatography ($CHCl_3$–MeOH = 10:1) to yield compound **5** as an orange solid (530 mg, 88 %). Mp 128 °C, R_f = 0.53 ($CHCl_3$–MeOH = 9:1); ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 1.78 (2H, qi, ^3J = 5.6, CH_2CH$_2$OH), 2.65–2.72 (6H, m, 3 x CH_2N), 3.21 (4H, t, ^3J = 4.8, CH_2N), 3.26 (4H, t, ^3J = 5.0, CH_2N), 3.83 (2H, t, ^3J = 5.3, CH_2OH), 3.86 (4H, t, ^3J = 4.8, CH_2O), 6.63 (1H, t, ^4J = 2.1, 2-H), 7.23 (1H, t, ^4J = 2.0, H_{Ar}), 7.26 (1H, t, ^4J = 2.0, H_{Ar}). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 27.3 (*C*H$_2$CH$_2$O), 48.9, 49.1, 53.2, 58.7 (4 x CH_2N), 64.5, 66.8 (2 x CH_2O), 101.9, 102.6 (C-6/4), 108.1 (C-2), 150.3 (C-5), 152.5, 152.7 (C-1/3); MS (ESI$^+$): *m/z* 351 (100, M$^+$+H); elemental calcd. for $C_{17}H_{26}N_4O_4$ (350.41): C, 58.27, H, 7.48, N, 15.99; found C, 58.06, H, 7.51, N, 16.13.

3-(-4-*tert*-Butoxycarbonyl)piperazino-5-morpholinoaniline **6**

Compound **2** (930 mg, 2.37 mmol) was dissolved in $CHCl_3$/MeOH (20 mL, v:v = 1:1) and Pd/C (500 mg) was added. The resulting mixture was treated with H_2 (approx. 1 bar) at rt for 24 h. Afterwards, the catalyst was filtered and washed with $CHCl_3$ (10 mL). The solvent of the combined filtrates was removed under reduced pressure to yield compound **6** (819 mg, 95%) as colorless solid. Mp 173 °C; R_f = 0.06 (PE–EtOAc = 1:1); ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 1.47 (9H, s, CH_3), 3.07–3.12 (8H, m, CH_2N), 3.54 (4H, t, ^3J = 5.2, CH_2N), 3.82 (4H, t, ^3J = 4.9, CH_2O), 5.84 (2H, d, ^4J = 1.7, 2/6-H), 5.93 (1H, t, ^4J = 1.7, 4-H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 28.6 (CH), 49.7, 49.8 (2 x CH_2N), 67.1 (CH_2O), 80.0 (C_q), 96.0, 96.2 (C-2/4), 96.6 (C-6), 148.1 (C-3), 153.5, 153.6 (C-1/5), 154.9 (C=O); MS (ESI$^+$): *m/z* 363 (100, M$^+$+H); elemental calcd. for $C_{19}H_{30}N_4O_3$ (362.47): C, 62.96, H 8.34, N, 15.46; found: C, 63.07, H, 8.40, N, 15.38%.

3-(4-(3-Fluoropropyl)piperazin-1-yl)-5-morpholinoaniline **7**

Compound **4** (479 mg, 1.49 mmol) was dissolved in MeOH (50 mL) and Pd/C (150 mg) was added. The resulting mixture was treated with H_2 (approx. 1 bar) at rt for 24 h. Afterwards, the catalyst was filtered and washed with $CHCl_3$ (10 mL). The solvent of the combined filtrates was removed under reduced pressure to yield compound **7** (438 mg, 100%) as brown syrup. R_f = 0.37 ($CHCl_3$–MeOH = 10:1); ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 1.83–1.99 (2H, m, CH_2CH$_2$F), 2.52 (2H, t, ^3J = 7.4, CH_2N), 2.58 (4H, t, ^3J = 5.0, CH_2N), 3.10 (4H, t, ^3J = 4.8, CH_2N), 3.15 (4H, t, ^3J = 5.1, CH_2N), 3.82 (4H, t, ^3J = 4.8, CH_2O), 4.52 (2H, dt, $^2J_{H,F}$ = 47.1, ^3J = 6.0, CH_2F), 5.81 (1H, t, ^4J = 1.9, H_{Ar}), 5.85 (1H, t, ^4J = 1.9, H_{Ar}), 5.93 (1H, t, ^4J = 1.9, H_{Ar}); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 28.0 (d, $^2J_{C,F}$ = 19.7, *C*H$_2$CH$_2$F), 49.4, 49.8, 53.4 (3 x CH_2N), 54.4 (d, $^4J_{C,F}$ = 5.5, CH_2N), 67.1 (CH_2O), 82.6 (d, $^1J_{C,F}$ = 164.4, CH$_2$*C*H$_2$F), 95.6, 95.8, 96.2 (3 x Ar-CH), 148.0, 153.4, 153.5 (3 x Ar-C_q); ^{19}F NMR (376 MHz, $CDCl_3$): δ = –220.4; MS (ESI$^+$): *m/z* 345 (9, M$^+$+Na), 323 (46, M$^+$+H); elemental calcd. for $C_{17}H_{27}FN_4O$ (322.42): C, 63.33, H, 8.44, N, 17.38; found: C, 63.03, H, 8.41, N, 17.44.

