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Labeling of benzodioxin piperazines with fluorine-18 as prospective radioligands for selective imaging of dopamine D_4 receptors

Fabian Kügler,[a,b] Johannes Ermert,[a]* and Heinz H. Coenen[a]

The D_4 receptor is of high interest for research and clinical application but puts high demands on appropriate radioligands to be useful tools for investigation. Search for adequate radioligands suitable for *in vivo* imaging is therefore still in progress. The potential neuroleptic drug 6-(4-[4-fluorobenzyl]piperazin-1-yl)benzodioxin shows high affinity and selectivity to the D_4 receptor. Derivatization of this lead structure by adding hydrophilic moieties was carried out in order to lower its lipophilicity what led to three new putative dopamine receptor D_4 ligands. A comprehensive description of the syntheses of standard compounds and corresponding labeling precursors is given which were obtained in satisfactory yields. Furthermore, the radiosyntheses by direct ^{18}F-labeling and build-up synthesis were compared. All derivatives of 6-(4-[4-fluorobenzyl]-piperazin-1-yl)benzodioxin were successfully synthesized in ^{18}F-labeled form with radiochemical yields of 9–35% and molar activities of 30–60 GBq/μmol using one-pot procedures.

Keywords: dopamine D_4 receptor; radioligand; n.c.a. [^{18}F]fluoride; one-pot radiosynthesis; positron emission tomography

Introduction

The dopamine D_4 receptor belongs to the type of receptors that consistently cause problems in their determination when using labeling techniques (antibody, mRNA, or ligand labeling). Therefore, hitherto, there exists neither an adequate radioligand for autoradiographic studies nor for the molecular imaging using positron emission tomography (PET) or single photon emission computed tomography (SPECT), but it would be a powerful tool allowing noninvasive D_4 receptor imaging. The most probable reason for the lack of a suitable radiotracer is the extremely low concentration of this receptor subtype in the mammalian brain. In consequence, even the concentration of this receptor is not very well known what is also true for its localization and function as widely discussed in the literature.[1,2] The dopamine D_4 receptor is part of the small group of dopaminergic receptors, which show comparatively close similarities in their pharmacology. There is evidence that this receptor system is associated with functions and pathophysiological processes of the human brain in schizophrenia, substance abuse,[3] and neurobehavioral dysfunctions such as attention deficit hyperactivity disorder,[4,5] libido dysfunctions,[6,7] and novelty seeking.[8,9] Nevertheless, a confirmation of these postulates and their verification by *in vivo* molecular imaging methods in humans is missing due to lack of suitable radioligands.

Chemical structures of known ligands selective for the D_4 receptor mostly consist of a central backbone of piperazine, sometimes a piperidine, flanked with two aromatic moieties[10] (cf. Scheme 1). One of these aromatic groups is always an arylamine to which the other is linked by a methylene group. Longer linkers cause an increased D_2 and then D_3 selectivity. These findings are meanwhile also confirmed by CoMFA and cloning studies.[8] The simplest structure that fulfills the minimum requirements of the aforementioned criteria is 1-phenyl-4-benzylpiperazine. The fact that this compound is very nonselective and has even higher affinity for D_2 receptors underlines the differences between known theory and reality. However, a hydroxyl substitution in 4-position of the *N*-aryl ring lowers D_2 and increases D_4 affinity. To this end, a methoxy substituent in this position leads to a compound that shows moderate D_4 affinity, whereas it is inactive at the D_2 and D_3 receptor (cf. Scheme 1).[11]

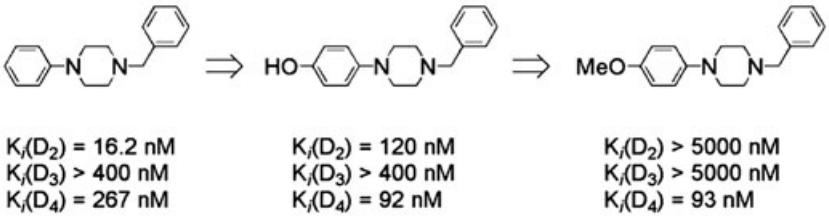
Further systematic development by the Thurkauf group[12] led to the fluorinated benzodioxin D_4 antagonist 6-(4-[4-fluorobenzyl]-piperazin-1-yl)benzodioxin (**1**), which shows a good D_4 subtype selectivity with a $D_{2;3}/D_4$ ratio of >1250. As one of the rare selective D_4 antagonists available, it may be helpful to verify the D_4 hypothesis of schizophrenia or, in case of verification, be used for selective blocking of D_4 receptors as an antipsychotic drug for treatment of this disease.[12]

Although it is known that affinity and selectivity requirements for an extremely low-expressed receptor are very demanding, problems developing suitable radioligands for PET or SPECT are often not due to a lack of selective compounds. Rather, a high lipophilicity of the radioligand causing adhesion to proteins and lipids tends to increase nonspecific binding, an essentially

[a]*Institute of Neurosciences and Medicine, INM-5: Nuclear Chemistry, Forschungszentrum Jülich GmbH, Jülich, Germany*

[b]*Present address: Nuklearmedizinische Klinik und Poliklinik, Technische Universität München, Ismaninger Straße 22, D-81675 München, Germany*

**Correspondence to: Johannes Ermert, Institute of Neurosciences and Medicine, INM-5: Nuclear Chemistry, Forschungszentrum Jülich GmbH, Germany. E-mail: j.ermert@fz-juelich.de*



Scheme 1. Change of the dopamine receptor affinity in dependence of substitution in 4-position of the *N*-aryl of the piperazine D_4 ligand backbone.

nonsaturable component of the total tissue uptake. Here, lipophilicity can become the more critical parameter regarding the effectiveness of a radioligand.[13] The calculated lipophilicity of the previously described D_4 antagonist **1** fits with a cLog *P* value of 3.35 to a generally accepted range for suitable cerebral neuroligands. However, with regard to the very small concentrations of D_4 receptors such a relatively high value is particularly critical if the relative ratio of receptor-binding to nonspecific binding becomes very low. Therefore, a series of derivatives were developed starting from **1** as lead structure with a lower lipophilicity in the range of log*D* of 1.8 to 2.3 at pH 7.4.

Generally, many criteria besides a suitable lipophilicity have to be fulfilled in order to develop a successful imaging agent for *in vivo* human studies using PET or SPECT. Thus, for the compounds of interest here, a detailed pharmacological evaluation of their *in vitro* affinity and lipophilicity, as well as their *in vivo* binding behavior, membrane permeability, and biodistribution including metabolic stability was recently published.[14] Here, the syntheses of the non-radioactive standard compounds and the ^{18}F-radiosyntheses are described in detail together with appropriate analytical conditions for the identification of radiolabeled compounds.

Experimental

General

All reagents and anhydrous solvents were purchased from Aldrich (Steinheim, Germany) or Fluka (Buchs, Switzerland) and used without further purification. 6-Fluoronicotinaldehyde **6d** was obtained from Alfar Aesar (Karlsruhe, Germany) and 4-fluoro-3-hydroxybenzaldehyde **6c** from ABCR (Karlsruhe, Germany).

Sep-Pak C-18 plus-cartridges were acquired from Waters (Eschborn, Germany); EN cartridges and Li-Chrolut glass columns (65 × 10 mm) from Merck (Darmstadt, Germany). Thin layer chromatography (TLC) was run on precoated plates of silica gel 60 F_{254} or Alumina N from Merck (Darmstadt, Germany). The compounds were detected by UV at 256 nm. HPLC was performed on the following system from Dionex (Idstein, Germany): an Ultimate 3000 LPG-3400A HPLC pump, an Ultimate 3000 VWD-3100 UV/VIS-detector (272 nm), a UCI-50 chromatography interface, and an injection valve P/N 8215. Reversed-phase HPLC was carried out using a Gemini 5 mm C18 110A column, for analytical separations with a dimension of 250 × 4.6 mm (flow 1 mL/min) and for semi-preparative applications 250 × 10 mm (flow 5 mL/min) from Phenomenex (Aschaffenburg, Germany). Determination of molar activities was carried out according to standard procedures by means of the previously described semi-preparative HPLC, comparing UV-signals under radioactivity peaks with a calibration UV/mass-curve, as described earlier.[15] Radio TLC chromatograms were analyzed by UV detection and on a Packard Instant Imager. ^{1}H, ^{13}C, and ^{19}F NMR spectra were recorded on a Bruker DPX Avance 200 spectrometer with samples dissolved in $CDCl_3$ or DMSO-d_6.

All chemical shifts are given in the succeeding text in δ ppm using the signals of the appropriate solvent as a reference. Mass spectra were obtained from a Finnigan Automass Multi mass spectrometer with an electron beam energy of 70 eV. High resolution electron spray mass spectra were recorded on an LTQ FT Ultra (Thermo Fischer). Melting points are uncorrected and were determined on a Mettler FP-61 apparatus in open capillaries.

