Positron-Emitting *N*-[^{18}F]Fluoroalkyl and [^{18}F]Fluoropyrrolidinyl Analogues of Eticlopride as Potential *in Vivo* Radioligands for Dopamine D2 Receptors

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N-Fluoroalkyl and 4-fluoropyrrolidinyl eticlopride analogues with high affinity toward central nervous system dopamine D2 receptors *in vitro* were labelled with positron emitting fluorine-18 ($t_{1/2}$=110 min), and their *in vivo* biodistribution was investigated in rats. *N*-[^{18}F]Fluoro-ethyl and -propyl eticlopride derivatives showed poor *in vivo* selectivity in the rat brain. On the other hand, 4-[^{18}F]fluoropyrrolidinyl eticlopride exhibited almost constant and relatively high striatal concentration. The striatal/cerebellar radioactivity ratio, which corresponds to the ratio of a brain D2 receptor-rich to poor region, gradually increased to 5.2—6.4, 90 min after the injection. The striatal accumulation was selectively inhibited by pre-injection of haloperidol, a dopamine D2 antagonist, without affecting accumulation in other tissues. Thus, the selective striatal accumulation of 4-[^{18}F]fluoropyrrolidinyl eticlopride in striatal tissue appears to be due to the specific binding to dopamine D2 receptors.

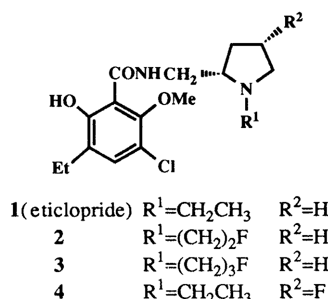
Keywords [^{18}F]fluoroeticlopride; specific radioligand; positron emission tomography; dopamine D2 receptor; *in vivo* binding experiment

Dopamine neurotransmitter systems are thought to be involved in various neuropsychiatric disorders, such as Parkinson's disease, Huntington's chorea, and schizophrenia.[1] *In vivo* imaging of dopamine receptors using radioligands with positron emission tomography (PET) has been expected to be clinically useful to investigate such disorders of the central nervous system (CNS) in the living human. Based on the proposal that the antipsychotic effect of neuroleptic drugs is mediated by blockade of CNS D2-dopamine receptors, a variety of positron emitting radioligands with high affinity toward D2 receptors have been prepared and employed in *in vitro* and *in vivo* investigations.[2-5] Nevertheless, it is difficult to correlate *in vivo* PET imaging with neuropsychiatric pathophysiology. Thus, it is of significant importance to develop potential radioligands with specific and high affinity toward CNS D2 receptors. Fluorine-18 has been utilized in many positron emitting D2 ligands,[3,4] since it has a relatively long half-life ($t_{1/2}$=110 min) compared to carbon-11 ($t_{1/2}$=20.4 min) and it is accepted as a desirable radioisotope for PET studies of slow ligand–receptor binding.[2] Benzamide drugs such as raclopride and eticlopride are highly potent and selective dopamine D2 antagonists,[6] which have recently attracted much attention as a lead compound to develop potential CNS D2 radioligands.[3-5,7,8] However, the incorporation of fluorine-18 to the pyrrolidine *N*-alkyl portion of raclopride or eticlopride has resulted in nonspecific binding *in vivo*.[5] During the development of potential radioligands for CNS D2 receptors, we have synthesized *N*-fluoroalkyl (**2**, **3**)[7] and 4-fluoropyrrolidinyl (**4**)[8] eticlopride analogues (**1**), (*S*)-(−)-5-chloro-3-ethyl-*N*-[(1-ethyl-2-pyrrolidinyl)-methyl]-6-methoxysalicylamide, which retain significant affinities toward dopamine D2 receptors in *in vitro* binding experiments (summarized in Table I). In this paper, we describe the synthesis of ^{18}F-labelled eticlopride analogues corresponding to **2**, **3**, and **4**, and their *in vivo* biodistribution in rats. In these studies 4-[^{18}F]fluoropyrrolidinyl eticlopride ([^{18}F]**4**) has been found to display specific binding to dopamine D2 receptors *in vivo*.