3-(4-(3-Amino-5-morpholinophenyl)piperazin-1-yl)propan-1-ol **8**

Compound **5** (1.10 g, 3.14 mmol) was dissolved in $CHCl_3$ (20 mL) and Pd/C (200 mg) was added. The resulting mixture was treated with H_2 (approx. 1 bar) at rt for 24 h. Afterwards, the catalyst was filtered and washed with $CHCl_3$ (10 mL). The solvent of the combined filtrates was removed under reduced pressure to yield compound **8** (887 mg, 88%) as light brown syrup. R_f = 0.22 ($CHCl_3$–MeOH = 10:1); ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 1.80 (2H, qi, ^3J = 5.2, CH_2CH$_2$OH), 2.70–2.80 (6H, m, 3 x CH_2N), 3.10 (4H, t, ^3J = 4.8, CH_2N), 3.21 (4H, t, ^3J = 4.8, CH_2N), 3.79–3.86 (6H, m, CH_2O/CH$_2$OH), 5.84 (2H, d, ^4J = 2.0, 2-/5-H), 5.92 (1H, t, ^4J = 2.0, 4-H). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 27.2 (*C*H$_2$CH$_2$O), 49.5, 49.8, 53.5, 58.9 (4 x CH_2N), 64.5, 67.1 (2 x CH_2O), 95.9, 96.0,

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96.4 (3 x Ar-CH), 148.1, 153.3, 153.5 (3 x Ar-C_q). MS (ESI^+): *m/z* 321 (100, M^++H); elemental calcd. for $C_{17}H_{28}N_4O_2$ (320.43): C, 63.72, H, 8.81, N, 17.48; found: C, 63.88, H, 8.66, N, 17.28.

N^2-(3-(4-(3-Fluoropropyl)piperazin-1-yl)-5-morpholinophenyl)-N^4-(1-(1-ethoxyethyl)-1*H*-indazol-4-yl)-N^4-methylpyrimidin-2,4-diamine **10**