Chemistry

The following compounds were synthesized according to reported literature methods: 4-*N*,*N*-dimethylaminobenzaldehyde and 4-*N*,*N*-dimethylamine-3-methoxybenzaldehyde were prepared by amination of corresponding fluorine compounds as described by Wilson *et al.*;[16] 4-formylphenyltrimethylammonium triflate **12**,[17] 3-benzyloxy-4-formylphenyltrimethylammonium triflate **12b**,[18] 3-benzyloxy-4-fluorobenzaldehyde **6b**,[18] 3-benzyloxy-4-nitrobenzaldehyde **12b′**,[18] and 1-(1,4-benzodioxin-6-yl)piperazine **2**[12] were prepared as described in the corresponding literature.

4-Formyl-3-methoxyphenyltrimethylammonium triflate ***12a***

4-Formyl-3-methoxyphenyltrimethylammonium triflate **12a** was obtained from 3 g (19.46 mmol) 4-fluoro-3-methoxybenzaldehyde as a white solid (5.9 mg, 17.2 mmol, 88.4%) according to a method by Wilson *et al.*.[16] mp 155.5–156.4 °C, ^{1}H NMR (DMSO-d_6, 200.13 MHz) δ 3.69 (s, 9H), 3.73 (s, 3H), 7.77–7.82 (m, 3H), 10.09 (s, 1H) ppm. FT-MS (ESI): 195.33 *m/z* (100) $[M+H]^+$, elemental analysis: found: %C, 41.98; %H, 4.70; %N, 4.05; expected: %C, 41.98; %H, 4.70; %N, 4.08.

1-(1,4-Benzodioxin-6-yl)piperazine ***2***

Under an atmosphere of dry argon, a mixture of 1.27 g (8.4 mmol) of 6-amino-1,4-benzodioxine and 1.5 g (8.4 mmol) of bis(2-chloroethyl)amine hydrochloride in 3 mL of dry ethylenglycol was heated at 150 °C over night. After cooling to room temperature, methanol was given to the mixture in order to precipitate a white crystalline solid. The solid was treated with a saturated sodium carbonate solution and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate, and concentrated in vacuo to give a pure white solid. The mother liquor was further reduced in vacuo to get rid of methanol and extracted with a saturated sodium carbonate solution as described previously. The product was purified by column chromatography ($CHCl_3$/methanol 5:1) to obtain a slight beige solid (1.17 g, 5.3 mmol, 63%). mp 169–171 °C. TLC ($CHCl_3$/methanol, 5:1): R_f = 0.18. ^{1}H NMR (200.13 MHz, DMSO-d_6): δ 2.78–2.92 (m, 8H), 4.17 (m, 4H), 6.43 (m, 2H), 6.70 (d, 1H) ppm. FT-MS (ESI): *m/z* 221.21 $[M+H]^+$, HRMS (221.1290) found: 221.1284.

1-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-4-(4-fluorobenzyl)-piperazine **1**

A mixture of 350 mg of **2** (1.6 mmol), 394 mg of 4-fluorobenzylbromid (2.08 mmol), 443 mg of K_2CO_3 (3.2 mmol), and 345 mg of potassium iodide (2.08 mmol) was suspended in 20 mL of dry acetonitrile and heated at 85 °C for 18 h. After cooling to room temperature, the mixture was treated with 50 mL of water and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over sodium sulfate and concentrated in vacuo. The product was purified by column chromatography (*n*-hexane/ethyl acetate 1:2) to obtain a white solid (240 mg, 0.73 mmol, 46%). mp 120 °C. TLC (*n*-hexane/ethyl acetate, 1:2): R_f = 0.7. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.56 (t, *J* = 4.8 Hz, 4H), 3.06

(t, J = 4.8 Hz, 4H), 3.50 (s, 2H), 4.19 (m, J = 4.5 Hz, 2H), 4.21 (m, J = 4.6 Hz, 2H), 6.43 (m, 2H), 6.75 (m, 1H), 6.98 (m, 1H), 7.29 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$, 50.33 MHz) δ 50.2, 53.0, 62.2, 64.2, 64.6, 105.6, 110.4, 115.0, 117.3, 130.6, 133.7, 137.3, 143.6, 146.5, 162.0 ppm. ^{19}F NMR (188.28 MHz, DMSO-d_6) δ −115.6 ppm. FT-MS (ESI): *m/z* 329.166 $[M+H]^+$. Elemental analysis: found: %C, 69.32; %H, 6.44; %N, 8.29; expected: %C, 69.49; %H, 6.45; %N, 8.53.

*General procedure for preparation of amides **9a**, **9b**, and **9c***

The corresponding aromatic carboxylic acid (3.54 mmol) was dissolved in 20 mL of anhydrous dichloromethane (DCM) followed by a small amount of DMF (20 μL) and oxalylchloride (5.3 mmol, 1.5 equiv). The mixture was stirred at room temperature until evaporation of gas had stopped (2–3 h), and the solvent was evaporated in vacuo to obtain the corresponding acid chloride.

Under an argon atmosphere, 1-(1,4-benzodioxin-6-yl)piperazine (**2**) was dissolved in DCM, and triethylamine (2.2 mmol, 0.6 equiv) was added. The solution was cooled in an ice bath at 0 °C and the respective acid chloride redissolved in 5 mL of DCM, which was added dropwise. The reaction mixture was warmed to room temperature and stirred overnight for at least 10 h. To quench the reaction, 20 mL of saturated sodium bicarbonate solution was added. The solution was extracted with DCM, washed with water and saturated sodium chloride solution, and dried over sodium sulfate. After evaporation of the solvent in vacuo, the residue was purified by flash chromatography.

(4-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)piperazine-1-yl)(4-fluorophenyl)methanone **9a**

Obtained from 4-fluorobenzoic acid (286 mg, 2.04 mmol) as white foamy solid (460 mg, 1.34 mmol, 66%). TLC (*n*-hexane/ethyl acetate 1:2): R_f = 0.56. ^{1}H NMR ($CHCl_3$, 400 MHz) δ 3.04 (b, 4H, $4CH_2$); 3.7 (b, 4H, $4CH_2$); 4.19 (m, 2H, $2CH_2$); 4.22 (m, 2H, $2CH_2$); 6.44–6.45 (m, 2H, Ar-CH); 6.77 (m, 1H, Ar-CH); 7.08 (m, 2H, Ar-CH); 7.42 (m, 2H, Ar-CH) ppm. ^{13}C NMR ($CDCl_3$, 50.33 MHz) δ 42.3, 50.8, 64.2, 64.5, 106.5, 11.0, 115.6, 117.5, 129.4, 131.6, 138.1, 143.7, 145.9, 163.4, 169.4 ppm. ^{19}F NMR (188.28 MHz, DMSO-d_6) δ −110.1 ppm. FT-MS (ESI): *m/z* 343.19 (100) $[M+H]^+$. Elemental analysis: found: %C, 66.90; %H, 5.85; %N, 7.97; expected: %C, 66.66; %H, 5.59; %N, 8.18.

(4-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)piperazine-1-yl)(4-nitrophenyl)methanone **9b**

Obtained from 4-nitrobenzoic acid (267 mg, 1.6 mmol) as a yellow solid (520 mg, 1.41 mmol, 88%). TLC (*n*-hexane/ethyl acetate 1:2): R_f = 0.49. ^{1}H NMR ($CHCl_3$, 400 MHz) δ 2.92 (b, 2H); 2.97 (b, 2H); 3.13 (b, 2H); 3.45 (b, 2H); 4.19 (m, 2H); 4.22 (m, 2H) 6.44–6.45 (m, 2H); 6.77 (d, 1H); 7.58 (dt, J = 8,7 Hz; 2H); 8.28 (dd, J = 8,3 Hz, 2H) ppm. ^{13}C NMR ($CDCl_3$, 50.33 MHz) δ 42.3, 47.6, 50.6, 51.1, 64.2, 64.6, 106.6, 11.1, 117.6, 123.9, 128.1, 138.3, 141.7, 143.7, 145.6, 148.4, 167.9 ppm. FT-MS (ESI): *m/z* 370.14 (90) $[M+H]^+$. Elemental analysis: found: %C, 61.49; %H, 5.50; % N, 11.16; expected: %C, 61.78; %H, 5.18; %N, 11.38.

