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^{11}C-Fallypride: radiosynthesis and preliminary evaluation of a novel dopamine D2/D3 receptor PET radiotracer in non-human primate brain☆

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Abstract—Fallypride [benzamide, 5-(3-fluoropropyl)-2,3-dimethoxy-*N*-[(2*S*)-1-(2-propenyl)-2-pyrrolidinyl]methyl]-, CAS RN 166173-78-0] is a selective dopamine D2/D3 receptor antagonist. Carbon-11 labeled fallypride may serve as a radiotracer for use in biomedical imaging technique positron emission tomography (PET). The precursor, 5-(3-fluoropropyl)-2-hydroxy-3-methoxy-*N*-[(2*S*)-1-(2-propenyl)-2-pyrrolidinyl]methyl]benzamide was synthesized from 2-hydroxy-3-methoxy-5-(2-propenyl)benzoic acid, methyl ester in seven steps with approximately 10% overall chemical yield. Using this precursor ^{11}C-fallypride was produced by radiolabeling with ^{11}C-methyl iodide in 25–40% radiochemical yields with specific activities of 200–1000 Ci/mmol. PET imaging studies in nonhuman primates with ^{11}C-fallypride showed radiotracer localization in dopaminergic brain regions such as caudate, putamen, thalamus and cortex. This regional localization of ^{11}C-fallypride is similar to that observed previously for ^{18}F-fallypride. The results suggest ^{11}C-fallypride is a useful PET radiotracer for imaging dopamine D2/D3 receptors.

1. Introduction

Fallypride [benzamide, 5-(3-fluoropropyl)-2,3-dimethoxy-*N*-[(2*S*)-1-(2-propenyl)-2-pyrrolidinyl]methyl]-, CAS RN 166173-78-0; Fig. 1 **1a**] is a well-known dopamine D2/D3 antagonist. Fallypride radiolabeled with fluorine-18 (Fig. 1 **1b**) is currently being used as a dopamine D2/D3 receptor imaging agent in positron emission tomographic (PET) studies.[1] The high affinity ($K_i = 30$ pM for D2 receptor sites), selectivity and rapid clearance from nonspecific binding sites enables visualization of a range of receptor concentrations—from very small levels in the cortex to the very high levels in the putamen. This has enabled human and non-human quantitative PET studies using fluorine-18 labeled fallypride.[1,2]

Carbon-11 labeled radiotracers have a potential advantage of back-to-back same-day studies. This can be of value when pharmacological or behavioral challenges are being studied. It can avoid movement of the subject from the PET scanner. Also, if drug effects are being explored, then repeat studies can be performed within 2–3 h of the first study. This is not the case of fluorine-18 labeled radiotracers. It will generally take an overnight wait to repeat a study with fluorine-18. Carbon-11 radiotracers allow good imaging statistics only for about an hour to 90 min. Thus, the radiotracer ought to achieve optimal binding kinetics within this time period. In the case of fallypride, optimal binding kinetics may be achieved in the extrastriatal regions (such as thalamus, amygdala and cortical regions) in 90 min, but not in the striatal regions (caudate, putamen and ventral striatum). We have previously shown that amphetamine-induced dopamine release is able to compete with the binding of ^{18}F-fallypride in both striatal and extrastriatal brain regions.[3,4] We thus postulated that there may be a potential use of ^{11}C-fallypride in same-day repeat studies of extrastriatal brain regions. This will allow the study of changes in ^{11}C-fallypride binding resulting from pharmacological or behavioral challenges.

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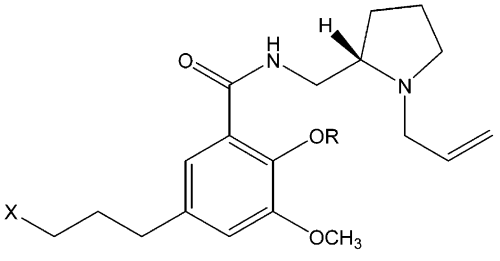
Fallypride is a substituted benzamide that contains a dimethoxy substituent group in the benzamide ring. The 2-methoxy group is a common feature in this class of compounds which can be replaced with a carbon-11 label. This has been done with ^{11}C-raclopride and ^{11}C-FLB 457 successfully.[5,6] Other potential sites on fallypride for introduction of a carbon-11 label include the 3-methoxy group, the *N*-allyl group and the *C*-fluoroalkyl group. Incorporation of a carbon-11 label in the latter two positions will be more difficult in comparision with that of the methoxy group. Radiolabeling of the 2-methoxy group was considered more viable than the 3-methoxy group because of synthetic ease in obtaining the phenolic precursor. We therefore decided to introduce carbon-11 label as a methyl group in the methoxy group at the 2-position (Fig. 1, **1c**) to make ^{11}C-fallypride. We report here the radiosynthesis of ^{11}C-fallypride and preliminary ^{11}C-fallypride-PET imaging studies in a non-human primate.

2. Results and discussion

2.1. Chemistry

In order to synthesize ^{11}C-fallypride by *O*-^{11}C-methylation, a precursor containing a free phenolic group had to be synthesized. Although either of the two *O*-methyl groups found in fallypride could be demethylated using boron tribromide or other demethylating agents, the carbon–fluorine bond was found to be unstable, resulting in the loss of fluorine (Mukherjee et al., unpublished observations). Therefore, an alternate, longer synthesis route of the precursor containing the phenolic group was essential in order to ensure stability of the molecule.