Table I. *In Vitro* Receptor Binding[a)]

Compound	IC_{50} (nM)[b)]
1	2.9[7)]
2	23[7)]
3	17[7)]
4	1.9[8)]

a) Data taken from the literature, in which bovine[7)] or rat[8)] striatal tissue homogenates were used *in vitro*. *b)* IC_{50} represents the concentration required to inhibit specific binding of [^{3}H]spiperone by 50%.



Results and Discussion

Chemistry We have already reported the syntheses of unlabelled ligands (**2**—**4**),[7,8] which were used as high performance liquid chromatographic (HPLC) standards in radiosynthesis. In the radiosynthesis of [^{18}F]**2**, [^{18}F]**3**, [^{18}F]**4**, the corresponding mesylates were chosen as precursors to introduce an [^{18}F]fluorine atom through nucleophilic substitution using the [^{18}F]fluoride anion.

The mesylate precursors (**13** and **14**) were prepared from L-(*S*)-prolinamide, as shown in Chart 1. The mesylate **15** for the synthesis of [^{18}F]**4** was prepared starting from L-(*S*)-hydroxyproline *via* its derivative **7**, as outlined in Chart 1, and has already been described in detail.[8] L-(*S*)-Prolinamide was alkylated with 1-bromo-2-tetrahydropyranyloxyethane to give **5**, which was reduced with $LiAlH_4$ to produce the corresponding diamine. It was then condensed with 5-chloro-3-ethyl-6-methoxysalicylic acid (**8**) using dicyclohexylcarbodiimide (DCC) to afford **9**. The

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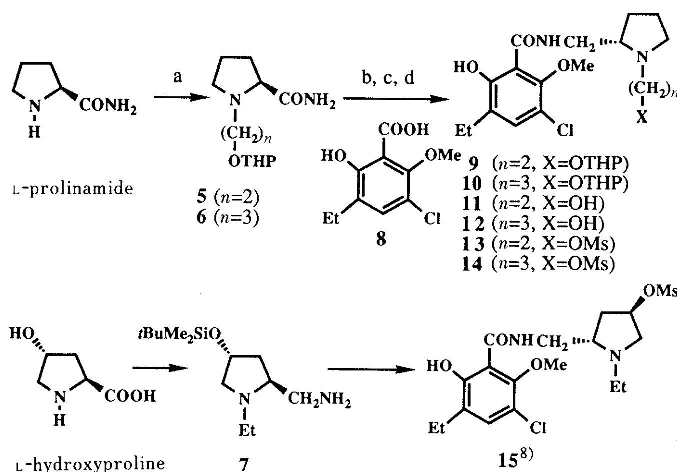

a) $Br(CH_2)_nOTHP$ (n=2 or 3), TEA in CH_3CN b) i) $LiAlH_4$ in THF, ii) *N*, *N'*-dicyclohexylcarbodiimide in CH_2Cl_2 c) 1 N HCl d) **13** : methanesulfonyl chloride, TEA, in ether **14** : methanesulfonyl chloride, TEA, in CH_2Cl_2 TEA : triethylamine THP : tetrahydropyranyl THF : tetrahydrofuran *t*BuMe₂Si : *tert*-butyldimethylsilyl

Chart 1. Syntheses of the Mesylate Precursors **13**, **14**, and **15**

mesylate **13** was obtained in 59% yield from **9** by hydrolysis of **9** following mesylation of the resulting alcohol **11**. The mesylate **13** being relatively labile at room temperature, it was immediately used for the radiosynthesis of [^{18}F]**2**. The mesylate **14** was synthesized by a similar procedure *via* (*S*)-*N*-(3'-tetrahydropyranyloxypropyl)-2-prolinamide **6**, which was prepared from 1-bromo-3-tetrahydropyranyloxypropane as an alkylating reagent and (*S*)-prolinamide.

Radiochemical syntheses of [^{18}F]**2**, [^{18}F]**3**, and [^{18}F]**4** are summarized in Chart 2. The desired radioligand was identified by HPLC co-injection studies with the corresponding unlabelled ligand. Chemical and radiochemical purities of the isolated radioligands were determined by analytical radio-HPLC with a reverse phase column. Aqueous [^{18}F]fluoride was produced by the ^{18}O(p, n)^{18}F nuclear reaction using an [^{18}O]H_2O enriched target which was used to prepare K^{18}F/Kryptofix222[9)] as an [^{18}F]-fluorinating agent for nucleophilic substitution reactions with the mesylate precursors.