(4-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)piperazine-1-yl)(6-fluoropyridine-3-yl)-methanone **9c**

Obtained from 2-fluoronicotinic acid (450 mg, 3.2 mmol) as a white solid (680 mg, 2 mmol, 62%). TLC (*n*-hexane/ethyl acetate, 1:3): R_f = 0.48. ^{1}H NMR ($CHCl_3$, 400 MHz) δ 3.06 (br, 2H); 3.12 (br, 2H); 3.52 (br, 2H); 3.80 (br, 2H); 4.21 (m, 2H); 4.25 (m, 2H); 6.52 (s, 1H); 6.53 (dd, J = 2.8 Hz/ 8.6 Hz, 1H); 6.79 (d, J = 9.2 Hz, 1H); 7.36 (dd, J = 2.5 Hz/8.4 Hz, 1H); 8.15 (ddd, J = 2.5 Hz/8.2 Hz/8.2 Hz, 1H); 8.42 (d, J = 2.0 Hz, 1H) ppm. ^{13}C NMR ($CDCl_3$, 50.33 MHz) δ 41.4, 46.9, 49.3, 49.6, 63.7, 64.1, 105.4, 109.5, 109.9, 116.9, 130.1, 137.0, 141.2, 143.3, 145.5, 146.2, 162.9, 165.6 ppm. ^{19}F NMR (188.28 MHz, DMSO-d_6) δ −66.7 ppm. FT-MS (ESI): *m/z* 344.14 (100) $[M+H]^+$, HRMS (344.14104) found: 344.1403.

*General procedure for the preparation of 3-substituted 6-(4-benzylpiperazine-1-yl)benzodioxin derivatives **7a–e***[19]

In a 100-mL two-neck flask, 1-(1,4-benzodioxine-6-yl)piperazine (**2**) (1.816 mmol, 1 equiv) and corresponding benzaldehyde (**3a–e**, 2.724 mmol, 1.5 equiv) were dissolved in 20 mL of dry methanol. After an addition of acetic acid (96%, 5.45 mmol, 3 equiv.), sodium cyanoborohydride (2.724 mmol, 1.5 equiv.) was added in small portions and rinsed with methanol. The reaction was heated for 12–24 h at 60 °C. After cooling, the reaction was quenched by addition of 50 mL of saturated sodium bicarbonate solution, extracted three times with chloroform, and washed with brine. The combined organic layer was dried over sodium sulfate and evaporated to dryness after filtration. The residue was purified by chromatography over a silica gel column (*n*-hexane/ethyl acetate 1:2).

1-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-4-(4-fluoro-3-methoxybenzyl)piperazine **7a**

The methoxy derivative was obtained from the commercially available 4-fluoro-3-methoxybenzaldehyde (**6a**) (420 mg, 2.724 mmol) as a pale yellow crystalline solid (551 mg, 1.54 mmol, 85%). mp 100 °C. TLC (*n*-hexane/ethyl acetate, 1:2): R_f = 0.44. ^{1}H NMR ($CHCl_3$, 400 MHz) δ 2.56 (t, J = 4.8 Hz, 4H), 3.06 (t, J = 4.8 Hz, 4H), 3.48 (s, 2H), 3.88 (s, 3H), 4.18 (m, J = 1.6 Hz, 2H), 4.20 (m, J = 1.6 Hz, 2H), 6.44 (m, 1H), 6.45 (m, 1H), 6.75 (m, 1H), 6.82 (m, 1H), 6.98 (m, 2H) ppm. ^{13}C NMR ($CDCl_3$, 50.33 MHz) δ 50.2, 53.1, 56.2, 62.5, 64.3, 64.6, 105.6, 110.3, 113.9, 115.5, 117.3, 121.3, 134.5, 137.3, 143.6, 146.5, 147.4, 151.5 ppm. ^{19}F NMR (188.28 MHz, DMSO-d_6) δ −137.8 ppm. FT-MS (ESI): *m/z* 359.18 $[M+H]^+$. HRMS (359.177) found: 359.17642.

1-(3-(Benzyloxy)-4-fluorobenzyl)-4-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)piperazine **7b**

Obtained from 3-benzyloxy-4-fluorobenzaldehyde (**6b**) (160 mg, 0.7 mmol) as a white crystalline solid (175 mg, 0.41 mmol, 58%). mp 94 °C. TLC (*n*-hexane/ethyl acetate, 1:2): R_f = 0.74. ^{1}H NMR ($CHCl_3$, 400 MHz) δ 2.40 (m, 4H); 2.92 (m, 4H); 3.41 (s, 2H); 4.11 (m, 2H); 4.14 (m, 2H); 5.14 (s, 2H); 6.36 (d, J = 2.7 Hz, 1H); 6.38 (dd, J = 2.9 Hz/8.6 Hz; 1H); 6.66 (d, J = 8.7 Hz, d); 6.84 (m,1H); 7.14 (m, 2H); 7.31–7.42 (m, 5H) ppm. ^{13}C NMR ($CDCl_3$, 50.33 MHz) δ 49.7, 52.9, 61.8, 64.2, 64.7, 70.6, 105.2, 109.8, 116.1, 116.3, 117.4, 121.8, 128.3, 128.4, 128.9, 135.3, 137.0, 137.1, 143.8, 146.2, 146.5, 151.3 ppm. ^{19}F NMR (188.28 MHz, DMSO-d_6) δ −137.1 ppm. FT-MS (ESI): *m/z* 345.21 $[M+H]^+$. Elemental analysis: found: %C, 71.80; %H, 6.13; expected: %C, 71.87; %H, 6.26.

5-((4-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)piperazine-1-yl)methyl)-2-fluorophenol **7c**

The hydroxyl derivative was obtained from 4-fluoro-3-hydroxybenzaldehyde (**6c**) (70 mg, 0.5 mmol) as a white crystalline solid (112 mg, 0.35 mmol, 66%). mp 186–187 °C. TLC (*n*-hexane/ethyl acetate, 1:2): R_f = 0.35. ^{1}H NMR ($CHCl_3$, 400 MHz) δ 2.57 (m, 4H); 3.06 (m, 4H); 3.45 (s, 2H); 4.18 (m, 2H); 4.20 (m, 2H); 6.44 (m, 2H); 6.74 (m, 1H); 6.79 (m, 1H); 6.98 (m, 2H); 9.84 (br, 1H) ppm. ^{13}C NMR ($CDCl_3$, 50.33 MHz) δ 50.1, 52.9, 62.2, 64.2, 64.6, 105.7, 110.4, 115.1, 117.3, 118.1, 121.3, 134.5, 137.4, 143.4, 143.6, 146.4, 150.3 ppm. ^{19}F NMR (188.28 MHz, DMSO-d_6) δ −142.6 ppm. FT-MS (ESI): *m/z* 345.19 (100) $[M+H]^+$. Elemental analysis: %C, 66.02; %H, 6.26; %N, 7.68; expected: %C, 66.26; %H, 6.15; %N, 8.13.

1-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-4-((6-fluoropyridine-3-yl)-methyl)piperazine **7d**

Method A: Obtained from 6-fluoronicotinaldehyde (**6d**) (100 mg, 0.8 mmol) as a white crystalline solid (35 mg, 0.11 mmol, 14%).

Method B: A 25 mL flask with a silicone septum was twice heated under vacuo while filled with argon. After cooling, 1 mL of borane (1 M in THF) was filled into the flask and 150 mg of **7b** (0.44 mmol) dissolved in 4 mL of dry THF were added afterwards. The reaction was heated to 65 °C for 7 h and a second

portion of borane (0.7 mL, 1 M in THF) was added. After another 22 h, the reaction was quenched by addition of small amounts of ice, extracted three times with chloroform and washed with brine. The combined organic layer was dried over sodium sulfate and evaporated to dryness after filtration. The residue was purified over a silica gel column (chloroform/methanol 8:1) to obtain a white solid (43 mg, 0.132 mmol, 30%). mp 94 °C. TLC (chloroform/methanol 8:1): $R_f = 0.63$; (ethyl acetate/methanol/diethyl amine 96:2:2): $R_f = 0.78$. ^{1}H NMR ($CHCl_3$, 400 MHz) δ 2.52 (br, 4H); 3.00 (br, 4H); 3.47 (s, 2H); 4.14 (m, 2H); 4.16 (m, 2H); 6.38 (m, 2H); 6.71 (m, 1H); 6.84 (dd, $J = 2.8$ Hz/8.4 Hz, 1H); 7.74 (ddd, $J = 2.3$ Hz/8.0 Hz/8.1 Hz, 1H); 8.09 (d, $J = 1.9$ Hz, 1H) ppm. ^{13}C NMR ($CDCl_3$, 50.33 MHz) δ 49.7, 52.9, 58.5, 64.2, 64.7, 105.2, 109.8, 117.4, 124.4, 133.7, 137.1, 140.8, 143.8, 146.4, 149.4, 150.5 ppm. ^{19}F NMR (188.28 MHz, DMSO-d_6) δ −71.2 ppm. FT-MS (ESI): *m/z* 330.16 $[M+H]^+$, HRMS (330.16172) found: 330.16124.