The precursor 5-(3-fluoropropyl)-2-hydroxy-3-methoxy-*N*-[(2*S*)-1-(2-propenyl)-2-pyrrolidinyl]methyl]benzamide (**10**) was synthesized as shown in Figure 2. The starting material 5-allyl-3-methoxysalicylic acid, methyl ester **2** was prepared in three steps from 3-methoxysalicylic acid using reported procedures.[7] The phenolic group at the 2-position was protected using a 2-methoxyethoxymethyl (MEM) ether group. The allyl group was converted to the corresponding alcohol **4** by treatment with dihydroborane followed with hydrogen peroxide and base as reported previously.[8] In order to convert the alcohol to the corresponding fluoride, either treatment with diethylaminosulfur trifluoride (DAST) or conversion to the tosylate followed by treatment with tetrabutylammonium fluoride (TBAF) may be possible and has been reported by us and others.[7,8] Here, we converted the alcohol to the tosylate and subsequently reacted with TBAF in order to obtain the corresponding fluoride **6**. These reactions proceeded in moderate to high yields.



1a. X = F; R = CH_3 Fallypride
1b. X = ^{18}F; R = CH_3 ^{18}F-Fallypride
1c. X = F; R = $^{11}CH_3$ ^{11}C-Fallypride

Figure 1. Chemical structure of fallypride and fluorine-18 and carbon-11 radiolabeled isoforms.

Saponification of the ester group yielded the acid **7**. This acid was then used to couple to the amine **8** in an amide linkage. The amide could be formed by a variety of procedures. We used DAST to convert the acid **7** to the corresponding acid fluoride which was then coupled to the amine. This proceeded smoothly and gave high yields similar to what has been reported for related compounds.[9] Deprotection of the MEM group using titanium chloride provided the final product **10** in good yields. However, titanium chloride also resulted in exchanging with the fluorine to provide the corresponding chlorinated analogue (approximately 30–40% of the product was chlorinated). This created a problem due to the inability to completely remove the chlorinated product from the desired fluorinated product **10**. Alternatively, the deprotection of the MEM group was carried out by using trifluoroacetic acid (TFA). As shown in Figure 2, the MEM protected acid was treated with TFA to provide the deprotected phenol **11**. This was then directly coupled with the amine **8** by the use of benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP). This avoided formation of the chlorinated side-product and the desired product **10** was obtained in approximately 50% yield. Unlabeled fallypride was synthesized as reported previously.[8]

2.2. Radiochemistry

Radiolabeling of the phenolic precursor was carried out by using methods reported previously for preparing ^{11}C-raclopride and ^{11}C-FLB 457.[5,6] Carbon-11 methyl iodide was efficiently prepared by delivering ^{11}C-CO_2 from the cyclotron to the molecular sieve trap in the General Electric methyl iodide unit (GE box; Fig. 3a). The trapped ^{11}C-CO_2 was then reduced to ^{11}C-CH_4 and eventually to ^{11}C-CH_3I in the GE box. We have previously used this approach for the production of ^{11}C-methionine, ^{11}C-choline and ^{11}C-flumazenil all of which require ^{11}C-methylation.[10] The conversion of ^{11}C-CO_2 to ^{11}C-CH_4 avoids the use of lithium aluminum hydride (LAH; converts ^{11}C-CO_2 to ^{11}C-CH_3OLi) which has been considered to be a factor in lowering specific activities of the ^{11}C-methylated derivatives. However, in our hands, in the radiosynthesis of ^{11}C-methionine, the specific activity was found to be similar using the LAH method or the ^{11}C-CH_4 method. The specific activities of the various compounds have generally been around 500 Ci/mmol. The ^{11}C-CH_4 method is expected to provide higher specific activities; however it is possible that contents in the target or the GE box may be affecting the specific activity of ^{11}C-CO_2 which eventually affects the specific activity of the final product. An approach

