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Research Article

^{18}F-labelling of a potent nonpeptide CCR1 antagonist: synthesis of 1-(5-chloro-2-{2-[(2R)-4-(4-[^{18}F]fluorobenzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea in an automated module

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Summary

The synthesis of 1-(5-chloro-2-{2-[(2R)-4-(4-[^{18}F]fluorobenzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea (**[^{18}F]4**), a potent nonpeptide CCR1 antagonist, is described as a module-assisted two-step one-pot procedure. The final product was obtained utilizing the reductive amination of the formed 4-[^{18}F]fluorobenzaldehyde (**2**) with a piperazine derivative **3** and sodium cyanoborohydride. After HPLC purification of the final product **[^{18}F]4**, its solid phase extraction, formulation and sterile filtration, the isolated (not decay-corrected) radiochemical yields of **[^{18}F]4** were between 7 and 13% ($n = 28$). The time of the entire manufacturing process did not exceed 95 min. The radiochemical purity of **[^{18}F]4** was higher than 95%, the chemical purity $\geqslant 60\%$ and the enantiomeric purity $> 99.5\%$. The specific radioactivity was in the range of 59–226 GBq/μmol at starting radioactivities of 23.6–65.0 GBq [^{18}F]fluoride. Copyright © 2006 John Wiley & Sons, Ltd.

Key Words: positron emission tomography; Alzheimer's disease; 4-[^{18}F]fluorobenzaldehyde; reductive amination; CCR1 antagonist; automated module synthesis

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Introduction

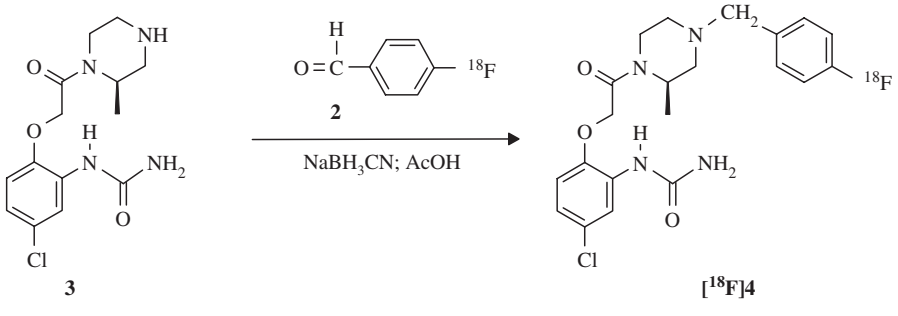
The hydrochloric acid salt of 1-(5-chloro-2-{2-[(2R)-4-(4-fluorobenzyl)-2-methyl-piperazin-1-yl]-2-oxoethoxy}phenyl)urea with the abbreviation BX 471 was described as a potent, selective and orally active nonpeptide antagonist of the CC chemokine receptor-1 (CCR1).[1] The CCR1 is a prime therapeutic target for treating autoimmune diseases, e.g. chronic inflammatory diseases. It was further discovered that CCR1 is upregulated in dystrophic neurites found in and around the amyloid plaques of Alzheimer's disease.[2] These CCR1-positive plaques were seen in very early stages of dementia and were also found to increase as the degree of dementia increased.[2] In healthy brain, expression of CCR1 was negligible.[2]

These data have encouraged us to develop a radiolabelled derivative of the CCR1 antagonist BX 471, mentioned above as a possible imaging agent for the reliable and early diagnosis of Alzheimer's disease by positron emission tomography (PET).[3] Accordingly, we have searched for a method for labelling BX 471 with the positron-emitting radionuclide ^{18}F ($t_{1/2} = 109.8$ min), which can be carried out as one-pot reaction in an automated module. The ^{18}F-labelled compound 1-(5-chloro-2-{2-[(2R)-4-(4-[^{18}F]fluorobenzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea (**[^{18}F]4**) has been evaluated in a human study for its potential to image Alzheimer's disease by PET.