*1-((6-Chloropyridine-3-yl)methyl)-4-(2,3-dihydrobenzo[b][1,4]-*dioxin-6-yl)piperazine **7e**

Also obtained by the general amination procedure from the commercially available 6-chloronicotinaldehyde (**6e**) (380 mg, 2.68 mmol) as a white crystalline solid (445 mg, 1.29 mmol, 71%). mp 94 °C. TLC (*n*-hexane/ethyl acetate, 1:2): $R_f = 0.53$. ^{1}H NMR ($CHCl_3$, 400 MHz) δ 2.45 (m, 4H); 2.94 (m, 4H); 3.51 (s, 2H); 4.10 (m,2H); 4.14 (m, 2H); 6.36 (d, $J = 2.5$ Hz, 1H); 6.38 (dd, $J = 2.7$ Hz/8.6 Hz, 1H); 6.66 (d, $J = 8.6$ Hz, 1H); 7.46 (d, $J = 8.2$ Hz, 1H); 7.77 (dd, $J = 2.3$ Hz/8.1 Hz, 1H); 8.31 (d, $J = 2.2$ Hz, 1H) ppm. ^{13}C NMR ($CDCl_3$, 50.33 MHz) δ 50.2, 53.1, 61.9, 64.2, 64.6, 105.7, 110.4, 117.4, 124.0, 135.0, 137.5, 137.6, 143.6, 146.3, 148.2, 150.3 ppm. FT-MS (ESI): *m/z* 346.13 $[M+H]^+$, HRMS (346.1322) found: 346.1315.

*4-Formyl-2-benzyloxy-N,N-*dimethylaniline **14** (alternative method to Langer *et al.*[18])

Under an atmosphere of argon 4-bromo-2-benzyloxy-*N,N*-dimethylanilin (**13**) (500 mg, 1.65 mmol) was dissolved in 6 mL of anhydrous diethylether and cooled in a carbon dioxide/acetone bath at −78 °C. A 1.75 mL of s-BuLi (2.45 mmol, 1.4 M in cyclohexane) was added cautiously, and the reaction was stirred for 45 min at −78 °C. Subsequently, DMF (200 μL, 2.6 mmol) was added all at once, and the reaction mixture was stirred for 1 h at room temperature. For quenching, the solution was diluted with 15 mL of water and 15 mL of saturated aqueous ammonium chloride and extracted with diethylether. After washing with saturated sodium chloride solution, the organic phase was dried over sodium sulfate, filtered, and evaporated in vacuo to dryness. The crude product was purified by flash chromatography on silica gel (*n*-hexane/ethyl acetate: 4:1) and obtained as a clear, light yellow liquid (240 mg, 0.95 mmol, 57%). TLC (*n*-hexane/ethyl acetate: 4:1): $R_f = 0.44$. ^{1}H NMR (DMSO-d_6, 200 MHz) δ 2.91 (s, 6H); 5.18 (s, 2H); 6.97 (d, 1H); 7.46 (m, 7H); 9.79 (s, 1H) ppm. MS (ESI): *m/z* 256.21 $[M+H]^+$.

Radiochemistry

N.c.a. [^{18}F]fluoride was produced via the ^{18}O(*p*,*n*)^{18}F nuclear reaction by bombardment of an isotopically enriched [^{18}O]water target[20] with 17 MeV protons at the JSW cyclotron BC 1710 (INM-5, Research Center Juelich). The aqueous [^{18}F]fluoride solution (10–50 μL, 75–375 MBq) was filled into a 5 mL conical vial (Reactivial) containing 1 mL of acetonitrile, 10 mg of Kryptofix 2.2.2 and 13 μL of an aqueous 1 M potassium carbonate solution.[21] The solvent was evaporated under a stream of argon at 80 °C and 600 mbar. This azeotropic drying was repeated twice using 1 mL of dry acetonitrile, followed by evaporation at 8–15 mbar for 5 min.

*6-(4-[4-[^{18}F]Fluorobenzyl]piperazine-1-yl)benzodioxin [^{18}F]**1***

Method A: A solution of precursor **9b** (8 mg, 22 μmol) in 0.5 mL of anhydrous DMSO was added to the vial containing the dried [^{18}F]fluoride at 160 °C. Monitoring of the reaction progress was carried out by radio HPLC from aliquots of about 30–50 μL (Gemini 5 μ RP18 A110, 250 × 4.6 mm, 1 mL/min, isocratic 60:40:0.1 v/v/v CH_3CN/H_2O/TEA) and by radio TLC (1:2 *n*-hexane/ethyl acetate) determining RCYs and the optimal reaction time. For reduction of the intermediate compound, the reaction mixture was diluted with 20 mL of water, passed through a Sep Pak C18 cartridge, washed with 5 mL of water, and dried with air. The cartridge was eluted through a drying cartridge charged with 4 Å molecular sieve and 270 mg dry sodium sulfate using 5 mL absolute dichloromethane. After removal of the solvent in vacuo (800 up to 300 mbar) at room temperature, 0.2 mL of BH_3THF (1 M, 0.2 mmol) solution and 0.7 mL of dry THF were added, and the solution was stirred for 10 min at 65 °C. The reaction mixture was cooled in an ice bath, carefully quenched with 20 mL of water, and passed through a second Sep Pak C18 cartridge. After washing with water, drying in air and eluting with 1 mL of acetonitrile, the solution was injected on a semi-preparative HPLC system (Gemini 5 μ RP18 A110, 250 × 10 mm, 4 mL/min, isocratic 30:70:0.1 v/v/v CH_3CN/H_2O/TFA).

Method B: A solution of precursor **12** (8 mg, 25.5 μmol) in 0.5 mL of anhydrous DMSO was added to a reaction vial at 110 °C, and the reaction was monitored via radio HPLC (Gemini 5 μ RP18 A110, 250 × 4.6 mm, 1 mL/min, isocratic 60:40:0.1 v/v/v CH_3CN/H_2O/TEA). A solution of intermediate **1** (8.7 mg, 40 μmol) in 40 μL acetic acid and 50 μL DMSO and a solution of sodium cyanoborohydride (4 mg, 64 μmol) in 50 μL DMSO were added successively. The reaction mixture was stirred for 15 min, diluted with water, and passed through a SepPak C18 cartridge, washed with water (5 mL) and dried with air. The cartridge was eluted with 1 mL of acetonitrile and injected on a semi-preparative HPLC system (Gemini 5 μ RP18 A110, 250 × 10 mm, 4 mL/min, isocratic 30:70:0.1 v/v/v CH_3CN/H_2O/TFA). The separated fraction was diluted with 15 mL of water and passed through a second SepPak C18 cartridge. After washing with water and drying in an argon stream, the cartridge was eluted with 5 mL of diethylether, which was evaporated in vacuo (800 up to 330 mbar). For *in vitro* studies, [^{18}F]**1** was formulated in 300 μL ethanol/saline (5:1).

*1-(2,3-Dihydrobenzo[b][1,4]*dioxin-6-yl)-4-(4-[^{18}F]fluoro-3-methoxybenzyl)piperazine [^{18}F]**7a**

Analogous to method B of the radiosynthesis of [^{18}F]**1**, a solution of **12a** (7 mg, 20 μmol) in 0.5 mL of anhydrous DMSO was added to the reaction vial containing the dried [^{18}F]fluoride at 110 °C, and the progress of reaction was monitored as described previously by radio HPLC (Gemini 5 μ RP18 A110, 250 × 4.6 mm, 1 mL/min, isocratic 60:40:0.1 v/v/v CH_3CN/H_2O/TEA). Without further separation, a solution of **2** (8.7 mg, 40 μmol) in 40 μL of acetic acid and 50 μL of DMSO and a solution of sodium cyanoborohydride (4 mg, 64 μmol) in 50 μL of DMSO were rapidly added to the reaction vial at the same temperature. The mixture was stirred for 15 min, diluted with water, and passed through a SepPak C18 cartridge, washed with water (5 mL) and dried with air. The cartridge was eluted with 1 mL of acetonitrile and the eluate injected on a semi-preparative HPLC system (Gemini 5 μ RP18 A110, 250 × 10 mm, 4 mL/min, isocratic 30:70:0.1 v/v/v CH_3CN/H_2O/TFA). The separated product fraction was diluted with 15 mL of water, passed through a SepPak C18 cartridge again, washed with water (5 mL), and dried in a stream of argon. The cartridge was eluted with 4 mL of diethylether, and the solvent was evaporated in vacuo (800 up to 330 mbar). For further experimental use in *in vitro* autoradiography studies, [^{18}F]**7a** was formulated in 300 μL ethanol/saline (5:1).