Compound **7** (358 mg, 1.11 mmol) and compound **9** (405 mg, 1.22 mmol) were dissolved in anhydrous dioxane (10 mL), *p*-TsOH·H_2O was added in catalytic amounts and the resulting mixture was stirred at 90°C for 24 h. After cooling to rt, saturated hydrogencarbonate solution was added (30 mL), the aqueous layer was extracted with EtOAc (3 x 20 mL), the combined organic layers were dried over Na_2SO_4 and the solvent was removed. Purification was performed by flash chromatography ($CHCl_3$–MeOH = 25:1) to yield compound **10** as a brownish solid (428 mg, 62%). R_f = 0.63 ($CHCl_3$–MeOH = 4:1); ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 7.82 (d, $^5J_{3,5}$ = 0.7 Hz, 1H, H-3), 7.80 (d, $^3J_{6',5'}$ = 6.0 Hz, 1H, H-6'), 7.68 (d, $^3J_{7,6}$ = 8.6 Hz, 1H, H-7), 7.42 (dd, $^3J_{6,5}$ = 7.4, $^3J_{6,7}$ = 8.6 Hz, 1H, H-6), 7.07 (dd, $^3J_{5,6}$ = 7.4 Hz, $^5J_{5,3}$ = 0.7 Hz, 1H, H-5), 6.97 (s, 1H, NH), 6.90 - 6.88 (m, 2H, H-2"/6"), 6.19 (t, $^4J_{4'',2''} = {}^4J_{4'',6''}$ = 2.0 Hz, 1H, H-4"), 5.91 (q, ^3J = 6.1 Hz, 1H, CH), 5.71 (d, $^3J_{5',6'}$ = 6.0 Hz, 1H, H-5'), 4.53 (dt, ^3J = 5.9 Hz; $^2J_{H,F}$ = 47.3 Hz, 2H, CH_2F), 3.85 (t, 3*J* = 4.9 Hz, 4H, CH_2N), 3.63 (s, 3H, CH_3), 3.49 (dq, ^{2}J = 9.3 Hz, ^3J = 7.1 Hz, 1H, CH_2CH_3), 3.32 (dq, ^2J = 9.3 Hz, ^3J = 7.1 Hz, 1H, CH_2CH_3), 3.23 (t, ^3J = 4.9 Hz, 4H, CH_2N), 3.17 (t, ^3J = 4.9 Hz, 4H, CH_2N), 2.61 (t, ^3J = 4.9 Hz, 4H, CH_2N), 2.53 (t, ^3J = 7.4 Hz, 2H, CH_2N), 1.99 - 1.86 (m, 2H, $CH_2CH_2CH_2$), 1.82 (d, ^3J = 6.1 Hz, 3H, $CHCH_3$), 1.17 (t, ^3J = 7.1 Hz, 3H, CH_2CH_3). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 163.0 (C-4'), 159.8 (C-2'), 155.8 (C-6'),152.9, 152.6 (C-3''/-5''), 140.8 (C-7a) 140.1 (C-4), 136.8 (C-1''), 131.2 (C-3), 127.4 (C-6), 122.1 (C-3a), 119.4 (C-5), 110.6 (C-5'), 100.8 (C-7), 100.3 (C-4''), 99.8, 97.3 (C-2''/-6''), 87.6 (CH), 82.4 (d, $^1J_{C,F}$ = 164.5 Hz, CH_2F), 67.1 (CH_2O), 64.1 (CH_2), 54.5 (d, $^3J_{C,F}$ = 5.4 Hz, CH_2N), 53.3, 49.9, 49.4 (3 x CH_2N), 38.6 (CH_3), 27.7 (d, $^2J_{C,F}$ = 18.8 Hz, CH_2CH_2F) 21.1, 15.0 (2 x CH_3). ^{19}F NMR (376 MHz, $CDCl_3$): δ = – 220.2. MS (ESI+): *m/z* (%) = 618 (30) [M^++H], 546 (100) [M^++H–EOE].

N^2-(3-(4-(3-Hydroxypropyl)piperazin-1-yl)-5-morpholinophenyl)-N^4-(1-(1-ethoxyethyl)-1*H*-indazol-4-yl)-N^4-methylpyrimidin-2,4-diamine **11**

Compound **8** (159 mg, 0.50 mmol) and compound **9** (181 mg, 0.55 mmol) were dissolved in anhydrous dioxane (7 mL), *p*-TsOH·H_2O was added in catalytic amounts and the resulting mixture was stirred at 90°C for 24 h. After cooling to rt, saturated hydrogencarbonate solution was added (20 mL), the aqueous layer was extracted with EtOAc (3 x 15 mL), the combined organic layers were dried over Na_2SO_4 and the solvent was removed. Purification was performed by flash chromatography ($CHCl_3$–MeOH = 20:1→10:1) to yield compound **11** as a brownish oil (90 mg, 29%). R_f = 0.52 ($CHCl_3$–MeOH = 4:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = = 7.81 (d, $^5J_{3,5}$ = 0.7 Hz, 1H, H-3), 7.79 (d, $^3J_{6',5'}$ = 6.0 Hz, 1H, H-6'), 7.68 (d, $^3J_{7,6}$ = 8.6 Hz, 1H, H-7), 7.41 ("t", $^3J_{6,5}$ = 7.4 Hz, $^3J_{6,7}$ = 8.6 Hz, 1H, H-6), 7.20 (s, 1H, NH), 7.07 (dd, $^3J_{5,6}$ = 7.4 Hz, $^5J_{5,3}$ = 0.7 Hz, 1H, H-5), 6.91 (t, ^3J = 2.0 Hz, 1H, HAr), 6.88 (t, ^3J = 2.0 Hz, 1H, HAr), 6.17 (t, $^4J_{4'',2''} = {}^4J_{4'',6''}$ = 2.0 Hz, 1H, H-4"), 5.91 (q, ^3J = 6.1 Hz, 1H, CH), 5.71 (d, $^3J_{5',6'}$ = 6.0 Hz, 1H, H-5'), 3.85 - 3.80 (m, 6H, CH_2O, CH_2OH), 3.62 (s, 3H, CH_3), 3.49 (dq, ^2J = 9.3 Hz, ^3J = 7.1 Hz, 1H, C$\underline{H}_2$CH$_3$), 3.31 (dq, ^2J = 9.3 Hz, ^3J = 7.1 Hz, 1H, C$\underline{H}_2$CH$_3$), 3.22 (t, ^3J = 5.0 Hz, 4H, CH_2N), 3.16 (t, ^3J = 4.8 Hz, 4H, CH_2N), 2.69–2.66 (m, 6H, CH_2N), 1.82 (d, ^3J = 6.1 Hz, 3H, CHC$\underline{H}_3$), 1.78 - 1.73 (m, 2H, $CH_2C\underline{H}_2CH_2$), 1.17 (t, ^3J = 7.1 Hz, 3H, $CH_2C\underline{H}_3$). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 162.9 (C-4'), 159.5 (C-2'), 155.1 (C-6'),152.9, 152.7 (C-3''/-5''), 150.5 (C-1''), 141.6 (C-7a) 140.1 (C-4), 131.6 (C-3), 127.4 (C-6), 122.3 (C-3a), 119.4 (C-5), 110.0 (C-5'), 100.4 (C-7), 100.0 (C-4''), 99.3, 97.5 (C-2''/-6''), 87.4 (CH), 67.1, 64.5 (2 x CH_2O), 64.0 (CH_2), 58.9, 53.5, 50.0, 49.7 (4 x CH_2N), 38.3 (CH_3), 27.2 ($CH_2\underline{C}H_2CH_2$), 21.1, 15.0 (2 x CH_3). MS (ESI+): *m/z* (%) = 616 (40) [M^++H], 544 (100) [M^+–EOE].