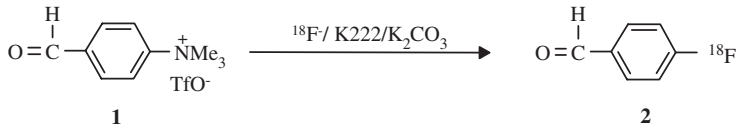
Results and discussion

Starting from a suitable precursor, the 1-(5-chloro-2-{2-[(2R)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea (**3**)[3], **[^{18}F]4** was synthesized using a published method of reductive amination of 4-[^{18}F]fluorobenzaldehyde (**2**)[4,5] with the piperazine derivative **3** and sodium cyanoborohydride according to Scheme 1.

The first step of this labelling way is the synthesis of the important ^{18}F-labelling moiety **2** by aromatic nucleophilic substitution of [^{18}F]fluoride on

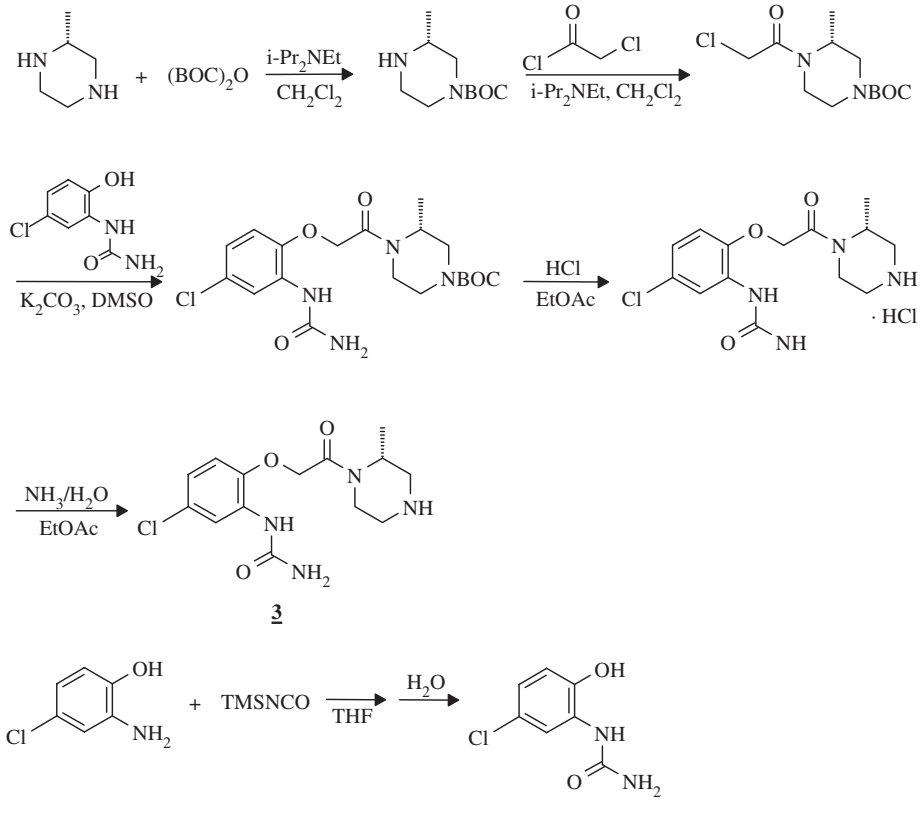


Scheme 1. Synthesis of [^{18}F]4



1 2

Scheme 2. Synthesis of 4-[^{18}F]fluorobenzaldehyde (2)



Scheme 3. Synthesis of the piperazine derivative 3

4-trimethylammonium-benzaldehyde triflate (**1**) according to Scheme 2 using the phase transfer catalyst Kryptofix 222 and potassium carbonate.[4–7]

The starting material **1** was prepared by conversion of 4-dimethylamino-benzaldehyde with methyl triflate as given by Wilson *et al.*,[4] with slight modifications.