*6-(4-[4-[^{18}F]Fluoro-3-hydroxybenzyl]piperazine-1-yl)benzodioxin [^{18}F]**7c***

The benzyl-protected precursor **12b** (3 mg, 7 μmol) dissolved in 0.5 mL of anhydrous DMSO was added to the vial containing dried [^{18}F]fluoride and heated at 110 °C, and the progress of the reaction was determined by radio HPLC (Gemini 5 μ RP18 A110, 250 × 4.6 mm, 1 mL/min, isocratic 80:20 v/v CH_3CN/H_2O). A solution of **2** (6.5 mg, 30 μmol) in 40 μL acetic acid and 50 μL DMSO and a solution of sodium cyanoborohydride

(4 mg, 64 μmol) in 50 μL DMSO were immediately added to the reaction vial and stirred for 7 min at 110 °C. Subsequently, the solution was diluted with 20 mL of water and passed through an EN cartridge. After washing with 5 mL of water and drying in a stream of argon for 8 min, the product was eluted from the cartridge and then passed through an unconditioned Alumina N cartridge with 5 mL of anhydrous diethylether in a second reaction vial. The ether was evaporated in vacuo, and the residue was diluted with 1 mL of anhydrous methanol. Radio HPLC analysis showed a nearly quantitative conversion of [^{18}F]**6b** to [^{18}F]**7b**. Palladium (black) (20 mg) and 250 mg of ammonium formate were added to the solution, and the resulting suspension was stirred for 8 min at 60 °C. The start of the reaction could be observed by a strong evolution of gas. The mixture was cooled in an ice bath, filtered through a small glass column with two frits (Merck: pore size 4), and washed with a small amount of methanol. After evaporation in vacuo, the residue was diluted with 1 mL of methanol/phosphate buffer and injected on a semi-preparative HPLC system (Gemini 5 μ RP18 A110, 250 × 10 mm, 4 mL/min, isocratic 60:40 v/v MeOH/phosphate buffer pH 8.5). The separated product fraction was diluted with 15 mL of water, passed through a SepPak C18 cartridge, washed with water (5 mL), and dried in a stream of argon. The cartridge was eluted with 4 mL of diethylether, and the solvent was evaporated in vacuo (800 up to 330 mbar). For further *in vivo* autoradiography studies, [^{18}F]**7c** was formulated in 300 μL of ethanol/saline (5:1).

1-(2,3-Dihydrobenzo[b][1,4]dioxin-6-yl)-4-((6-[^{18}F]fluoropyridin-3-yl)-methyl)-piperazine [^{18}F]**7d**

Method A: The precursor for direct labeling **7e** (13.8 mg, 40 μmol) dissolved in 0.5 mL of anhydrous DMSO was added to the vial containing the [^{18}F]fluoride complex, and the solution was stirred at 160 °C for 20 min. Progress of the reaction was monitored by radio HPLC (Gemini 5 μ RP18 A110, 250 × 4.6 mm, 1 mL/min, isocratic 50:50:0.1 v/v/v CH_3CN/H_2O/TEA). Water was added to the solution, and it was passed through a Sep Pak C18 cartridge, washed with water, and dried with air. The cartridge was eluted with 2 mL of acetonitrile, and the solvent was evaporated in vacuo. The residue was diluted with 1 mL acetonitrile/water (50/50) and injected on a semi-preparative HPLC system (Gemini 5 μ RP18 A110, 250 × 10 mm, 4 mL/min, isocratic 40:60:0.1 v/v/v CH_3CN/H_2O/TEA).

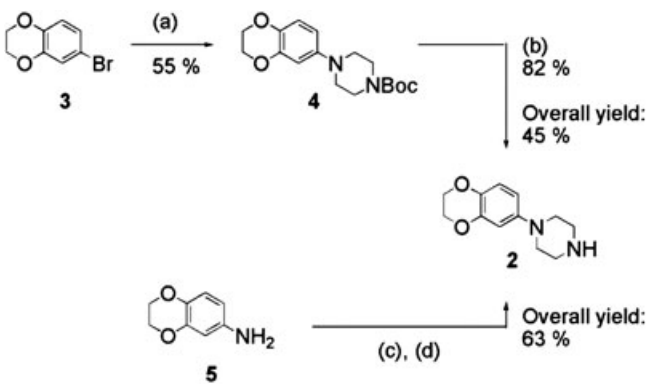
Method B: The radiochemical synthesis of [^{18}F]**7d** by reductive amination was conducted in an identical manner as described for [^{18}F]**1**, [^{18}F]**7a**, and [^{18}F]**7b**, starting from precursor **6e** (5 mg, 35.5 μmol) dissolved in 0.5 mL of anhydrous DMSO and stirred for 2 min at 110 °C. The RCY of [^{18}F]**6d** was determined by radio HPLC (Gemini 5 μ RP18 A110, 250 × 4.6 mm, 1 mL/min, isocratic 60:40:0.1 v/v/v CH_3CN/H_2O/TEA). After reaching the optimal yield at 2 min, a solution of **2** (6.5 mg, 30 μmol) in 40 μL of acetic acid and 50 μL of DMSO and a solution of sodium cyanoborohydride (4 mg, 64 μmol) in 50 μL of DMSO were added immediately, and the reaction mixture was stirred for further 8 min. After addition of 20 mL of water, the solution was passed through a Sep Pak C18 cartridge followed by washing with 5 mL water and drying with air. The cartridge was eluted with 1 mL of ethanol/water (80/20) and injected on a semi-preparative HPLC system (Gemini 5 μ RP18 A110, 250 × 10 mm, 4 mL/min, isocratic 40:60:0.1 v/v/v CH_3CN/H_2O/TEA). The separated fraction was diluted with 15 mL of water, passed through a SepPak C18 cartridge, washed with 5 mL of water, and dried in a stream of argon. The cartridge was eluted with 4 mL of diethylether, and the solvent was evaporated in vacuo (800 up to 330 mbar). For further experimental use, [^{18}F]**7d** was formulated in 300 μL of saline (1% Tween 80).

Results and discussion

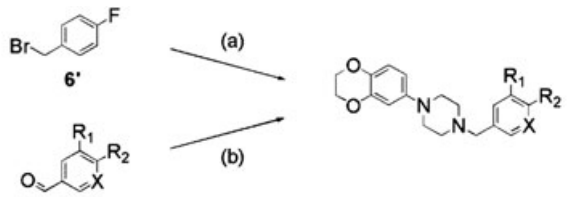
Chemistry

The first objective was to synthesize three further derivatives of lead structure **1**, all substituted at the benzyl moiety. Therefore, benzyl- and benzodioxinpiperazine moieties were separately synthesized as intermediates and coupled afterwards. Analogous to the description of Liu *et al.*,[22] the latter intermediate 1-(1,4-benzodioxin-6-yl)piperazine (**2**) was synthesized via cyclization of the commercially available aminodioxin 2,3-dihydrobenzo[*b*]-[1,4]dioxin-6-amine with bis(2-chloroethyl)amine hydrochloride in diethylene glycol or diethylene glycol monomethyl ether[15] (cf. Scheme 2). Thereby, higher yields were found by saving one reaction step in contrast to the palladium catalyzed coupling with the corresponding dioxine-6-bromide as described earlier by Hodgetts *et al.*[12] Double nucleophilic substitution on anilines at high temperature (Prelog cyclization) constitutes the easiest method to generate arylpiperazines when appropriate anilines are available and higher temperatures are tolerable. This concerns not only yield and reaction time but also the purchase of suitable compounds as well as product purification.

The intermediate **2** is the common key structure for all final radioactive and inactive compounds with the exception of those for direct labeling syntheses. Isolating the hydrochloride product by precipitating the salt from methanol and liberation of the free base with Na_2CO_3 resulted in a pure white solid. Alternatively, the product was obtained from the supernatant by flash chromatography, yielding a light yellowish solid that, however, showed no differences, neither in analytical properties nor in its reaction behavior.

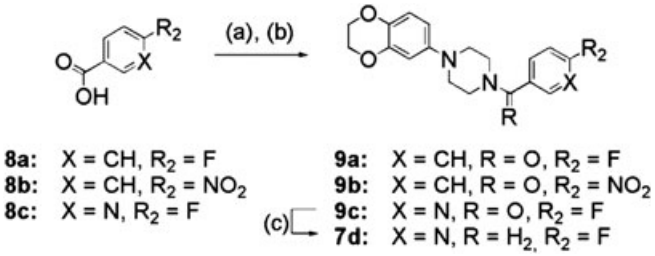


Scheme 2. Two considered synthesis routes for the preparation of the intermediate **2**. Reaction conditions: (a) $Pd_2(dba)_3$, P(*o*-tolyl)$_3$, NaOtBu, 1-boc-piperazine, toluene, 100 °C; (b) TFA, CH_2Cl_2, rt; (c) C_4H_9NCl HCl, $C_2H_6O_2$, 150 °C, 12 h; (d) Na_2CO_3.