N^2-(3-(4-(3-Fluoropropyl)piperazin-1-yl)-5-morpholinophenyl)-N^4-(1*H*-indazol-4-yl)-N^4-methylpyrimidin-2,4-diamine **14**

Compound **10** (384 mg, 0.62 mmol) was treated with HCl (12 mL, 1 M) and stirred at rt for 1.5 h. Afterwards, a saturated hydrogencarbonate solution was added for neutralization, the aqueous layer was extracted with chloroform (3 x 20 mL), the combined organic layers were dried over Na_2SO_4 and the solvent was removed. Purification was performed by flash chromatography ($CHCl_3$–MeOH = 30:1) to yield compound **14** as a brownish oil (288 mg, 85%). R_f = 0.35 ($CHCl_3$–MeOH = 9:1); ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 10.56 (s, 1H, NH), 7.91 (d, $^5J_{3,5}$ = 0.8 Hz, 1H, H-3), 7.81 (d, $^3J_{6',5'}$ = 6.0 Hz, 1H, H-6'), 7.46 - 7.41 (m, 2H, H-6/-7), 7.10 (s, 1H, NH), 7.08 (dd, $^3J_{5,6}$ = 6.5 Hz, $^5J_{5,3}$ = 0.8Hz, 1H, H-5), 6.90 - 6.88 (m, 2H, H-2"/-6"), 6.19 (t, $^4J_{4'',2''} = {}^4J_{4'',6''}$

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= 2.1 Hz, 1H, H-4"), 5.71 (d, $^3J_{5',6'}$ = 6.0 Hz, 1H, H-5'), 4.53 (dt, ^3J = 6.0 Hz; $^2J_{H,F}$ = 47.3 Hz, 2H, CH_2F), 3.85 (t, ^3J = 4.8 Hz, 4H, CH_2O), 3.63 (s, 3H, CH_3), 3.22 (t, ^3J = 4.8 Hz, 4H, CH_2N), 3.16 (t, ^3J = 4.8 Hz, 4H, CH_2N), 2.61 (t, ^3J = 4.8 Hz, 4H, CH_2N), 2.54 (t, ^3J = 7.4 Hz, 2H, CH_2N), 1.99 - 1.86 (m, 2H, CH_2). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 163.0 (C-4‘), 159.9 (C-2‘), 155.7 (C-6‘),152.9, 152.9 (C-3’’/-5’’), 141.8 (C-7a) 141.7 (C-4), 137.5 (C-1‘‘), 133.2 (C-3), 127.8 (C-6), 120.7 (C-3a), 119.3 (C-5), 109.1 (C-5’), 100.2 (C-7), 99.7 (C-4’’), 99.1, 97.4 (C-2’’/-6’’), 82.7 (d, $^1J_{C,F}$ = 164.5 Hz, CH_2F), 67.2 (CH_2O), 54.5 (d, $^3J_{C,F}$ = 5.5 Hz, CH_2N), 53.4, 50.0, 49.7 (3 x CH_2N), 38.3 (CH_3), 28.0 (d, $^2J_{C,F}$ = 19.8 Hz, CH_2CH_2F). ^{19}F NMR (376 MHz, $CDCl_3$): δ = – 220.0 ppm. MS (ESI+): *m/z* (%) = 546 (100) [M^++H].