The synthesis of **2** and its reductive amination with a secondary amine can be carried out as a one-pot process. Such conversions are described for ^{18}F-labelling of fluorodexetimides[4] and fluorotropaprides[5]. We adapted this method for our purposes.

The second precursor, the piperazine derivative **3**, was prepared by a multi-step synthesis starting from (R)-(−)-2-methylpiperazine (Scheme 3)[3] via the

hydrochloric acid salt of **3**, followed by liberation of the free base **3** by treatment with ammonium hydroxide solution.

Manual labelling procedures

Similar to the conditions of the one-pot experiments given by Wilson *et al.*[4] (reactions of 7–8.6 μmol **1** with dried [^{18}F]fluoride/K_2CO_3/K222 mixtures) we have tested the reductive amination of the formed **2** with the piperazine precursor **3** using sodium cyanoborohydride and acetic acid in DMSO (120°C, 10 min reaction time). Unfortunately, the reaction did not yield **[^{18}F]4** and **2** remained unchanged.

DMF was the solvent of choice for the two-step one-pot procedure, i.e. the optimization of the reaction conditions was carried out in DMF. DMF was also successfully used by Hamacher *et al.*[8] for the synthesis of **2** using electrochemically separated [^{18}F]fluoride, Kryptofix 222/K_2CO_3 and 4-nitrobenzaldehyde as precursor.

To reach satisfactory yields of **2**, the nucleophilic substitution had to be carried out using a relatively large amount of **1** (27–34 μmol) in 1 ml of DMF and heating at 120°C for 10 min. In this way 76–78% **2** were obtained. The crude **2** thus produced was subsequently reductively aminated with the piperazine precursor **3**, using sodium cyanoborohydride and acetic acid, in the same vessel at 120°C for 10 min. Starting from 76–78% **2**, the radiochemical yield of **[^{18}F]4** in the reaction mixture was up to 52–53%. An equimolar ratio between precursor **1** and **3** proved to be favourable for higher yields. Probably the aldehyde group of the unconverted **1** is also reductively aminated with the piperazine **3** as competitive reaction. Henceforth this procedure was adapted to an automated module.

Labelling procedures in the module

A scheme of the automated module used for the synthesis of **[^{18}F]4** is illustrated in Figure 1. The capability of this module includes the radio-synthesis of **2** and its reductive amination with the piperazine derivative **3** to **[^{18}F]4**, the HPLC purification of the final product **[^{18}F]4**, its solid phase extraction and formulation.

In order to improve HPLC purification, the amounts of the precursors **1** and **3** were reduced to 22–23 μmol compared with the manual synthesis. The amounts of the solvents for precursor **3** (HOAc) and $NaBH_3CN$ (DMF) were increased to reduce the losses of the transfer to the reactor (see Experimental).

Best purification of crude **[^{18}F]4** was obtained with a semipreparative C18 Kromasil column (Knauer) using a mixture of MeCN : EtOH : phosphate buffer (pH7) = 45 : 5 : 50 as eluent. The separated HPLC fraction of **[^{18}F]4** was diluted with water and collected by solid phase extraction[9] on an