6:	X = CH, R_1 = H, R_2 = F	**1:**	X = CH, R_1 = H, R_2 = F
6a:	X = CH, R_1 = OMe, R_2 = F	**7a:**	X = CH, R_1 = OMe, R_2 = F
6b:	X = CH, R_1 = OBn, R_2 = F	**7b:**	X = CH, R_1 = OBn, R_2 = F
6c:	X = CH, R_1 = OH, R_2 = F	**7c:**	X = CH, R_1 = OH, R_2 = F
6d:	X = N, R_1 = H, R_2 = F	**7d:**	X = N, R_1 = H, R_2 = F
6e:	X = N, R_1 = H, R_2 = Cl	**7e:**	X = N, R_1 = H, R_2 = Cl

Scheme 3. General synthesis scheme of title structures and reference compounds. Reaction conditions: (a) **2**, K_2CO_3, KI, CH_3CN, reflux, 18 h; (b) **2**, CH_3OH, CH_3COOH, $NaBH_3CN$, 60 °C, 24 h.

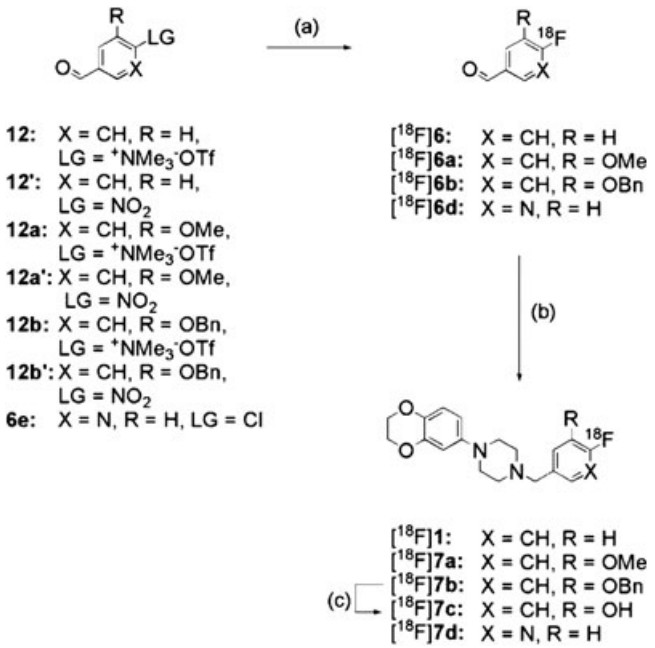


Scheme 4. Synthesis of the precursor **9b** for direct radiolabeling and its reference compound **9a** and the alternative synthesis of title compound **7d**. Reaction conditions: (a) $C_2O_2Cl_2$, DMF, CH_2Cl_2, 50 °C, 2 h; (b) **2**, CH_3Cl, $(CH_3)_3N$ o. C_5H_5N, rt, 3–24 h; (c) BH_3THF, C_4H_8O, reflux, 24 h.

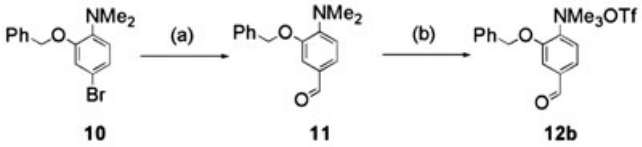
The coupling reaction of the starting intermediate **2** can be performed with corresponding benzylhalides, which led in case of 4-fluorobenzylbromide (**6′**) to good yields of up to 55% of **1** (cf. Scheme 3a). Reductive amination with the aldehydes **6–6e**, however, was selected as the preferred method because of the easier synthesis and also better reproducibility. Thus, a series of new 3-substituted 6-(4-[4-fluorobenzyl]piperazine-1-yl)benzodioxin derivatives were obtained in 20–85% yield (cf. Scheme 3b). They were all used as standard reference compounds with the exception of the pyridine compound **7e**, which makes the direct labeling precursor to obtain [^{18}F]**7d**.

As displayed in Scheme 4 the nitrobenzylamide **9b** and its corresponding fluorine analog **9a** as standard were synthesized from the corresponding carboxylic acids via formation of acid chlorides. The fluoropyridine standard analog (4-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)piperazine-1-yl)(6-fluoro-pyridine-3-yl)methanone) (**7d**) was also synthesized that way with the intention of subsequent reduction and thereby achieving a shorter route of production. However, problems with the reduction procedure occurred as described in the succeeding text.

Alternatively, reductive amination using 6-fluoro-pyridine-3-carbaldehyde **6d** yielded **7d** as described for the other previously



Scheme 5. Radiosynthesis of [^{18}F]**1**, [^{18}F]**7a**, and [^{18}F]**7c–d** by a two-step, one-pot synthesis. Reaction conditions: (a) [^{18}F]F^-, K ⊂ 2.2.2., K_2CO_3, DMSO, 110 °C; (b) **2**, $NaBH_3CN$, DMSO, 110 °C; (c) Pd(black), $HCOONH_4$, MeOH, 60 °C, 10 min.



Scheme 6. Synthesis of (3-benzyloxy-4-formylphenyl)-trimethyl-ammonium triflate (**15b**). Reaction conditions: (a) 1 sec-BuLi, $C_4H_{10}O$, −78 °C, 45 min; 2. DMF, rt, 1 h; (b) $CF_3SO_3CH_3$, CH_2Cl_2, 40 °C, 7 h.

mentioned aldehydes (cf. Scheme 3). Despite an extended reaction pathway and a low amination yield due to the high sensitivity of **6d**, an overall yield of about 40% was obtained. Thus, this method can be compared with the amide formation described earlier.

All aldehydes used as precursors for radiofluorination are listed in Scheme 5. The aldehydes (4-formylphenyl)-trimethylammonium triflate (**12**) and (4-formyl-3-methoxyphenyl)-trimethylammonium triflate (**12a**) were synthesized by the well-known amination of commercially available corresponding fluorine compounds with dimethylamine hydrochloride and subsequent quaternization with methyltrifluoromethane sulfonate.[16,17] The benzyl protected precursor (3-benzyloxy-4-formylphenyl)-trimethylammonium triflate (**12b**) was synthesized from 2-(benzyloxy)-4-bromo-*N*,*N*-dimethylaniline (**10**) (Scheme 6), which was obtained in four steps from 4-bromo-*N*,*N*-dimethylaniline according to literature.[23] The key step was the formation of the aldehyde group from the bromo compound **11** with dimethylformamide (DMF) and *sec*-butyllithium. The nitro precursors **12′** and **12a′** were commercially available, whereas the nitro-analog **12b′** was synthesized by a benzylation of 4-nitro-3-hydroxybenzaldehyde at a temperature of 110 °C following a procedure described by Langer *et al.*[18]

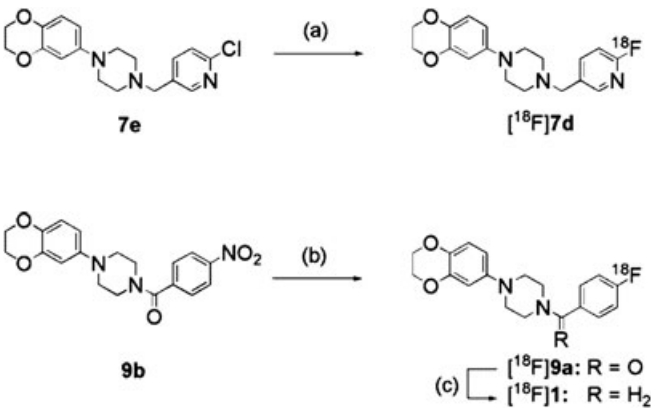
A four-step route starting from 4-fluoro-3-methoxybenzaldehyde was utilized to prepare the corresponding hydroxy and benzyloxy reference compounds (**7b**, **7c**) of the respective radiolabeled compounds by a Lewis acid assisted cleavage of the methyl group from **7a**. The series of compounds obtained included the reference compounds of the desired radiolabeled analogs (**1**, **7a**, **7c** and **7d**), the precursors for aromatic ^{18}F-for-Cl and ^{18}F-for-NO_2 substitution (**7e** and **9b**), and precursor compounds **12–12b** and **6e** suitable for a two-step radiosynthesis of [^{18}F]**1** and [^{18}F]**7a–d** by reductive amination.

Radiochemistry

Direct labeling

For direct labeling of arenes with n.c.a. [^{18}F]fluoride, it is principally necessary that they are activated for nucleophilic substitution. Synthesis of n.c.a. 4-[^{18}F]fluoro-phenylpiperazines is therefore only practicable by labeling of the corresponding amides followed by a radioactive reduction (cf. Scheme 7). The nitro group was selected as the best adaptable leaving group for an S_NAr reaction when trimethylammonium salts are not available. Trimethylammonium triflate as leaving group cannot be generated with the compounds of interest due to the competition of the two tertiary arylamines (dimethyl and arylpiperazine group) with very similar nucleophilicities.