The mesylate **13** was heated with K^{18}F/Kryptofix222 in acetonitrile at 80 °C for 10 min to give [^{18}F]**2** in a good radiochemical yield. Since the radioactivity from [^{18}F]**2** was still contaminated with ultraviolet (UV)-absorbing impurities after HPLC using a normal phase column (Whatman Partsil 5 PAC RAC), further purification by HPLC using a reverse phase column (YMC YMC-Pack ODS-AQ) was needed to obtain satisfactory radiochemical and chemical purity. Isolated radiochemical yields of [^{18}F]**2** ranged from 3—10% with specific activities of 1.1—1.9 TBq (30—50 Ci)/mmol at the end of the 90 min synthetic period. The [^{18}F]derivative **3** was prepared from **14** by a similar procedure involving successive purification by normal phase HPLC (Whatman Partsil 5 PAC RAC) and reverse phase HPLC (YMC YMC-Pack ODS-AQ). This resulted in 13—18% radiochemical yields with specific activities of 1.1—1.9 TBq (30—50 Ci)/mmol at the end of the 120 min synthetic period.

More drastic conditions were needed to prepare 4-[^{18}F]fluoropyrrolidinyl eticlopride ([^{18}F]**4**). The mesylate

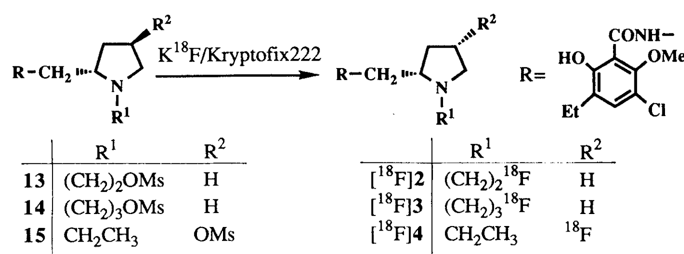

Chart 2. Radiochemical Syntheses of [^{18}F]**2**, [^{18}F]**3**, and [^{18}F]**4**

TABLE II. *In Vivo* Tissue Distribution of [^{18}F]**2**[a)]

Tissue	30 min	60 min	120 min
Brain[b)]	0.17±0.06	0.21±0.03	0.20±0.04
Striatum	0.23±0.05	0.25±0.02	0.19±0.02
Cerebellum	0.18±0.05	0.20±0.03	0.17±0.02
Cerebral cortex	0.18±0.01	0.21±0.06	0.19±0.03
Blood	0.22±0.06	0.25±0.01	0.28±0.04
Bone	0.11±0.04	0.15±0.03	0.26±0.04
Lung	0.34±0.08	0.46±0.04	0.28±0.03
Liver	2.66±2.57	1.55±0.55	1.36±0.12
Kidney	0.41±0.16	0.50±0.06	0.44±0.02
Heart	0.21±0.01	0.24±0.04	0.22±0.04
Small intestine	0.79±0.1	1.34±1.16	1.72±0.80
Striatum/cerebellum[c)]	1.28	1.25	1.12
Striatum/cerebral cortex[c)]	1.28	1.19	1.00
Brain/blood[c)]	0.77	0.84	0.71

a) A saline solution containing [^{18}F]**2** was injected into the tail vein of rats. Tissue radioactivity was expressed as %dose/g with means±S.D. from three rats. *b*) Whole brain. *c*) %dose/g ratio.

15[8)] and K^{18}F/Kryptofix222 were heated without solvent.[10)] Reflux condition with several solvents did not result in appreciable or reproducible radiofluorination. When smaller amounts of the mesylate precursor **13** and K_2CO_3-Kryptofix222 were used in radiofluorination and normal phase HPLC column (Whatman Partsil 5 PAC RAC) was equipped with a guard column (Waters HP CN), the radioactive peak of [^{18}F]**4** was resolved from UV-absorbing impurities. Sufficiently pure [^{18}F]**4** could be obtained by a single HPLC purification. Isolated radiochemical yields of [^{18}F] **4** ranged from 4.3—6.2% with specific activities of 0.6—5.6 TBq (15—150 Ci)/mmol at the end of the 40 min synthetic period.