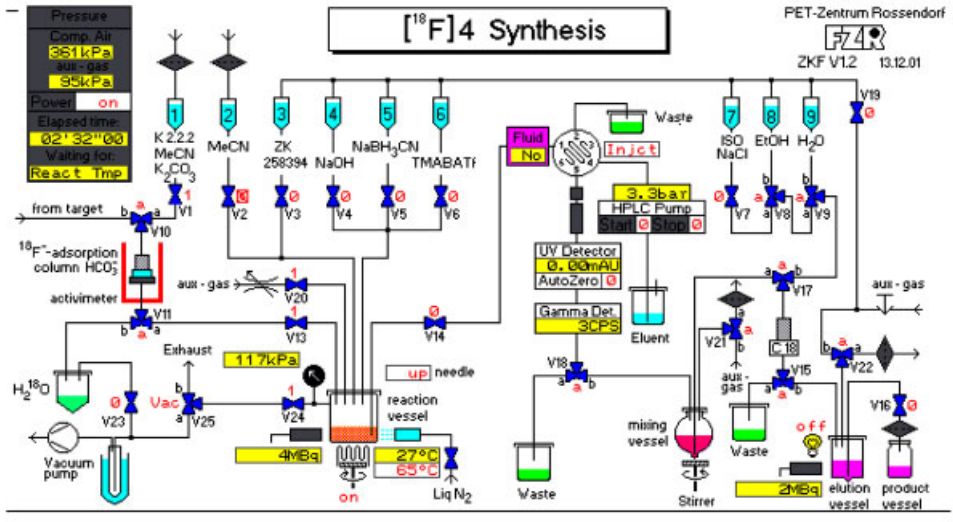


Figure 1. Schematic diagram of the module for synthesizing [^{18}F]4

RP-18-cartridge. After washing the cartridge with water, **[^{18}F]4** was eluted with ethanol. Then the ethanolic solution of **[^{18}F]4** and a saline solution were transferred successively through a sterile filter to obtain the formulated final solution of **[^{18}F]4**. The colourless, sterile and pyrogene-free solution was ready for injection.

The isolated (not decay-corrected) radiochemical yield of **[^{18}F]4** ranged from 7 to 13% ($n = 28$) in its final formulation. The synthesis time of the entire manufacturing process did not exceed 95 min. The radiochemical purity of **[^{18}F]4** was higher than 95%. The chemical purity ranged from 60 to 90% based on UV absorption at 240 nm. Nontoxicity of **[^{18}F]4** solutions with such a composition was confirmed by a toxicity study. The specific radioactivity was in the range of 59–226 GBq/μmol at starting radioactivities of 23.6–65.0 GBq [^{18}F]fluoride.

1-(5-chloro-2-{2-[(2R)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea (**3**) was used as starting material to yield the *R*-enantiomerically pure **[^{18}F]4**. Although racemization was not expected during the reductive amination of **2** with the piperazine derivative **3**, we checked this issue by HPLC with a special chiral column. Therefore, the *S*-enantiomer of **4** (1-(5-chloro-2-{2-[(2S)-4-(4-fluorobenzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy}-phenyl)urea) was synthesized and used as reference substance in addition to the desired *R*-enantiomer of **4** (1-(5-chloro-2-{2-[(2R)-4-(4-fluorobenzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea). Briefly, both **4** (**R and S**) were synthesized by 4-fluorobenzylation of the piperazine precursor **3** in its *R*-(−) or *S*-(+) form

starting form (R)-(−)-2- or (S)-(+)-2-methylpiperazine which were commercially available.[3,10]

No racemization was observed after synthesis of **[^{18}F]4**. The enantiomeric purity of the final **[^{18}F]4** was >99.5%.

Experimental

^{1}H-NMR spectra were recorded on a Bruker AC 300 system. IR-data were collected on a Nicolet NIC 710 IR spectrometer with KBr compound pellets. MS spectra were measured on a VG Micromass Autospec system.

To determine the extent of the reaction conversion, the radiochemical and chemical purity of the reaction products and the specific radioactivity of **[^{18}F]4**, an HPLC system (JASCO) was used, including a pump, a Rheodyne injector with a 20 µl loop, a LiChrospher WP300 RP-18 column (5 µm, 250 mm × 3 mm + precolumn 4 mm × 4 mm, Merck) and a UV detector (240 nm) coupled in series with a radioactivity detector FLO-ONE\Beta 150TR (Canberra Packard). The HPLC analyses were carried out at a flow rate of 0.5 ml/min with the following eluent: 0–20 min: 0.2% TFA in MeCN:water = 23:77, 20–30 min: 0.2% TFA in MeCN. The specific radioactivity of **[^{18}F]4** was calculated by means of a calibration curve using different concentrations of the nonradioactive *R*-enantiomer of **4** in relation to their UV absorbance response and the final radioactivity of **[^{18}F]4**.