N^2-(3-(4-(3-Methylsulfonyloxypropyl)piperazin-1-yl)-5-morpholinophenyl)-N^4-(1-(1-ethoxyethyl)-1*H*-indazol-4-yl)-N^4-methylpyrimidin-2,4-diamin **12**

Compound **11** (65 mg, 0.11 mmmol) was dissolved in anhydrous dichloromethane (10 mL), MsCl (18 mg, 0.16 mmol) and DIPEA (27 mg, 0.21 mmol) were added and the resulting mixture was stirred for 1 h at rt. Afterwards, water (10 mL) was added and the aqueous layer was extracted with dichloromethane (3 x 5 mL), the combined organic layers were dried over Na_2SO_4 and the solvent was removed at temperatures below 30°C to yield compound **12** as a light brownish oil (64 mg, 87%). R_f = 0.55 ($CHCl_3$–MeOH = 10:1); ^{1}H NMR: (400 MHz, $CDCl_3$): δ = 7.81 (d, $^5J_{3,5}$ = 0.8 Hz, 1H, H-3), 7.79 (d, $^3J_{6',5'}$ = 6.0 Hz, 1H, H-6'), 7.68 (d, $^3J_{7,6}$ = 8.5 Hz, 1H, H-7), 7.41 ("t", $^3J_{6,7}$ = 8.5 Hz, $^3J_{6,5}$ = 7.4 Hz, 1H, H-6), 7.12 (s, 1H, NH), 7.07 (dd, $^3J_{5,6}$ = 7.4 Hz, $^5J_{5,3}$ = 0.8 Hz, 1H, H-5), 6.89 (m, 2H, H_{ar}), 6.18 (t, $^4J_{4'',2''}$ = $^4J_{4'',6''}$ = 2.0 Hz, 1H, H-4"), 5.92 (q, ^3J = 6.1 Hz, 1H, CH), 5.71 (d, $^3J_{5',6'}$ = 6.0 Hz, 1H, H-5'), 4.32 (t, ^3J = 7.0 Hz, 2H, CH2N), 3.85 - 3.83 (m, 4H, CH_2O), 3.62 (s, 3H, CH_3), 3.49 (dq, ^2J = 9.3 Hz, ^3J = 7.0 Hz, 1H, C<u>H</u>$_2$CH$_3$), 3.31 (dq, ^2J = 9.3 Hz, ^3J = 7.0 Hz, 1H, CH_2CH_3), 3.21 (t, ^3J = 5.0 Hz, 4H, CH_2N), 3.17 (t, ^3J = 5.0 Hz, 4H, CH_2N), 3.01 (s, 3H, CH_3S), 2.59 (t, ^3J = 5.0 Hz, 4H, CH_2N), 2.51 (t, ^3J = 7.0 Hz, 2H, CH_2N), 1.99 - 1.93 (m, 2H, CH_2), 1.81 (d, ^3J = 6.1 Hz, 3H, CHC<u>H</u>$_3$), 1.17 (t, ^3J = 7.0 Hz, 3H, CH_2C<u>H</u>$_3$). ^{13}C NMR (101 MHz, $CDCl_3$): δ = 162.9 (C-4‘), 159.8 (C-2‘), 155.7 (C-6‘),152.9, 152.8 (C-3’’/-5’’), 150.5 (C-1’’), 141.7 (C-7a) 140.1 (C-4), 131.6 (C-3), 127.4 (C-6), 122.3 (C-3a), 119.4 (C-5), 109.9 (C-5’), 100.2 (C-7), 99.6 (C-4’’), 99.0, 97.5 (C-2’’/-6’’), 87.4 (CH), 68.5, 67.1 (2 x CH_2O), 64.0 (CH_2), 54.1, 53.4, 50.0, 49.7 (4 x CH_2N), 38.2, 37.4 (2 x CH_3), 26.6 (CH_2), 21.1, 15.0 (2 x CH_3). MS (ESI+): *m/z* (%) = 636 (100) [M^++H–EOE].