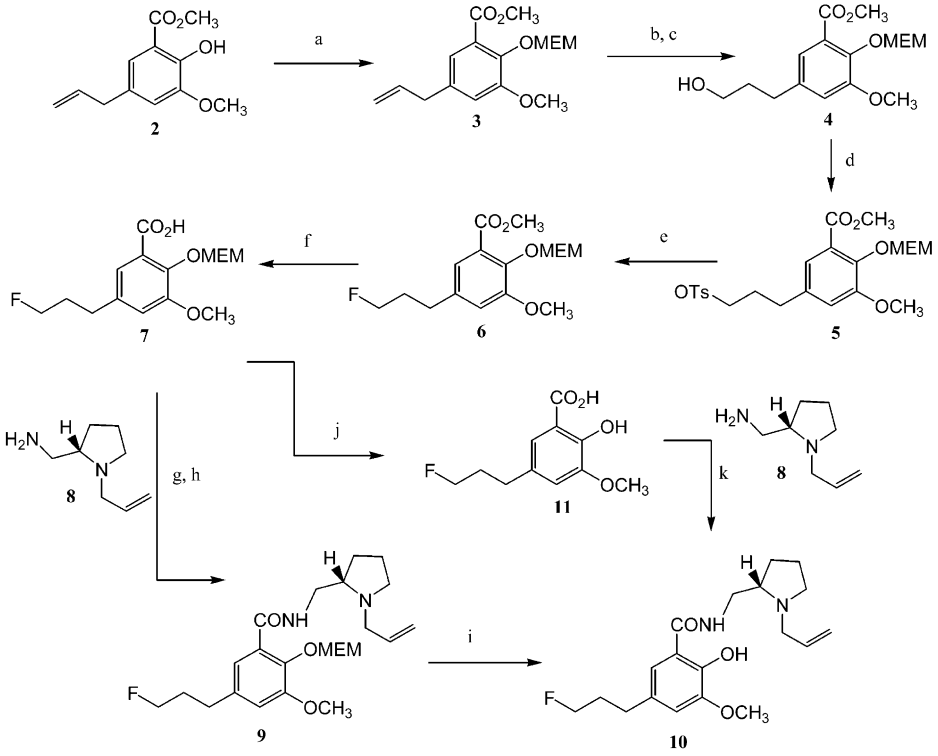


Figure 2. Reaction scheme for synthesis of the precursor, 5-(3-fluoropropyl)-2-hydroxy-3-methoxy-*N*-[(2*S*)-1-(2-propenyl)-2-pyrrolidinyl]methyl]benzamide: (a) MeM chloride, NaH, THF; (b) BH_3, THF; (c) 30% H_2O_2, 1 M NaOH; (d) TsCl, Et_3N, CH_2Cl_2; (e) $Bu_4N^+F^-$, THF; (f) 0.5 N NaOH, THF; (g) diethylaminosulfur trifluoride, CH_2Cl_2; (h) **8**, Et_3N, CH_2Cl_2; (i) $TiCl_4$, CH_2Cl_2; (j) TFA, CH_2Cl_2; (k) **8**, BOP, Et_3N, CH_3CN.

currently being employed is to discard the first batch of ^{11}C-CH_3I that is produced by the GE box. This possibly purges the unlabeled CO_2 that may be present in the system and therefore increases the specific activity in the subsequent runs.

This ^{11}C-CH_3I from the GE box was transferred to the Nuclear Interface ^{11}C-methylation unit (NI box; Fig. 3a) as reported previously.[10] Both the GE box and the NI box were interfaced with a PC controller and were remotely operated. The ^{11}C-CH_3I from the GE box was transferred to the NI box via a tubing linking the two boxes and controlled by PC operated valves.

The reaction Scheme used for the radiolabeling procedure is shown in Figure 3b. The reaction vessel in the NI box contained the phenolic precursor **10** dissolved in dimethylformamide (DMF) and tetrabutylammonium hydroxide (TBAH) was used as a base during the radiolabeling reaction. This is somewhat similar to the reaction conditions typically used for the production of ^{11}C-raclopride and ^{11}C-FLB 457 where the base is sodium hydroxide and reaction solvent is dimethylsulfoxide.[6] The amount of ^{11}C-CH_3I trapped in this reaction vessel typically ranged between 100 and 200 mCi. The radiolabeling reaction proceeded smoothly with the desired product, ^{11}C-fallypride as the principal radioactive species on the HPLC purification (Fig. 4). The precursor was well separated and the fluorine in the molecule did not interfere and was not affected by the radiolabeling conditions. Radiochemical yields were typically in the 25–40% range and is in the range of ^{11}C-methylation reactions using ^{11}C-methyl iodide. Higher yields have been reported when ^{11}C-methylations are carried out with ^{11}C-methyl triflate.[11]

Chromatographic separation (preparative HPLC, C18 reverse phase column, 56% of acetonitrile, 0.1% of triethylamine and water at 2.8 mL/min) provided ^{11}C-fallypride as the principal product (Fig. 4). The HPLC chromatogram also illustrates the excellent separation of the phenolic precursor from the radiolabeled product, ^{11}C-fallypride which eluted at approximately 10 min. The radiochemical yield of ^{11}C-fallypride was in the range of 25–40% decay-corrected from ^{11}C-methyl iodide. Initial specific activities were estimated to range between 200 and 300 Ci/mmol. Improvements in the production of ^{11}C-CH_3I has now raised the specific activities of ^{11}C-fallypride to 400–800 Ci/mmol. Thus far the best specific activity has been 1000 Ci/mmol.

2.3. PET imaging

Brain uptake and distribution of ^{11}C-fallypride in a rhesus monkey PET study demonstrated binding in dopaminergic regions. Example brain slices are shown in Figure 5. Receptor-rich regions such as putamen were clearly evident in all the slices. Regions that contain moderate amount of receptors such as the thalamus (about 5% of that in the putamen) were also clearly seen. Cortical regions are known to have small but significant amount of D2/D3 receptors as demonstrated in our previous studies with ^{18}F-fallypride.[2] Cortical binding was also evident with ^{11}C-fallypride.