***In Vivo* Tissue Biodistribution in Rats** A saline solution of ^{18}F-labelled ligand was injected into rat tail veins. The animals were anesthetized with ether, and were killed at different times after the injection. Their tissues were dissected, weighed, and analyzed for radioactivity. Brain regional distributions of radioactivity were investigated using a brain regional-dissection technique. Biodistributions of *N*-2-[^{18}F]fluoroethyl ([^{18}F]**2**) and *N*-3-[^{18}F]fluoropropyl eticlopride ([^{18}F]**3**) are summarized in Tables II and III, respectively.

Radioactivity of [^{18}F]**2** was retained in virtually all tissue studied except in the liver throughout the time investigated (Table II). The bone uptake increased gradually. The highest uptake was found in the liver (2.66% dose/g at 30 min), which decreased to 1.36% dose/g at 120 min. The low brain uptake indicates that [^{18}F]**2** has limited ability to penetrate through the blood–brain barrier. In contrast, [^{18}F]**3** exhibited a different biodistribution pattern (Table III).

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TABLE III. *In Vivo* Tissue Distribution of [^{18}F]**3**[a]

Tissue	5 min	30 min	60 min	120 min
Brain[b]	0.76±0.16	0.40±0.02	0.20±0.15	0.11±0.03
Striatum	0.87±0.25	0.56±0.08	0.23±0.15	0.13±0.05
Cerebellum	0.61±0.09	0.33±0.02	0.14±0.10	0.09±0.02
Cerebral cortex	0.83±0.02	0.47±0.06	0.19±0.13	0.11±0.03
Blood	0.13±0.03	0.15±0.04	0.09±0.06	0.07±0.02
Bone	0.26±0.04	0.38±0.15	0.35±0.20	0.70±0.54
Lung	3.93±1.46	1.53±0.42	0.87±0.83	0.46±0.07
Liver	1.36±0.31	1.22±0.19	0.96±0.44	0.77±0.12
Kidney	1.10±0.34	0.74±0.17	0.46±0.24	0.33±0.05
Heart	0.47±0.11	0.30±0.05	0.15±0.11	0.09±0.03
Small intestine	1.13±0.57	1.35±0.83	0.81±0.99	0.57±0.03
Striatum/cerebellum[c]	1.43	1.70	1.64	1.44
Striatum/cerebral cortex[c]	1.05	1.19	1.21	1.18
Brain/blood[c]	5.84	2.66	2.22	1.57

a) A saline solution containing [^{18}F]**3** was injected into the tail vein of rats. Tissue radioactivity was expressed as %dose/g with means ± S.D. from three rats. *b)* Whole brain. *c)* %dose/g ratio.

TABLE IV. *In Vivo* Tissue Distribution of [^{18}F]**4**[a]

Tissue	30 min	60 min	90 min
Brain[b]	0.25±0.14	0.16±0.05	0.13±0.02
Striatum	0.56±0.18	0.49±0.12	0.47±0.10
Cerebellum	0.20±0.09	0.11±0.03	0.09±0.01
Cerebral cortex	0.27±0.13	0.15±0.03	0.14±0.03
Blood	0.17±0.03	0.14±0.05	0.13±0.01
Bone	0.42±0.15	0.66±0.28	0.77±0.09
Lung	0.50±0.27	0.31±0.13	0.23±0.02
Liver	2.04±0.38	2.14±0.66	2.38±0.42
Kidney	0.47±0.14	0.38±0.11	0.42±0.06
Heart	0.21±0.07	0.14±0.04	0.13±0.01
Small intestine	0.47±0.07	1.00±0.80	2.47±1.35
Striatum/cerebellum[c]	2.80	4.45	5.22
Striatum/cerebral cortex[c]	2.07	3.27	3.36
Brain/blood[c]	1.47	1.14	1.00

a) A saline solution of [^{18}F]**4** containing 20—40% ethanol was injected into rat tail veins. Tissue radioactivity was expressed as %dose/g with means ± S.D. from four rats. *b)* Whole brain. *c)* %dose/g ratio.