N^2-(3-(4-(3-[^{18}F]Fluoropropyl)piperazin-1-yl)-5-morpholinophenyl)-N^4-(1*H*-indazol-4-yl)-N^4-methylpyrimidin-2,4-diamine [^{18}F]**14**

An anion-exchange cartridge (Waters, Sep-Pak Light Accell Plus QMA) was activated by rinsing with 10 mL of a 1M $NaHCO_3$ solution and 10 mL of deionized H_2O. [^{18}F]Fluoride was trapped on the cartridge, eluted with a Kryptofix K 2.2.2./K_2CO_3 solution (1.5 mL) into the reaction vessel and dried via azeotropic distillation with anhydrous acetonitrile (3 x 2 mL) under a stream of nitrogen at 100°C for approx. 30 min. Precursor **12** or **13** (~3 mg) was dissolved in 500 µL of anhydrous acetonitrile, and the mixture was added to the [^{18}F]fluoride-containing sealed vial. The resulting solution was heated at 100°C for 30 min. Afterwards, HCl (100 µL / 2 M) was added and the mixture was heated at 100°C for further 5 min. Next, the mixture was treated with 15 mL deionized H_2O and then passed through a C18 cartridge (LiChrolut RP-18/Merck). After elution with ethanol, samples for analytical radio-TLC and radio-HPLC were taken. Analytical radio-TLC: R_f = 0.37 (dichloromethane/methanol, 9/1). Analytical radio-HPLC: t_R = 11.9 min (eluent: acetonitrile/water 20/80 + 0.1% TFA). Purification was done via semi-preparative radio-HPLC.

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Table 1. Comparison of calculated vs. determined logP values.

compound	calcd. logP[47]	calcd. logP[48]	expt. logP
I	3.30	2.77	n.d.
II	3.77	3.11	n.d.
III	3.42	3.40	2.98
14	3.87	3.34	2.55

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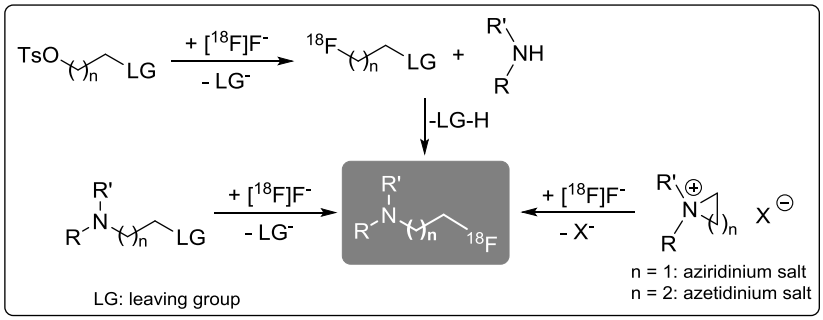

Figure 1. General strategies for the insertion of a [^{18}F]fluoroethyl or [^{18}F]fluoropropyl moiety.

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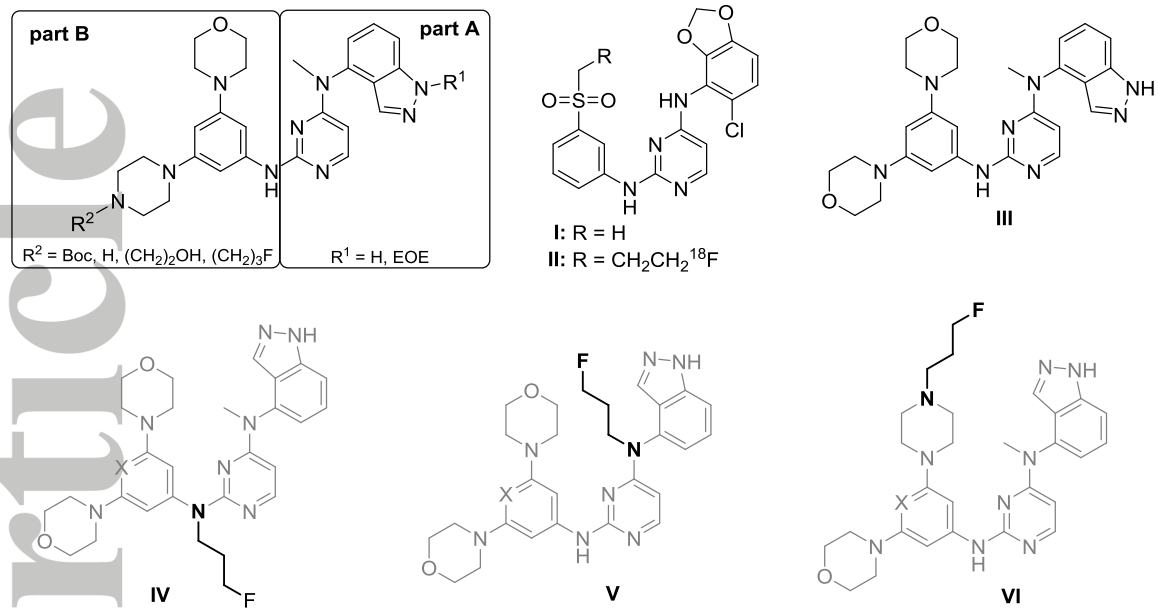

Figure 2. Overview of original inhibitors **I** and **III** as basis for the development of possible radiotracers **IV** – **VI** and recently prepared [^{18}F]tracer **II**.