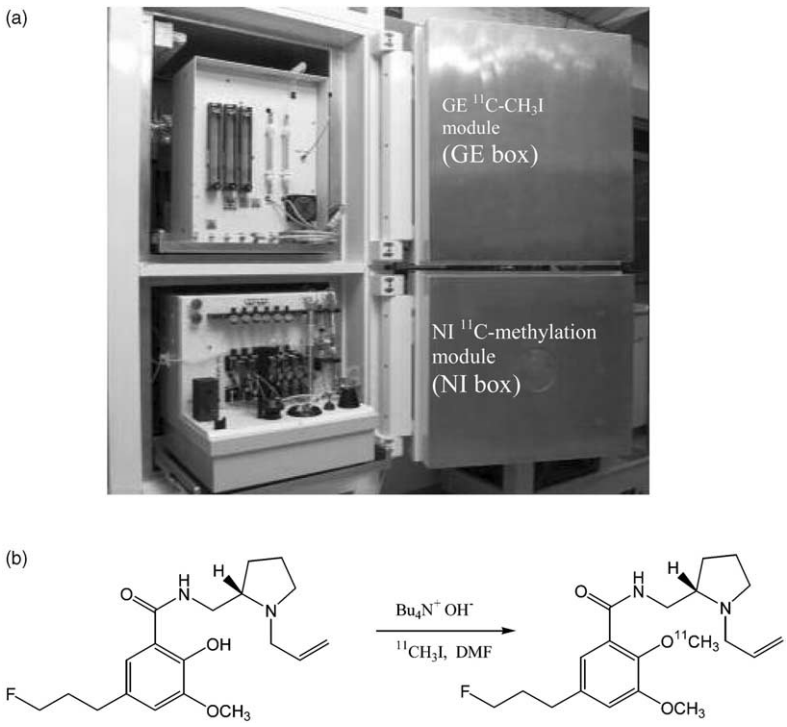


Figure 3. (a) GE ^{11}C-methyl iodide module (GE box) and NI ^{11}C-methylation module (NI box) within stacked mini-hot cells. The shielding door is shown in open position. The ^{11}C-CH$_3$I was produced in the GE box and transferred to the reaction vial in the NI box for the radiolabeling reaction; (b) radiosynthesis reaction scheme for ^{11}C-fallypride using ^{11}C-CH$_3$I.

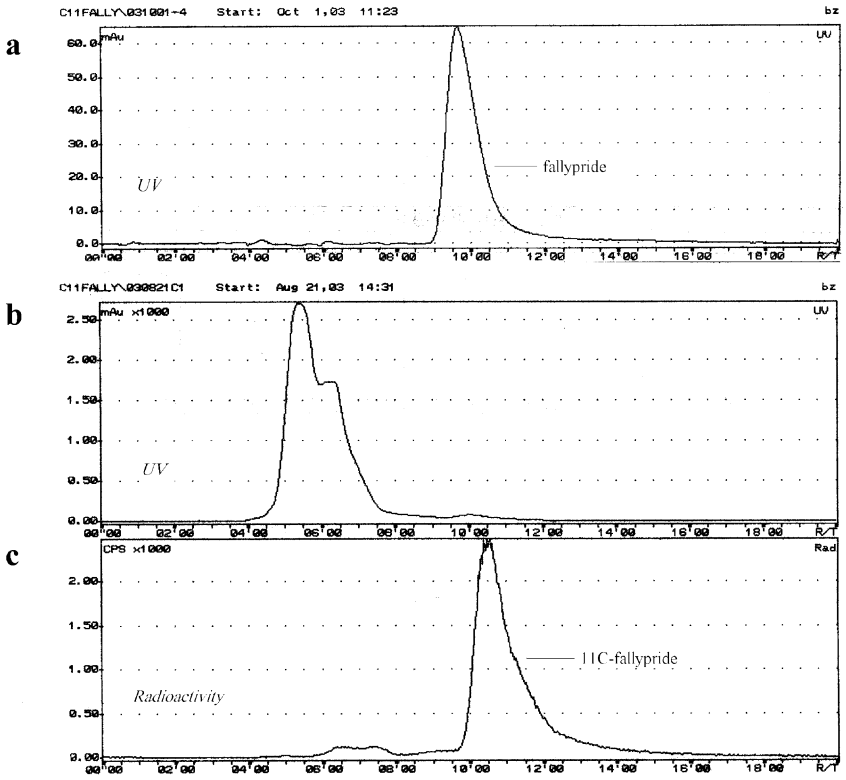


Figure 4. HPLC semi-preparative separation profile of fallypride and ^{11}C-fallypride product mixture on a C-18 column (250×10 mm) eluted with 56% acetonitrle and 44% water containing 0.25% triethylamine at a flow-rate of 2.8 mL/min: (a) UV (254 nm) trace of one fallypride reference standard (0.016 mg; 43.8 nmol); (b) UV (254 nm) trace of radiolabeling reaction mixture; and (c) radioactivity trace of ^{11}C-fallypride eluted at approximately 10 min and is well separated from the phenolic precursor appearing at 5 min.

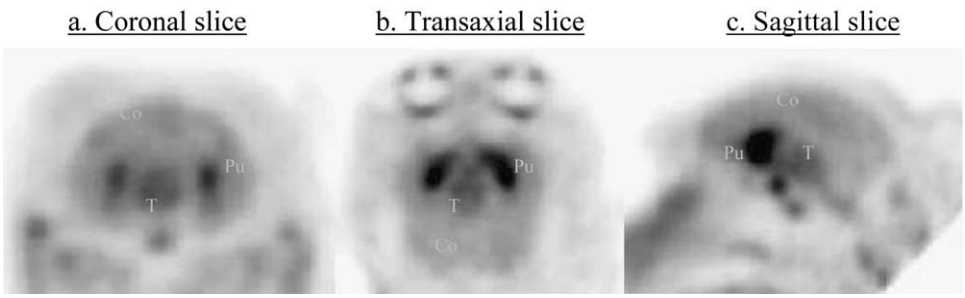


Figure 5. Select PET image slices (coronal, transaxial and sagittal planes) of the rhesus monkey brain using ^{11}C-fallypride. Dopaminergic regions showing ^{11}C-fallypride binding include putamen (Pu), thalamus (T) and cortex (Co).