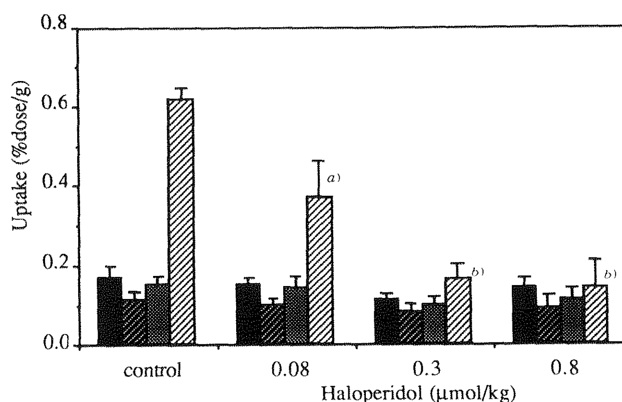

Fig. 1. Inhibition of [^{18}F]**4** Accumulation in Blood (■), Cerebellum (■), Cortex (■), and Striatum (▨) 90 min after *i.v.* Injection

Rats were pretreated with indicated doses of haloperidol 30 min before the injection of [^{18}F]**4**. Each bar represents the mean ± S.D. from three rats. Student's *t*-test with respect to the control, *a)* $p<0.01$, *b)* $p<0.001$.

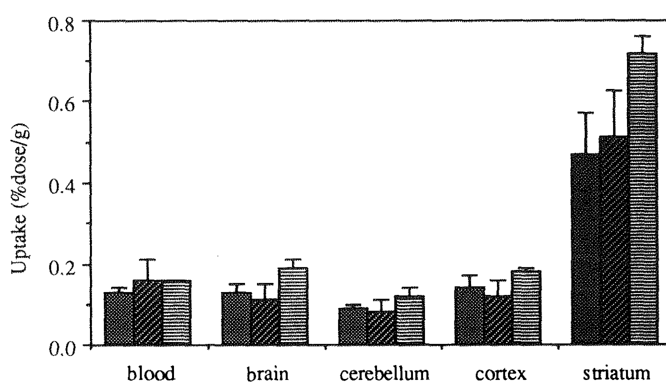

Fig. 2. Comparison of Tissue Distribution of [^{18}F]**4** 90 min after *i.v.* Injection of Three Different Saline Solutions Containing Ethanol (■ EtOH), Hydrochloric Acid (■ HCl), or Dimethylsulfoxide (▤ DMSO)

Each bar represents the mean ± S.D. from three rats, three rats, and two rats, respectively.

Initial uptake was cleared in almost all tissues except in the bone. The highest uptake was observed in the lungs (3.93% dose/g at 5 min), but thereafter the radioactivity dropped to 0.46% dose/g at 120 min. The bone activity increased gradually in both cases, indicating slow defluorination of these ligands *in vivo*. The brain/blood ratio was relatively high for [^{18}F]**3** at the early time point, but the striatum/cerebellum ratio, which illustrates selective binding to the D2 receptor-rich over -poor region, was not impressive in either case. Similar nonspecific *in vivo* binding in the regional brain was reported in *in vivo* biodistribution studies of fluorine-18 labelled *N*-fluoroalkyl raclopride and eticlopride derivatives. Although *N*-fluoroalkylation of the pyrrolidine nitrogen of benzamide neuroleptics decreases their *in vitro* affinity toward CNS D2 receptors,[7,8,11] detailed biological investigations have revealed that it may actually increase their affinity or that of ^{18}F-labelled metabolites for plasma proteins and result in nonspecific *in vivo* binding.[5] Our research, which was initiated independently from the above reports, confirms these findings. Thus, [^{18}F]**2** and [^{18}F]**3** are not useful *in vivo* D2 receptor radioligands.

Since [^{18}F]**4** is scarcely soluble in saline, it was dissolved in saline containing 20—40% ethanol. The *in vivo* tissue distribution of [^{18}F]**4** in rats is listed in Table IV. The liver uptake remained high until 90 min after the injection. The bone uptake (an index of metabolic defluorination) increased gradually to 0.77% dose/g at 90 min. Among the three ^{18}F-labelled ligands, the carbon–fluoride bond of [^{18}F]**4** is the most biologically labile *in vivo*. It should be noted that the striatal uptake remained relatively high, while the cerebellar uptake decreased gradually. As a result, the striatum/cerebellum ratio gradually increased with time: 2.8, 4.45, and 5.22 at 30, 60, and 90 min after the injection, respectively. These findings are similar to previous reports on [^{11}C]eticlopride biodistribution in the monkey measured by PET.[2a] These have indicated that dissociation rates of ligand–receptor complexes in the striatum are slower than those of nonspecific bindings. The striatum/cerebellum ratio of 5.22 obtained here was a bit smaller than that of about 10 reached using ^{3}H-labelled eticlopride in the rat brain 120 min after injection.[12]