Enantiomeric purity of **[^{18}F]4** was determined using a CHIRALCEL OJ-R column (5 µm, 150 mm × 4.6 mm + precolumn 10 mm × 4 mm, Daicel) eluted isocratically with 0.01 M $HCOONH_4$ in MeCN:water = 35:65 at a flow rate of 0.5 ml/min.

A module for nucleophilic fluorination from GE Medical Systems (former Nuclear Interface, Münster, Germany) was modified in terms of program and hardware and used for the study. This module, outlined in Figure 1, is an automatically operating, remote-controlled, closed system. It has been installed in a hot cell to process highest radioactivity levels of [^{18}F]fluoride.

Chemistry

*1-(5-chloro-2-{2-[(2R)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea (**3**).* The hydrochloric acid salt of **3** was synthesized according to the literature procedure,[3] starting from enantiomerically pure (R)-(−)-2-methylpiperazine. The free base **3** was generated as follows.

1-(5-chloro-2-(2-[(2R)-2-methylpiperazin-1-yl]-2-oxoethoxy)phenyl)urea hydrochloride (200 mg, 0.554 mmol) was suspended in 150 ml of water. Ammonium hydroxide solution (20 ml, 22%) was added and the aqueous phase was extracted three times with 50 ml of ethyl acetate. The combined organic extracts were dried over sodium sulphate, filtered and the solvent was evaporated. The dry precipitate was re-dissolved in acetonitrile and

recrystallized. Crystallization yielded 171 mg, 0.526 mmol (95%) of **3** as white precipitate. Melting point: 139.0–141.5°C; MS (CI): 327 (100%) M + H, ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.2 (d, 3H, -CH-C$\underline{H}_3$), 2.5–2.9 (m, 2H + 2H, N-CH_2-C$\underline{H}_2$NH-), 3.0 (t, 1H, N-C$\underline{H}_2$-CH_2NH-, ax), 3.75 (d, 1H, N-C$\underline{H}_2$-CH_2NH-, eq), 4.2 (q, 1H, N-C$\underline{H}$-CH_3), 4.8 (s, 2H, -O-C$\underline{H}_2$-CO), 5.9 (s, 2H, -N$\underline{H}_2$), 6.82 (dd, 1H, aryl), 6.88 (d, 1H, aryl), 7.95 (s,1H, -N$\underline{H}$), 8.12 (s, 1H, -N$\underline{H}$), 8.13 (s, 1H, aryl). IR (KBr, cm^{-1}): 3300–3500 (-NH_2 and –NH), 1670 (carbonyl (urea)), 1655 (carbonyl (amide)).

*1-(5-chloro-2-{2-[(2R or 2S)-4-(4-fluorobenzyl)-2-methylpiperazin-1-yl]-2-oxoethoxy}-phenyl)urea (***4** ***(R)*** **and 4** ***(S)*** **form)**. Compound **4** (**R** and **S**) was synthesized according to the literature procedures described in,[3,10] starting from the enantiomerically pure (R)-(−)-2- or (S)-(+)-2-methylpiperazine via precursor **3** (**R** or **S** form). The conversion of **3** (**R** or **S** form) with 4-fluorobenzyl chloride yielded **4** (**R** and **S**):

To a solution of 1-(5-chloro-2-{2-[(2R or 2S)-2-methylpiperazin-1-yl]-2-oxoethoxy}phenyl)urea (**3** (**R**) or **3** (**S**)) (150 mg, 0.46 mmol) in 20 ml CH_2Cl_2 was added diisopropylethylamine (119 mg, 0.92 mmol), sodium iodide (68 mg, 0.46 mmol) and 4-fluorobenzyl chloride (67 mg, 0.46 mmol). The resulting mixture was stirred at ambient temperature for 15 h. After the reaction was completed, the reaction mixture was washed with water (3 × 20 ml) and brine, then dried over Na_2SO_4, filtered and concentrated in vacuum to afford the raw product as a white solid. The material was taken up in methylene chloride/methanol and purified by column chromatography on silica gel (Mesh 60) and methylene chloride /methanol (v/v 95:5) as the mobile phase. Purification yielded a white solid (105 mg, 52% **4** as *R*-enantiomer); 101 mg, 51% **4** as *S*-enantiomer)).