Decay corrected time–activity curves of ^{11}C-fallypride in various brain regions of the rhesus monkey brain are shown in Figure 6 and indicate kinetics that are comparable to those observed for ^{18}F-fallypride.[2] Highest uptake is seen in the striatal regions (caudate and putamen exhibit uptake in the range of 0.04–0.06% injected dose/cc), with lower uptake in the thalamus and cortex. Binding in the thalamus and cortex was found to be somewhat lower than that measured for ^{18}F-fallypride. This is primarily due to the lower specific activity of ^{11}C-fallypride (200 Ci/mmol for this particular experiment) compared to that of ^{18}F-fallypride (2800 Ci/mmol). Theroetically however, the kinetics of the two tracers should be similar if the specific activity of the ^{11}C-fallypride can be improved. Similarly, cortical binding was also observed with ^{11}C-fallypride, but lower than that found for ^{18}F-fallypride. In the case of caudate and putamen, ^{11}C-fallypride may not allow enough time to attain equilibrium. Typically the scanning times of approx. 3 h are employed for ^{18}F-fallypride.[1,2]

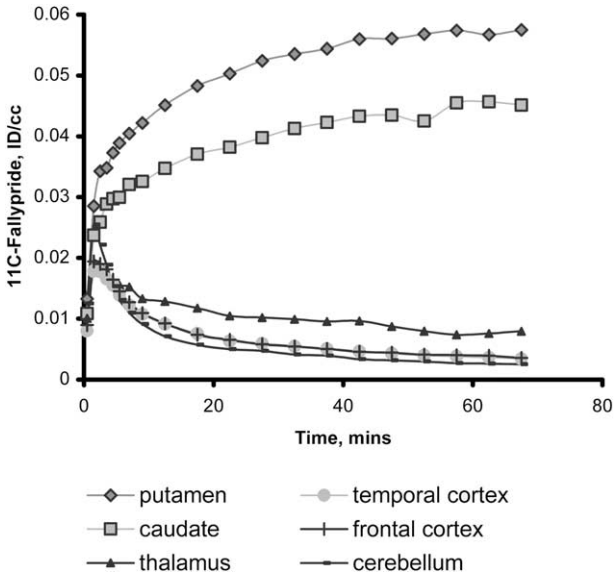


Figure 6. Time–activity curve in rhesus monkey brain after iv administration of 2.4 mCi ^{11}C-fallypride, specific activity 200 Ci/mmol.

3. Conclusions

The synthetic procedure of precursor 5-(3-fluoropropyl)-2-hydroxy-3-methoxy-*N*-[(2*S*)-1-(2-propenyl)-2-pyrrolidinyl]methyl]benzamide has been developed. The compound was found to be stable and suitable for radiolabeling with ^{11}C-methyl iodide. ^{11}C-Fallypride was prepared in 25–40% radiochemical yield and specific activities of 200–1000 Ci/mmol in a tandem GE ^{11}C-CH_3I synthesis module (GE box) and NI ^{11}C-methylation module (NI box). Preliminary ^{11}C-fallypride-PET imaging studies in rhesus monkeys indicate similar characteristics of uptake and binding to ^{18}F-fallypride. Our results suggest that ^{11}C-fallypride may be an excellent PET radiotracer for same day repeat studies of extrastriatal dopamine D2/D3 receptors.

4. Materials and methods

Commercial reagents and solvents (Aldrich Chemical Company, Milwaukee, WI, USA) of analytical grade were used without further purification. 2-Hydroxy-3-methoxy-5-(2-propenyl) benzoic acid, methyl ester **2**, (*S*)-2(aminomethyl)-*N*-allylpyrrolidine **8** and fallypride **1a** were prepared according to previously described methods using 3-methoxysalicylic acid and allyl bromide.[7,8] Reactions and extractions solutions were evaporated in a rotatory evaporator under reduced pressure as appropriate. Analytical and preparative thin layer chromatography (TLC) were performed using Baker-flex silica gel IB-F (Philipsburg, NJ, USA) and Alltech DC-Fertigplatten SIL G-200 UV254 plates (Deerfield, IL, USA), respectively.

High pressure liquid chromatography (HPLC) was carried out on a Gilson Gradient System (Middleton, WI, USA) consisting of two Gilson pumps and one UV detector with wavelength fixed at 254 nm. The system was used isocratically (varying compositions of acetonitrile and water containing triethylamine) for the purification of radiolabeled reaction mixtures. Semiprep (250×10 mm) C18 columns from Alltech were used for reverse-phase HPLC. Proton nuclear magnetic resonance (NMR, 500 MHz, TMS internal reference in $CDC1_3$) spectra were obtained on a Bruker-Advance 500 spectrometer. Electrospray mass spectra were obtained on a Model 7250 mass spectrometer (Micromass LCT).