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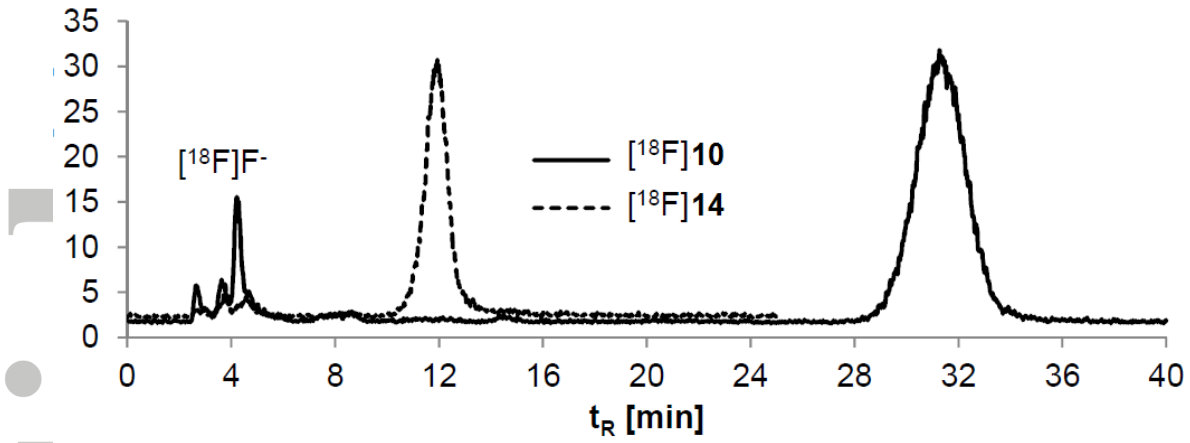

Figure 3. Radio-HPLC chromatogram of [^{18}F]**10** (EOE protected) and the final tracer [^{18}F]**14** (deprotected) after purification.

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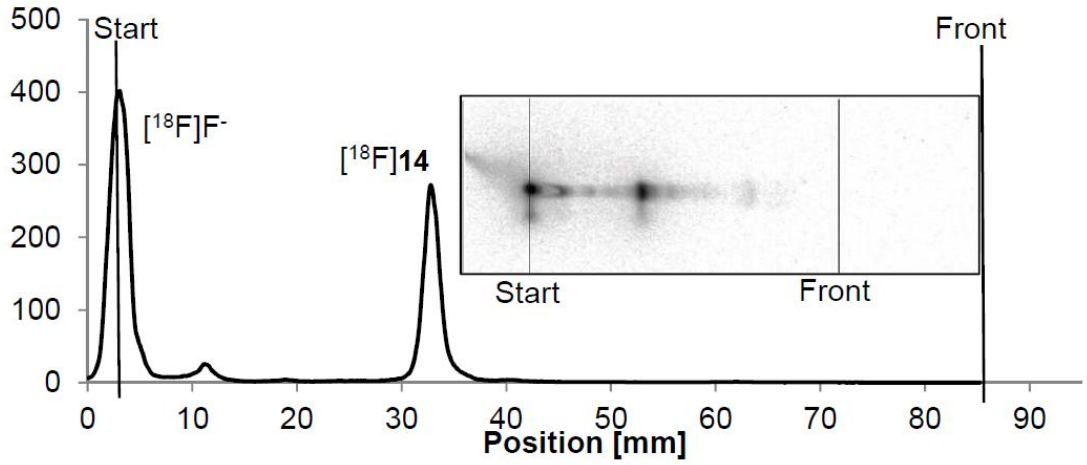

Figure 4. Radio-TLC chromatogram of the final tracer [^{18}F]**14** from the reaction mixture after deprotection.