Kryptofix 2.2.2 was used as anion activator for nucleophilic ^{18}F-substitution in dimethyl sulfoxide (DMSO) or DMF. In DMSO, substitution of the nitro group resulted in a radiochemical yield (RCY) of up to 50% after 15 min. As a typical feature of direct labeling of activated aromatic systems, adequate yields are only



Scheme 7. Direct labeling of [^{18}F]**7d** and [^{18}F]**9a** and its reduction to [^{18}F]**1**. Reaction conditions: (a) [^{18}F]F$^-$, K ⊂ 2.2.2., K_2CO_3, DMSO, 160 °C, 20 min; (b) [^{18}F]F$^-$, K ⊂ 2.2.2., K_2CO_3, DMSO, 160 °C, 15 min; (c) BH_3THF, THF, 65 °C, 15 min.

obtained with high reaction temperatures of at least 160 °C. DMF cannot be used because of its lower boiling point, although it leads to considerably higher RCYs at equal temperatures (cf. Figure 1). In this study, higher temperatures of DMF near its boiling point diminished the reproducibility of RCY.

Because of the absence of further reduction-sensitive moieties, at first, the strong reduction system $LiAlH_4/AlCl_3$ in diethylether or THF was applied on the amide [^{18}F]**9a**. However, the RCY varied from zero to 100% after 5 min reaction time, and no reproducibility could be achieved. Furthermore, rapid hydrogen evolution hampered the working performance. Using instead the BH_3/THF complex as reducing agent led to a conversion of 42 ± 4% but required 65 °C and 15 min reaction time. Despite an expected overall RCY of about 21% (calculated from the 50% and 42% yields of radiofluorination and reduction steps, respectively), the RCYs of [^{18}F]**1** were only just above 1% after separation with a semi-preparative HPLC column. This was especially caused by the threefold solid phase extractions, where each time a loss could be observed as well as losses during the HPLC separation. The poor RCYs consequently led to the decision to turn away from direct labeling to applying build-up radiosynthesis.

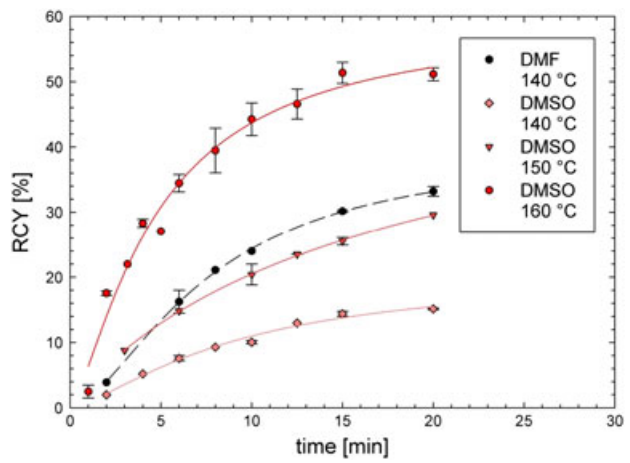


Figure 1. Time dependence of radiochemical yield of [^{18}F]**9a** at different reaction temperatures in DMSO (red) and DMF (black) by direct ^{18}F-substitution on **9b**. Reaction conditions: n.c.a. [^{18}F]F$^-$, [**9b**] = 40 mmol/L, [K ⊂ 2.2.2.] = 40 mmol/L, [K_2CO_3] = 20 mmol/L, solvent = 0.5 mL. Data are expressed as the mean ± SD (n = 2–3). This figure is available in colour online at wileyonlinelibrary.com/journal/jlcr

It was expected that direct nucleophilic ^{18}F-substitution on the pyridine derivative **7d** would proceed much easier. This is due to the fact that pyridines themselves induce an aromatic activation for nucleophilic substitution in *ortho*-position and *para*-position. However, despite this and the high RCYs of 2-[^{18}F]fluoropyridine amounting to 94% described in the literature,[24] only a poor RCY of [^{18}F]**7d** of less than 5% was obtained with the precursor **7e** at 160 °C in DMSO after 30 min.

Build-up synthesis

Since the first utilization of reductive amination of ^{18}F-labeled aldehydes by Wilson *et al.*,[16] the method has proven convenient to reliably produce corresponding n.c.a. benzylamines with fluorine-18 in an aryl position. The labeling conditions were a reaction temperature of about 130–150 °C, DMSO as solvent, and (K⊂2.2.2.)/carbonate as anion activator.[25] As displayed in Figure 2, RCYs obtained by precursors containing the trimethylammonium triflate leaving group (**12–12b**) reach up to 80% after 5–10 min and thus are about twice as high as RCYs obtained by the corresponding nitro precursors (**12′–12b′**) at the same reaction temperature (cf. Scheme 5 and Figure 2).

The general procedure is to separate the labeled aldehyde by solid phase extraction or HPLC purification and then to perform the amination reaction in a mixture of MeOH and CH_3CO_2H at temperatures of about 60 °C. As a variation of the Leuckart–Wallach reaction, sodium cyanoborohydride is commonly chosen as a reducing agent instead of an excess of formic acid. Tian *et al.*[26] showed that reductive amination reactions can also be performed in the presence of Kryptofix 2.2.2. and DMSO. Labeling and amination could therefore be performed in a one-pot reaction in DMSO at the same temperature of 110 °C, thus facilitating remote controlled syntheses.

In the amination reactions performed here, it was found unnecessary to add methanol to the reaction mixture in contrast to acetic acid that is obviously an essential reagent for the rapid intermediate formation of the immonium ion.

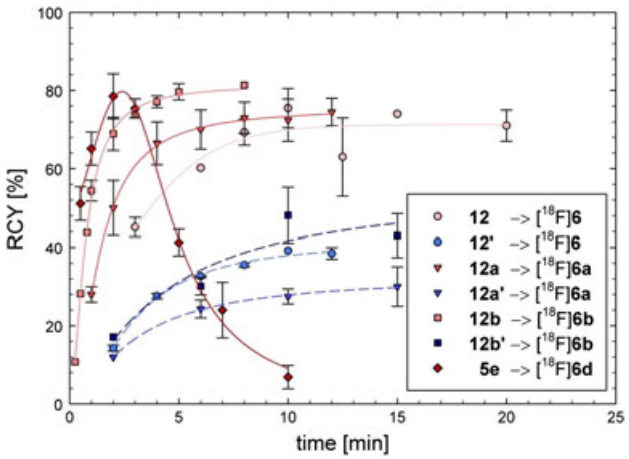


Figure 2. Time dependence of radiochemical yield of ^{18}F-labeling of benzaldehyde derivatives. For comparison, RCYs obtained from nitro precursors (**12′–12b′**) are displayed in dashed blue lines, whereas RCYs obtained from benzaldehyde trimethylammonium triflates (**12–12b**) are shown in solid red lines. 6-[^{18}F]Fluoropyridinealdehyde ([^{18}F]**6d**), obtained from the chloro analog, is also displayed in red. Reaction conditions: n.c.a. [^{18}F]F$^-$, [**12–12b**, **6e**] = 20–60 mmol/L, [K ⊂ 2.2.2.] = 40 mmol/L, [K_2CO_3] = 20 mmol/L, DMSO = 0.5 mL, 110 °C. Data are expressed as the mean ± SD (n = 2–4). This figure is available in colour online at wileyonlinelibrary.com/journal/jlcr

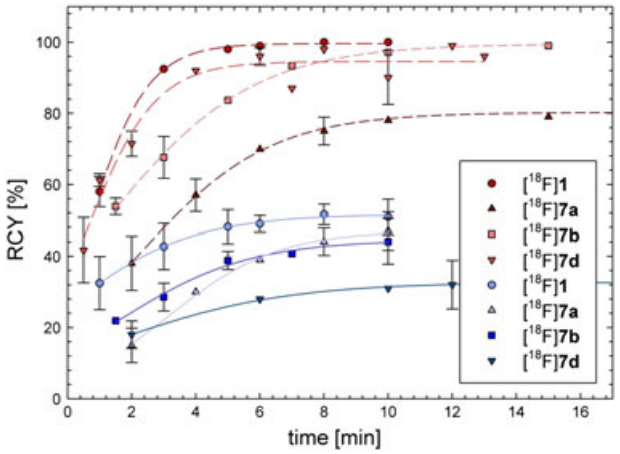


Figure 3. Time dependence of the total RCY of the amination products ([^{18}F]**1** and ([^{18}F]**7a–d**) related to [^{18}F]F^- (blue) as well as that related to the intermediate aldehyde compounds ([^{18}F]**6–6d**) (red). Reaction conditions: [**2**] = 46 mmol/L, $NaBH_3CN$ = 64 µmol, DMSO = 0.6 mL, AcOH = 40 µL, 110 °C. Data are expressed as the mean ± SD (n = 2–3). This figure is available in colour online at wileyonlinelibrary.com/journal/jlcr