4.1. 3-Methoxy-2-[(2-methoxyethoxy)methoxy]-5-(2-propenyl)benzoic acid, methyl ester (3)

2-Hydroxy-3-methoxy-5-(2-propenyl) benzoic acid, methyl ester **2** (6.00 g; 27 mmol), dissolved in THF (20 mL), was added drop wise to the suspended mixture of sodium hydride (2.0 g of a 60% (w/w) mineral oil suspension; 139 mmol) in 40 mL of THF at 0 °C and the reaction was allowed to stir for 30 min at 0 °C. Then (2-methoxyethoxy)methyl chloride (4 mL, 40.5 mmol) was added slowly. The reaction was allowed to warm to room temperature for 2 h. After that the reaction was quenched by slow addition of 1 M NaOH (40 mL) at 0 °C. The THF was removed by rotary evaporation, and the product was extracted using CH_2Cl_2 (3×50 mL), dried ($MgSO_4$), filtered, and concentrated. Purification of the crude material by column chromatography (gravity silica gel; EtOAc/hexane, 1:l) provided **3** (8 g, 96%; R_f 0.72 in 1:1 hexane/ethyl acetate) as a yellow liquid: ^{1}H NMR δ 7.16 (d, 1H, J = 2.0 Hz), 6.87 (d, 1H, J = 2.0 Hz), 5.93 (ddt, 1H, J = 16.7, 10.2, 6.7 Hz), 5.20 (s, 2H), 5.12 (dd, 1H, J = 16.7, 1.7 Hz), 5.08 (dd, 1H, J = 10.2, 1.6 Hz), 3.96 (m, 2H), 3.88 (s, 3H), 3.83 (s, 3H), 3.56 (m, 2H), 3.37 (s, 3H), 3.36 (d, 2H). Mass spectra (m/z, %): 333 ($[M + Na]^+$, 100%), 235 ($[M + Na]^+$–$C_3H_7O_2Na$, 15%).

4.2. 3-Methoxy-2-[(2-methoxyethoxy)methoxy]-5-(3-hydroxypropyl)benzoic acid, methyl ester (4)

To a cooled (0 °C) solution of alkene **3** (6.00 g; 19.4 mmol) in THF (20 mL) BH_3 in THF (1.5 M, 20 mL) was added, and the mixture was stirred at 0 °C for 30 min followed by 1 h at room temperature. The reaction mixture was cooled (0 °C) and then 1 M NaOH (50 mL) was added carefully, followed by 30% aqueous hydrogen peroxide (40 mL). The reaction mixture was stirred at 0 °C for 30 min then room temperature for 1 h. The THF was removed by rotary evaporation, and the aqueous solution was extracted with CH_2C1_2 (3×50 mL). The pooled dichloromethane layers were dried ($MgSO_4$), filtered, and evaporated to give 5.98g of a crude oil which was purified by column chromatography (silica gel; EtOAc/hexane, 1:1) to provide **4** (3.98 g, 63%; R_f 0.29 in 1:1 hexane: ethyl acetate) as a clear oil. ^{1}H NMR δ 7.17 (d, 1H, J = 2.0 Hz), 6.89 (d, 1H, J = 2.0 Hz), 5.2 (s, 2H) 3.96 (m, 2H), 3.87 (s, 3H), 3.84 (s, 3H), 3.68 (t, 2H, J = 6.3 Hz), 3.57 (m, 2H), 3.37 (s, 3H), 2.68 (t, 2H, J = 7.4 Hz), 1.88 (m, 2H). Mass spectra (m/z, %): 351 ($[M + Na]^+$, 100%).

4.3. 3-Methoxy-2-[(2-methoxyethoxy)methoxy]-5-(3-[(4-methylphenyl)sulfonyl]oxy]propyl) benzoic acid, methyl ester (5)

The alcohol **4** (1.11g; 3.38 mmol) in CH_2C1_2 (5 mL) was treated with pyridine (0.35 mL, 4.4 mmol) and *p*-toluenesulfonyl chloride (0.7 g, 3.67 mmol). The reaction vessel was kept at room temperature for overnight. The reaction solvent was evaporated, and the residue was treated with 0.25 M HCl (20 mL) and extracted with Et_2O (2×25 mL). The pooled organic layers were dried ($MgSO_4$), filtered, concentrated, and chromatographed (silica gel column; EtOAc/hexane, 1:1) to yield **5** (1.54 g, 94%; R_f 0.62 in 1:1 hexane: ethyl acetate) as a colorless oil: ^{1}H NMR δ 7.78 (d, 2H, J = 8.1 Hz), 7.33 (d, 2H, J = 8.3 Hz), 7.19 (d, 1H, J = 2.0 Hz), 6.84 (d, 1H, J = 2.0 Hz), 5.18 (s, 2H), 4.04 (t, 2H, J = 6.0 Hz), 3.95 (s, 3H), 3.87 (s, 3H), 3.83 (s, 2H), 3.57 (m, 2H), 3.37 (s, 3H), 2.63 (m, 2H), 2.45 (s, 3H), 1.96 (m, 2H). Mass spectra (m/z, %): 505 ($[M + Na]^+$, 100%), 407 ($[M + Na]^+$–$C_3H_7O_2Na$, 32%).