Inhibition Experiments To confirm that the striatal uptake of [^{18}F]**4** is mediated by specific binding to dopamine D2 receptors, uptake inhibition experiments were carried out with various amounts of haloperidol, a neuroleptic drug which blocks CNS dopamine D2 receptors. A saline solution containing 0.08, 0.3, or 0.8 μmol/kg of haloperidol was injected in rat tail veins 30 min before the

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injection of a saline solution of [^{18}F]**4** containing 40% ethanol. The animals were anesthetized with ether and were killed 90 min after an [^{18}F]**4** injection, and its tissue distribution was investigated. Figure 1 illustrates the influence of haloperidol on [^{18}F]**4** uptake in the regional brain and blood, and indicates that only the striatal accumulation was inhibited. The striatal uptake was decreased to 60%, 26%, and 23% of control by haloperidol doses of 0.08, 0.3, and 0.8 μmol/kg, respectively. Thus, the dose-dependent uptake inhibition clearly suggest that the striatal binding of [^{18}F]**4** is a specific process related to dopamine D2 receptors.

***In Vivo* Distribution *via* Different Injections** It has been shown that ethanol alters neuronal activity, and that [^{3}H]spiroperidol binding to dopamine receptor is inhibited by *in vitro* addition of ethanol.[13] During the present *in vivo* binding experiments with [^{18}F]**4**, the injection of a saline solution containing 20—40% ethanol produced a slight anesthesia in rats. It seemed important therefore to take into account ethanol's effect on the *in vivo* biodistribution. Thus [^{18}F]**4** was administered to rats *via* injections (omitting ethanol) prepared using hydrochloric acid (adjusted to pH 3.3) or 1% dimethyl sulfoxide (DMSO) as a cosolvent. DMSO is relatively nontoxic to animals and has been shown not to increase brain uptake of water-soluble tracers.[14] Figure 2 illustrates the difference in uptake in blood and brain 90 min after the injection of three different preparations. Although the injection containing DMSO produced slightly higher uptake in the striatum, uptake in the cerebellum and the cortex was also increased. No significant difference was observed in the striatum/cerebellum ratio, since ratios of 5.2, 6.4, and 5.9 were obtained with the injections containing ethanol, hydrochloric acid, or DMSO, respectively. These results suggest that ethanol contained in the injection may not interfere with the binding of [^{18}F]**4** to the dopamine D2 receptors.

In *in vivo* receptor binding experiments, radioligand doses must be less than that for receptor-saturation in regional brain. The density of dopamine D2 receptors in rat striatum was estimated to be 50 pmol/g from the *in vitro* measurement with ^{3}H-labelled eticlopride.[15] In our study, a typical dose was calculated to be 1.3 nmol based on the specific activity of 1.1 TBq (30 Ci)/mmol, and the maximum striatal uptake was 0.87% dose/g for [^{18}F]**3** (Table III, 5 min). These facts, together with the estimated density of dopamine D2 receptors in rats, indicate that in this study fewer than 23% of the receptors were bound to ^{18}F-labelled ligand, which suggests that the present *in vivo* binding experiments were performed at much lower doses than those producing receptor-saturation in the rat striatum.