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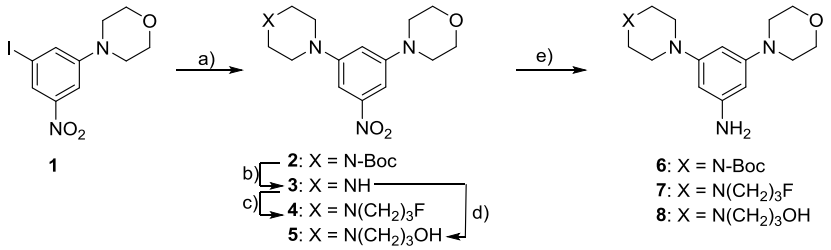

Scheme 1. Synthesis pathway for substructure B. *Reagents and conditions:* a) *N*-Boc-piperazine, Cs_2CO_3, xantphos, Pd_2dba_3, 90°C, 2 d; b) TFA, DCM, rt, overnight; c) 1-fluoro-3-iodopropane, Et_3N, THF, 60°C, overnight; d) 3-bromopropanol, NaI, Et_3N, THF, 60°C, overnight; e) Pd/C-H_2, MeOH-CH_2Cl_2, rt, 24 h.

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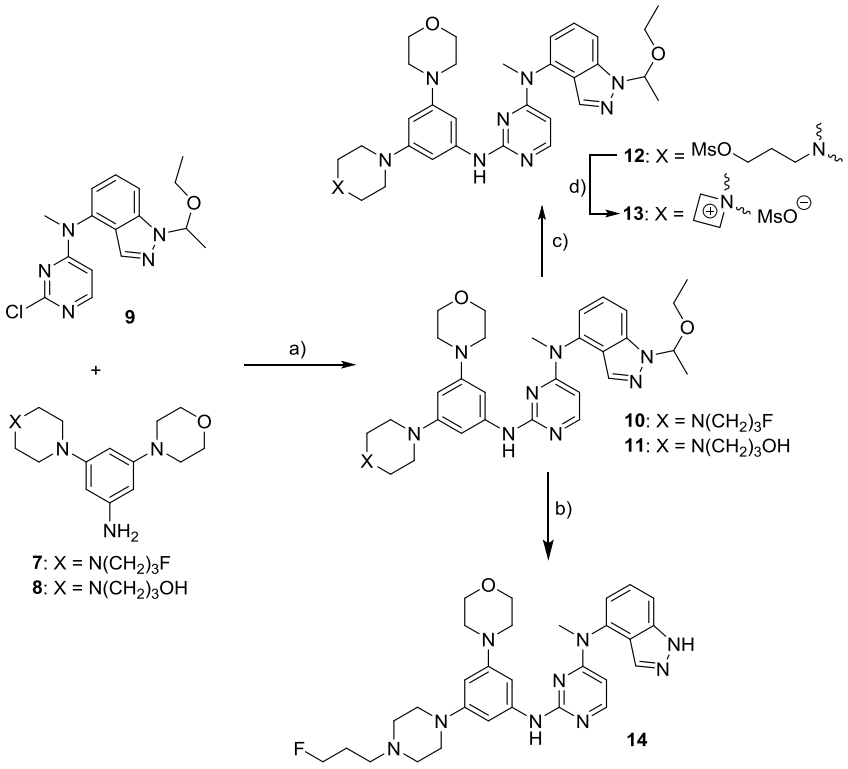

Scheme 2. Synthesis pathway for precursor **13** and reference compound **14**. *Reagents and conditions:* a) *p*-TsOH, dioxane, 90°C, overnight; b) HCl, EtOH, 1.5 h; c) MsCl, DCM, rt, 60 min; d) acetonitrile, 60°C, 3 h.

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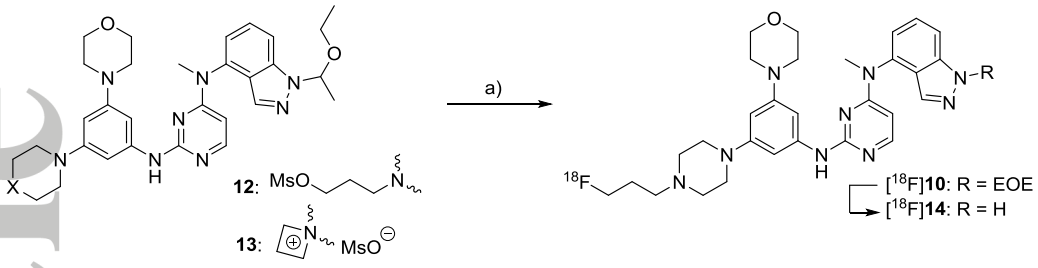

Scheme 3. General radiolabeling procedure for [^{18}F]**14** from precursors **12** and **13**. Reagents and conditions: a) K[^{18}F]F, K_2CO_3, K_{222}, acetonitrile, 100°C, 30 min.

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