4.4. 3-Methoxy-2-[(2-methoxyethoxy)methoxy]-5-(3-fluoropropyl)benzoic acid, methyl ester (6)

Tetra-*n*-butylammonium fluoride in THF (1 M, 5.1 mL, 5.1 mmol) was added to a solution of tosylate **5** (1.5g, 3.1 mmol) in THF (20 mL). The reaction mixture was warmed to 55–60 °C and stirred for 2 h. The solution was concentrated by rotary evaporation and then water (5 mL) was added and extracted repeatedly with ether (5×15 mL). The combined ether extracts were pooled together, dried ($MgSO_4$) and concentrated, and then purified by column chromatography (silica gel, EtOAc/hexane, 1:1) to yield **6** (0.79 g, 77%; R_f 0.67 in 1:1 hexane: ethyl acetate) as a brownish oil. ^{1}H NMR δ 7.17 (d, 1H, J = 2 Hz), 6.89 (d, 1H, J = 2 Hz), 5.2 (s, 2H), 4.50 (dt, 2H, J = 47.1, 5.8 Hz), 3.95 (m, 2H), 3.88 (s, 3H), 3.84 (s, 3H), 3.57 (m, 2H), 3.38 (s, 3H), 2.73 (t, 2H, J = 7.5 Hz), 2.01 (m, 2H). Mass spectra (m/z,%): 353 ($[M + Na]^+$, 100%).

4.5. 3-Methoxy-2-[(2-methoxyethoxy)methoxy]-5-(3-fluoropropyl)benzoic acid (7)

The ester **6** (0.75 g; 3.43 mmol) was dissolved in THF (20 mL), and then 0.5 M NaOH (60 mL) was added, and the solution was heated to 80 °C for 1 h. THF was removed and the aqueous reaction mixture was washed with ether (20 mL). The aqueous layer was acidified with H_3PO_4 to pH 2–3 and then extracted with CH_2Cl_2 (4×50 mL). The organic portion was dried ($MgSO_4$) and filtered, and the solvent was evaporated to give **7** (0.7 g, 100%; R_f 0.45 in 9:1 dichoromethane: methanol) as a colorless oil. ^{1}H NMR δ 7.54 (d, 1H, J = 2 Hz), 6.97 (d, 1H, J = 2 Hz), 5.2 (s, 2H), 4.47 (dt, 2H, J = 47.2, 5.8 Hz), 3.88 (s, 3H), 3.84 (s, 3H), 3.57 (m, 2H), 2.73 (m, 2H), 2.03 (m, 2H). Mass spectra (m/z, %), 339 ($[M + Na]^+$, 15%), 242 ($[M + H]$-$C_3H_7O_2]^+$, 100%)

4.6. (*S*)-*N*-[(1-Allyl-2-pyrolidinyl)methyl]-3-methoxy-2-[(2-methoxyethoxy)methoxy]-5-(3-fluoropropyl)benzamide (9)

3-Methoxy-2-[(2-methoxyethoxy)methoxy]-5-(3-fluoropropyl) benzoyl fluoride (prepared from compound **7** by using reported method),[9] (0.35 g, 1.1 mmol) and Et_3N (1.4 mmol, 200 μL) were taken in CH_2Cl_2 (4 mL). To this solution (*S*)-2-(aminomethyl)-*N*-allylpyrrolidine (0.15 g, 1.1 mmol) dissolved in CH_2Cl_2 (1 mL) was added drop wise at ambient temperature. The reaction mixture was allowed to stir at ambient temperature for 1 h. The organic layer was washed with saturated solution of sodium bicarbonate followed by water and was then dried over $MgSO_4$, filtered and the solvent removed in vacuo. The crude product was purified by preparative TLC (CH_2Cl_2/MeOH, 9:1) to yield **9** (0.24g, 50%; R_f 0.46 in 9:1 dichoromethane/methanol). ^{1}H

NMR d 7.46 (d, 1H, $J=2$ Hz), 6.85 (d, 1H, $J=2$ Hz), 5.92 (m, 1H), 5.3 (s, 2H), 5.18 (d, 1H), 5.21 (d, 1H), 4.46 (dt, 2H, $J=47.1$, 5.9 Hz), 3.82 (s, 3H), 3.50 (m, 2H) 3.35 (s, 3H), 3.27 (m, 2H), 3.09 (m, 2H), 2.89 (m, 1H), 2.74 (m, 2H). Mass spectra (m/z, %), 439 ($[M+H]^+$, 100%), 461 ($[M+Na]^+$, 51%).

4.7. (*S*)-*N*-[(1-Allyl-2-pyrolidinyl)methyl]-2-hydroxy-3-methoxy-5-(3-fluoropropyl)benzamide (10)

Benzamide **9** (0.19 g; 0.46 mmol) was dissolved in CH_2Cl_2 (5 mL) and $TiC1_4$ in CH_2Cl_2 (1 M, 2.5mL) was added at 0 °C. The mixture was stirred for 1 h at 0 °C. A 10% solution of $NaHCO_3$ (25 mL) was added slowly at 0 °C. The solution was then warmed to room temperature. The reaction mixture was extracted with CH_2Cl_2 (3×25 mL). The organic portions were pooled together, dried ($MgSO_4$), filtered, evaporated, and purified by preparative TLC (silica gel; CH_2Cl_2:MeOH, 9:1) to yield **10** (0.11 g, 76%; R_f 0.50 in 9:1 dichoromethane: methanol) as a yellow mass. ^{1}H NMR δ 8.05 (br, 1H), 7.09 (s, 1H), 6.83 (d, 1H, $J=1.7$ Hz), 5.98 (ddt, 3H, $J=16.8$, 10.1, 6.8 Hz), 4.47 (dt, 2H, $J=47.3$, 5.9 Hz), 3.88 (s, 3H), 3.52 (t, 2H, $J=6.3$ Hz) 3.35 (s, 3H), 3.27 (m, 2H), 3.09 (m, 2H), 2.89 (m, 1H), 2.74 (m, 2H). Mass spectra (m/z, %), 351 ($[M+H]^+$, 100%), 373 ($[M+Na]^+$, 3%).