In conclusion, the present experiments have shown that 4-[^{18}F]pyrrolidinyl eticlopride ([^{18}F]**4**) is a suitable ligand for *in vivo* PET radiotracer study of dopamine D2 receptors. Further investigation is underway to develop a new radioligand with higher brain uptake as well as biological stability.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are not corrected. Optical rotations were determined by a JASCO DIP-360 digital polarimeter. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were taken either at 100 MHz (JEOL FX-100) or at 270 MHz (JEOL GSX270), and chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane (δ 0.0). Infrared (IR) spectra were taken on a JASCO IRA-1 spectrometer. Low resolution electron impact (EI), field desorption (FD), and fast atom bombardment (FAB) mass spectra (respective abbreviations are as follows: EIMS, FDMS, FABMS) as well as high resolution EI mass spectra (HRMS) spectra were obtained on a JEOL JMS-D300 spectrometer. High resolution FAB mass spectra (HRFABMS) were obtained on a JEOL NMS-SX 102-SX 102 spectrometer. Kieselgel 60 (70—230 mesh, Merck) was used for column chromatography. Elemental analyses were performed by the staff of the microanalytical section of Kyushu University. No-carrier-added aqueous [^{18}F]fluoride was prepared in an [^{18}O]H_2O enriched target (8—16%) using ^{18}O(p, n)^{18}F nuclear reaction employing a cyclotron at the University Hospital, Kyushu University. Radio-HPLC was carried out with Waters M-5 system, and analyzed both by an absorbance detection at 254 nm (Waters model 441) and by a radioanalyzer (Aloka, model RCL550). Radio-thin-layer chromatography (TLC) was performed on a silica gel plate (Merck Kieselgel 60 F_{254}). Radioactivity was measured either on a radioisotope calibrator (Capintec CRC-30) or on a gamma counter (Packard auto gamma-500). Chemical and radiochemical purity of ^{18}F-labelled ligands were determined by HPLC, and the peak of the desired ligand was identified by HPLC co-injection studies. Specific activities were determined by UV spectrometer and a radioisotope calibrator.

Chemistry 1-(2′-Tetrahydropyranyloxyethyl)-2-pyrrolidinecarboxamide (5) 1-Bromo-2-tetrahydropyranyloxyethane (7.55 g, 36.1 mmol) was added dropwise into a solution of L-prolinamide hydrochloride (3.62 g, 24.1 mmol) and triethylamine (3.21 g, 48.2 mmol) in anhydrous acetonitrile (50 ml) at room temperature, and the reaction mixture was stirred for 47 h. The solvent was removed, and the residue was chromatographed on a silica gel column to yield crystals, which were recrystallized from hexane to afford colorless crystals (2.72 g, 47%). mp 71—72 °C. IR (Nujol) 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) (100 MHz) δ: 7.63 (1H, br), 5.49 (1H, br), 4.60 (1H, m), 3.78—3.91 (2H, m), 3.40—3.56 (2H, m), 3.09—3.31 (2H, m), 1.68—3.00 (7H, m), 1.51—1.87 (6H, m). EIMS *m/z*: 242 (M^{+}). *Anal.* Calcd for $C_{12}H_{22}N_2O_3$: C, 59.48; H, 9.15; N, 11.56. Found: C, 59.25; H, 9.05; N, 11.34.

1-(3′-Tetrahydropyranyloxypropyl)-2-pyrrolidinecarboxamide (6) Compound **6** was obtained as colorless crystals (1.2 g, 47%) by the same procedure as described above using L-prolinamide (1.50 g, 10 mmol), 1-bromo-3-tetrahydropyranyloxypropane (2.31 g, 1.3 mmol), and triethylamine (2.02 g, 20 mmol). mp 57—58 °C. IR (Nujol) 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) (100 MHz), δ: 7.61 (1H, br), 5.50 (1H, br), 4.63 (1H, m), 3.69—3.95 (2H, m), 3.01—3.60 (1H, m), 1.52—2.96 (14H, m). EIMS *m/z*: 256 (M^{+}). *Anal.* Calcd for $C_{13}H_{24}N_2O_3$: C, 60.91, H, 9.44; N, 10.93. Found: C, 60.78; H, 9.31; N, 10.86.

(*S*)-(−)-5-Chloro-3-ethyl-*N*-{[1-(2′-tetrahydropyranyloxyethyl)-2-pyrrolidinyl]methyl}-6-methoxysalicylamide (9) A solution of **5** (2.72 g, 11 mmol) in anhydrous tetrahydrofuran (THF, 25 ml) was added dropwise to a suspension of $LiAlH_4$ (1.28 g, 33 mmol) in anhydrous ether, and the whole mixture was heated at 40 °C for 8 h. The reaction mixture was diluted with ether (25 ml) and excess $LiAlH_4$ was quenched by the addition of aqueous saturated Na_2SO_4. The precipitates were filtered off through a celite pad. The filtrate was dried over anhydrous Na_2SO_4, and evaporated to give 2-aminomethyl-1-(2′-tetrahydropyranyloxyethyl)pyrrolidine as an oily product (2.2 g, 82%). ^{2}H-NMR ($CDCl_3$) (100 MHz) δ: 4.61 (1H, m), 3.79—3.93 (2H, m), 3.47—3.65 (2H, m), 2.93—3.22 (2H, m), 2.70—2.71 (2H, m), 2.23—2.54 (3H, m), 1.83—1.92 (2H, m), 1.69—1.82 (4H, m), 1.48—1.68 (6H, m). FABMS *m/z*: 229 (MH^{+}). HRMS *m/z*: 229.1914 calcd for $C_{12}H_{25}N_2O_2$. Found: 229.1915. This oil was used for the synthesis of **9** without further purification.