4, *R*-enantiomer: MS (EI): 434 (100%) M+, ^{1}H-NMR (DMSO-D_6, δ, ppm): 1.23 (d, 3H, -CH-C$\underline{H}_3$), 2.0 (t,d, 1H, N-CH_2-C$\underline{H}_2$NH-, ax), 2.12 (d,d, 1H, N-CH_2-C$\underline{H}_2$NH-, ax), 2.6 (d, 1H, N-CH_2-C$\underline{H}_2$NH-, eq), 2.78 (d, 1H, N-CH_2-C$\underline{H}_2$NH-, eq), 3.12 (t, 1H, N-C$\underline{H}_2$-CH_2NH-, ax), 3.41 (d, 1H, N-C$\underline{H}_2$-C(aryl)), 3.5 (d, 1H, N-C$\underline{H}_2$-C(aryl)), 3.87 (d, 1H, N-C$\underline{H}_2$-CH_2NH-, eq), 4.3 (q, 1H, N-C$\underline{H}$-CH_3), 4.82 (s, 2H, -O-C$\underline{H}_2$-CO), 6.0 (s, 2H, -N$\underline{H}_2$), 6.8 (dd, 1H, aryl), 6.88 (d, 1H, aryl), 7.1 (t, 1H + 1H, aryl), 7.33 (d,d, 1H + 1H, aryl) 8.0 (s,1H, -NH), 8.12 (s, 1H, -NH), 8.13 (s, 1H, aryl), 8.15 (d, 1H, aryl). IR (KBr, cm^{-1}): 3300–3500 (-NH_2 and –NH), 1690 (carbonyl (urea)), 1655 (carbonyl (amide)).

4, *S*-enantiomer: MS (EI): 434 (100%) M+, ^{1}H-NMR ($CDCl_3$, δ, ppm): 1.2–1.4 (m, 3H, -CH-C$\underline{H}_3$), 2.0–2.2 (m, 1H + 1H, N-CH_2-C$\underline{H}_2$NH-, ax + ax), 2.65 (d, 1H, N-CH_2-C$\underline{H}_2$NH-, eq), 2.83 (br, 1H, N-CH_2-C$\underline{H}_2$NH-, eq), 3.0–3.8 (m, 2H, N-C$\underline{H}_2$-C(aryl)), (m, 1H, N-C$\underline{H}_2$-CH_2NH-), 4.3–4.8 (m, 2H, -O-C$\underline{H}_2$-CO), (m, 1H, N-C$\underline{H}$-CH_3), 5.0 (s, 2H, -N$\underline{H}_2$), 6.8 (d, 1H, aryl), 6.85 (d,d, 1H,

aryl), 7.0 (t, 1H + 1H, aryl), 7.3 (d,d, 1H + 1H, aryl), 8.27 (d, 1H, aryl), 9.0 (s, 1H, -NH). IR (KBr, cm^{-1}): 3300–3500 (-NH_2 and –NH) 1690 (carbonyl (urea)), 1655 (carbonyl (amide)).

Radiochemistry

Production of ^{18}F. [^{18}F]Fluoride was produced utilizing the PET cyclotron 'Cyclone 18/9' (IBA, Belgium). [^{18}O]H_2O was irradiated with protons exploiting the $^{18}O(p,n)^{18}F$ nuclear reaction in a 1.5 ml target. The incident energy of the protons was 18 MeV, the beam current was about 30 μA. After irradiation, the [^{18}O]H_2O containing [^{18}F]F^- was transferred in a pneumatic transport system from the cyclotron to the radiopharmaceutical laboratory. The [^{18}F]F^- was separated from the [^{18}O]H_2O using an anion exchange cartridge (Sep-Pak® Light Waters Accell™ Plus QMA cartridge) in the HCO_3^- form.