Closer examination of this product revealed a mixture of the chloro- and fluoro-derivatives, which were inseparable on preparative TLC. An alternate method of deprotection of the MEM group involved the use of trifluoroacetic acid that is described below.

4.8. 2-Hydroxy-3-methoxy-5-(3-fluoropropyl)benzoic acid (11)

The acid **7** (0.2 g; 0.88 mmol) was dissolved in dichoromethane (1 mL) and 0.2 mL of trifluoroacetic acid was added. This mixture was allowed to stir overnight after which time the contents were dried in vacuo. The residue was taken up in 10% $NaHCO_3$ and acidified with 1 N HCl and extracted with dichoromethane. The organic portions were pooled together, dried ($MgSO_4$), filtered, evaporated, and purified by preparative TLC (silica gel; CH_2Cl_2:MeOH, 9:1) to yield **11** (0.10 g, 50%; R_f 0.49 in 9:1 dichoromethane: methanol) as a yellow oil. ^{1}H NMR δ 7.59 (d, 1H, $J=1.9$ Hz), 6.98 (d, 1H, $J=2$ Hz), 4.48 (dt, 2H, $J=47.1$, 5.9 Hz), 3.92 (s, 3H), 2.74 (m, 2H), 2.06 (m, 2H). Mass spectra (m/z, %), 229 ($[M+H]^+$, 3%), 251 ($[M+Na]^+$, 100%).

4.9. (*S*)-*N*-[(1-allyl-2-pyrolidinyl)methyl]-2-hydroxy-3-methoxy-5-(3-fluoropropyl)benzamide (10)

The acid **11** (0.1 g; 0.44 mmol) was dissolved in acetonitrile (1 mL) and to this was added (*S*)-2-(aminoethyl)-*N*-allylpyrrolidine (0.07g, 0.55 mmol). While stirring, BOP reagent (benzotriazol-1-yloxy-tris(dimethylamino)-phosphonium hexafluorophosphate) 0.195 g and 0.1 mL of triethylamine were added and the reaction mixture was stirred overnight. Solvents were removed in vacuo and the residue was taken up in dichloromethane and washed with water and $NaHCO_3$. The organic portions were pooled together, dried ($MgSO_4$), filtered, evaporated, and purified by preparative TLC (silica gel; CH_2Cl_2/MeOH, 9:1) to yield **10** (0.075 g; 48%) as a yellow mass. Spectral properties confirmed the product as described before.

4.10. Radiosynthesis

^{11}C-CO_2 from a RDS-112 cyclotron was converted to ^{11}C-CH_3I in a General Electric methyl iodide unit (GE box). This ^{11}C-CH_3I was then transferred to the reaction vessel in the Nuclear Interface methylation unit (NI box). The reaction vessel contained a freshly prepared mixture of the precursor **10** (1 mg, free base) and 1.0 μL of tetrabutylammonium hydroxide dissolved in 100 μL DMF and maintained at −10 °C. The reaction was carried out at 50 °C for 2 min and 55 °C for 4 min. The initial lower temperature of 50 °C (external temperature; note: bp of CH_3I is 41–43 °C) may avoid excessive vaporization of ^{11}C-CH_3I resulting in higher radiochemical yield. After the reaction, 0.25 mL of water was added to the reaction vessel and volatiles were pumped out. Subsequently, 1 mL of HPLC buffer was added to the reaction vessel and the contents of the vessel were loaded and injected onto a C18 column (Alltech Econosil C18 10U, 250×10 mm) eluted with a solvent containing 56% of acetonitrile, 0.1% of triethylamine and water at 2.8 mL/min. The radioactive fraction between 10 and 12 min, which corresponds to the reference fallypride under the same condition, was collected. This collected fraction was taken to near dryness in vacuo. Approximately 5–8 mL of sterile saline (0.9%, NaCl INJ, USP, 10 mL single-dose, Abbot Laboratories, Chicago, IL, USA) was added to the flask. The contents of the flask were then drawn into a 5- or 10-mL sterile syringe depending on volume. The contents of the syringe were then filtered through a 0.2 micron Millex-FG Millipore sterile filter (Millipore Corp., Bedford, MA, USA). This final dose is then used to determine specific activity using the fallypride standards.

4.11. PET imaging

The rhesus monkey was anesthetized using ketamine (10 mg/kg), xylazine (0.5 mg/kg) and was subsequently maintained on 0.5–1.5% isoflurane. Two intravenous catheters were placed, one on each arm, for purposes of administration of the radiopharmaceutical and for obtaining blood samples during the study. The head of the animal was placed in the gantry of a ECAT EXACT HR+ PET scanner and positioned in place with the use of adhesive tape as described previously.[2] A transmission scan using a Ge-68/Ga-68 rod source was acquired prior to administration of the radiopharmaceutical to correct for tissue attenuation of the coincident radiation. A dynamic sequence of scans for a total of approximately 70 min was acquired in the 3-D-mode immediately after administering approximately 2–3 mCi of ^{11}C-fallypride intravenously. Data in final form were expressed in units of percent-injected dose/cc or nCi/cc. Areas showing maximal radioligand binding within the caudate, putamen, thalamus, cortex and cerebellum were delineated in the images.

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