Furthermore, fewer radioactive side products were found by using DMSO instead of methanol. As graphically depicted in Figure 3, a nearly quantitative conversion of the ^{18}F-labeled aldehyde to the tertiary amine in DMSO could be observed upon radio-HPLC analysis. The total RCYs obtained over both reaction steps, that is, related to initial [^{18}F]fluoride, were about 30–50%. Performing the one-pot reaction with DMF, which is also a suitable solvent for radiofluorination of aromatic aldehydes, or using lower temperatures, however, led to an extreme loss of RCY. A general disadvantage of the one-pot synthesis, however, is the accumulation of inactive reactants and unidentified side products in the final reaction solution. Depending on the respective product, HPLC purification can therefore be obstructed. Nevertheless, after semi-preparative HPLC isolation, radiochemical purity of all products was >98% and their molar activities reached 60 GBq/µmol for [^{18}F]**1** and [^{18}F]**7a**, and about 30–40 GBq/µmol for [^{18}F]**7c** and [^{18}F]**7d**.

A higher amount of the precursor probably acted as a pseudo carrier and diminished the rate of decomposition as well as the rate of product formation (cf. Figure 4). The influence of both activating components (aldehyde and pyridine) seems to polarize the C–F binding so strongly that even weaker nucleophiles (every halide, hydride, or hydrogen) in low concentrations may displace [^{18}F]fluoride. Lower precursor concentrations than 40 mM are therefore problematic because deviation from the optimal time of quenching the reaction and starting the next step has a serious influence on RCY. Despite these findings, no comparable observation is described in literature for similar nicotinic compounds (*ortho* pyridine and *para* carbonyl) used for ^{18}F-fluorination. A back reaction with chloride as nucleophile, which is theoretically also conceivable, would lead to an equilibrium that, however, was not observed.

This defluorination procedure was also the critical side reaction when the amination reaction with [^{18}F]**6d** was performed. Any change of reaction parameters, however, such as reaction time, lowering of the amine concentration or reaction temperature or using weaker reduction systems, resulted in very poor RCYs.

For the production of the ^{18}F-labeled benzyl protected aldehyde compound [^{18}F]**6b**, ^{18}F-fluorination and amination were performed as described previously. To separate the intermediate product [^{18}F]**6b** from the reaction solution, adsorption on a polymer adsorbent LiChrolut EN cartridge offered an excellent way because the benzyl group led to a considerable higher lipophilicity. Thus, the aforementioned described problems with final HPLC purification are not valid for the hydroxyl derivative [^{18}F]**7c** upon reductive debenzylation of [^{18}F]**7b** in methanol with ammonium formate and palladium black at 60 °C under inert conditions. Before this cleavage, however, complete drying of the benzyl-intermediate is necessary by applying a stream of argon and elution through an AluminaN® cartridge. A water tolerating cleavage with strong acids such as trifluoroacetic acid that additionally would possibly save one separation step is not suitable because of a rapid degradation of the dioxin component and of enhancing even more side reactions. A similar degradation can be observed when cleavage of the methoxy substituent of [^{18}F]**7a** was attempted with BBr_3.

Despite the high debenzylation yield and the good pre-purification, the RCY of [^{18}F]**7c** obtained after semi-preparative radio HPLC separation was lower than with all other products [^{18}F]**1** and [^{18}F]**7**. Losses can be due to the additional reduction and extraction steps and due to adsorption processes during the cleaving step.

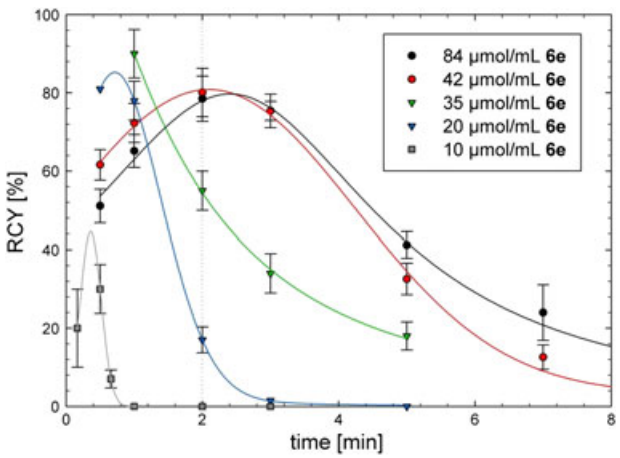


Figure 4. Time dependences of the total RCY of [^{18}F]**6d** with different concentrations of precursor **6e**. Data are expressed as the mean ± SD (n = 2–3). This figure is available in colour online at wileyonlinelibrary.com/journal/jlcr

Comparison of methods

Although direct labeling is the method of choice with short-lived radionuclides and build-up syntheses are normally reserved for cases when the former failed,[27,28] in direct comparison of both methods here, the latter led to better results. In both cases of direct labeling of **9b** and **7e**, only RCYs of about 1–5% were achieved at the end of synthesis (cf. Table 1), whereas build-up syntheses led to RCYs of 9–35% as summarized in Table 2.

Table 1. Radiochemical yields of [^{18}F]**1** and [^{18}F]**7d** by direct labeling in DMSO at 160 °C according to Scheme 7

Precursor	Final product	RCY (%)	Total synthesis time (min)
9b	[^{18}F]**1**	5 ± 2	120
7e	[^{18}F]**7d**	1 ± 0.5	50

Table 2. Total radiochemical yields of consecutive radiofluorination and amination to obtain [^{18}F]**1** and [^{18}F]**4a–d** in a one-pot reaction according to Scheme 5

Precursor	[^{18}F]Fluoro-aldehyde	RCY (%)	Time* (min)	Final product	RCY (%)	Total synthesis time (min)
12	[^{18}F]**6**	75 ± 5	10	[^{18}F]**1**	35 ± 5	~ 90
12′		38 ± 1.5	12			
12a	[^{18}F]**6a**	72 ± 5	10	[^{18}F]**7a**	20 ± 5	~ 90
12a′		30 ± 5	15			
12b	[^{18}F]**6b**	80 ± 2	5	[^{18}F]**7c**	9 ± 4	~ 120
12b′		48 ± 7	10			
6e	[^{18}F]**6d**	80 ± 6	2	[^{18}F]**7d**	15 ± 5	~ 80

*Time of radiofluorination.

Furthermore, the required reaction time of 120 min in case of [^{18}F]**1** could also not match up with the 80 to 90 min of the two-step radiofluorination/amination reaction.

This is due to the high ^{18}F-labeling yields of the trimethylammonium triflate benzaldehydes within short reaction times of 2 to 10 min. Further reasons include the insensitivity and, therefore, reproducibility of the reductive amination reaction and its high conversion rates that make this reaction type remarkably appropriate for n.c.a. ^{18}F-chemistry. Because of a possible one-pot operation, the two-step reaction is characterized by a short total reaction time and low product losses.

An exception is the pyridine chloride derivative **7e** because generation of the ^{18}F-labeled product [^{18}F]**7d** could be performed in a single step. However, ^{18}F-fluorination of the 6-chloronicotinic aldehyde **6e** leads to many inactive side products which complicate product isolation procedures and decrease the yields. On the other hand, the extremely poor yield obtained from direct labeling of the chloropyridine–methylpiperazine compound **7e** is surprising, as normally good results with nucleophilic substitution at the *ortho*-position or *para*-position of pyridines are achieved. Therefore, it would be worthwhile to examine other leaving groups such as the nitro substituent in further experiments. Using microwave irradiation, as described beneficial in many earlier ^{18}F-labeling procedures of pyridines, might be a second reasonable alternative.

Conclusion

Four derivatives of 6-(4-[4-fluorobenzyl]piperazin-1-yl)benzodioxin (**1**) were successfully ^{18}F-labeled in sufficient yields by using a reductive amination reaction of aldehydes after their previous n.c.a. nucleophilic radiofluorination. Because of the viability of both reaction steps without interim separation, radiosynthesis of the typical 1-phenyl-4-benzylpiperazine backbone of D_4 ligands, ^{18}F-labeled at the benzyl moiety, can be performed quickly and easily. These reaction conditions that enable the possibility of convenient one-pot radiosyntheses can be transferred to many different derivatives of [^{18}F]fluorobenzylpiperazine beyond those anticipated here for imaging dopaminergic receptors. This facilitates altering physicochemical parameters such as lipophilicity in order to achieve variations in binding behavior of radioligands.

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Conflict of interest

The authors did not report any conflict of interest.

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