Manual synthesis of [**^{18}F**]**4**. The [^{18}F]fluoride was eluted from the anion exchange cartridge into a 5 ml reaction vessel using a solution of Kryptofix 222 (15 mg; 40 μmol) and potassium carbonate (2.77 mg; 20 μmol) in aqueous acetonitrile (1.5 ml; 66% acetonitrile). The radioactive eluate was dried by means of a vacuum and a nitrogen stream at 95°C. The reaction mixture was carefully dried by repeated addition and evaporation of anhydrous acetonitrile (3 × 1 ml). After addition of a solution of **1** (8.5–10.8 mg; 27.1–34.5 μmol) in 1 ml anhydrous DMF, the well-sealed vessel was heated at 120°C for 10 min to yield **2**. Subsequently, the piperazine precursor **3** (9.2–12.4 mg; 28.1–38.0 μmol) in 200 μl of acetic acid and the reducing agent $NaBH_3CN$ (4.0 mg; 64 μmol) in 100 μl of DMF were added to the reaction vial. The reductive amination was performed by heating the reaction mixture at 120°C for 10 min.

Module-assisted one-pot synthesis of drug substance [**^{18}F**]**4**. Before starting the one-pot synthesis, a cleaning program for the module was carried out. Then the storage vessels 1–9 and the mixing vessel were supplied with the needed starting materials. The starting activity of the [^{18}F]fluoride deposited on the anion exchange cartridge was measured. The synthesis program was started with the elution of the [^{18}F]fluoride from the anion exchange cartridge into the reaction vessel using a solution of Kryptofix 222 (15 mg; 40 μmol) and potassium carbonate (2.77 mg; 20 μmol) in aqueous acetonitrile (1.5 ml; 66% acetonitrile). The radioactive eluate was dried by means of a vacuum and a nitrogen stream at 95°C. The reaction mixture was carefully dried by addition and evaporation of 3 ml anhydrous acetonitrile. After addition of a solution of **1** (7.0 mg; 22.3 μmol) in 1 ml anhydrous DMF, the mixture was heated at 120°C for 10 min. Then the solution of the piperazine precursor **3** (ZK258394; 7.5 mg; 23 μmol) in 500 μl acetic acid and a solution of $NaBH_3CN$ (4.0 mg;

64 μmol) in 500 μl DMF were added in succession. After heating at 120°C for 10 min the reaction mixture was neutralized with 6 ml 1 M sodium hydroxide and transferred to the HPLC injection loop. The purification was carried out by means of a semipreparative HPLC system (JASCO), including a pump, a Rheodyne injector with a 10 ml loop, a Knauer C18 column (Kromasil-100, 7 μm, Vertex, 300 mm × 8 mm with precolumn 30 mm × 8 mm) with the eluent MeCN : EtOH : phosphate buffer (pH7) = 45 : 5 : 50 (c[NaH_2PO_4] = 2.6 mM; c[Na_2HPO_4] = 5.1 mM) at a flow rate of 2.5 ml/min and based on a UV (240 nm) and a radioactivity detector.

The separated fraction of **[^{18}F]4** (retention time: about 36 min) from the HPLC purification was transferred into the mixing vessel containing 20 ml water. This water-diluted solution was passed through the reversed phase C18 cartridge (Chromafix C18 ec; Macherey-Nagel) for solid phase extraction of **[^{18}F]4**. After washing the cartridge with 15 ml water, **[^{18}F]4** was eluted with 1 ml ethanol into the elution vessel.

Formulation of the radiopharmaceutical under aseptic conditions. The ethanolic solution of **[^{18}F]4** (1 ml) and a solution of 19 ml sterile, isotonic NaCl were passed successively through a sterile filter 'Millex-GP' (Millipore) yielding the final solution of **[^{18}F]4** (product vessel) as a clear, colourless, sterile, pyrogen-free, isotonic NaCl solution with a total volume of 20 ml containing 5% ethanol.

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