N,N′-Dicyclohexylcarbodiimide (1.40 g, 6.58 mmol) was added to a solution of 5-chloro-3-ethyl-6-methoxysalicylic acid (1.53 g, 6.58 mmol)[16] in anhydrous CH_2Cl_2 (20 ml) at 0 °C, and the whole mixture was stirred for 15 min. A solution of the above oil (1.50 g, 6.58 mmol) in CH_2Cl_2 (20 ml) was added to the above mixture, and the reaction mixture was stirred at room temperature for 20 min. The solvent was removed, and the residue was chromatographed on a silica gel column ($CHCl_3$) to give a colorless oil (2.32 g, 80%). IR (neat) 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) (100 MHz) δ: 13.86 (1H, br), 8.86 (1H, br), 7.21 (1H, s), 3.87 (3H, s), 2.70 (2H, q, J=7.5 Hz), 1.38—4.56 (22H, m), 1.19 (3H, t, J=7.5 Hz). EIMS *m/z*: 440 (M^{+}). *Anal.* Calcd for $C_{22}H_{33}ClN_2O_5$: C, 59.95; H, 7.54; N, 6.26. Found: C, 59.72; H, 7.49; N, 6.26.

(*S*)-(−)-5-Chloro-3-ethyl-*N*-{[1-(3′-tetrahydropyranyloxypropyl)-2-pyrrolidinyl]methyl}-6-methoxysalicylamide (10) Compound **6** (1 g, 3.9 mmol) was reduced with $LiAlH_4$ (0.30 g, 11.3 mmol) by the same procedure

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as described for the synthesis of **5** to give the corresponding diamine (0.85 g, 90%), which was used for the next reaction without purification. 5-Chloro-3-ethyl-6-methoxy-salicylic acid (460 mg, 1.98 mmol)[16] and the amine obtained above (500 mg, 2.06 mmol) were condensed with N,N'-dicyclohexylcarbodiimide by the same procedure as described for the synthesis of **9**. Purification by column chromatography (silica gel, $CHCl_3$) afforded **10** as a colorless oil (563 mg, 62%). IR (neat) $1630\,cm^{-1}$. ^{1}H-NMR ($CDCl_3$) (100 MHz) δ: 13.87 (1H, br), 8.87 (1H, br), 7.21 (1H, s), 4.46 (1H, m), 3.88 (3H, s), 3.66—3.83 (3H, m), 3.19—3.58 (5H, m), 1.54—3.09 (17H, m), 1.19 (3H, t, $J=7.6$ Hz). EIMS m/z: 454 (M^+). *Anal.* Calcd for $C_{23}H_{35}ClN_2O_5$: C, 60.71; H, 7.75; N, 6.16. Found: C, 60.73; H, 7.74; N, 6.07.

(*S*)-(−)-5-Chloro-3-ethyl-*N*-{[1-(2′-hydroxyethyl)-2-pyrrolidinyl]methyl}-6-methoxysalicylamide (11) A solution of **9** (50 mg, 0.11 mmol) in 1 N HCl was stirred at room temperature for 1 h, and extracted with ether. The water layer was separated, and alkalinized to pH 10 with aqueous NH_3, then extracted with CH_2Cl_2. The extract was dried over anhydrous Na_2SO_4, and evaporated to give a crude oil, which was chromatographed on a silica gel column ($CHCl_3$) to afford a colorless oil (37 mg, 94%). $[\alpha]_D^{23}$ −95° ($c=1.5$, $CHCl_3$). IR (neat) 3260, $1630\,cm^{-1}$. ^{1}H-NMR ($CDCl_3$) (270 MHz) δ: 13.70 (1H, br), 8.78 (1H, br), 7.22 (1H, s), 3.89 (3H, s), 3.81—3.60 (3H, m), 3.22—3.38 (2H, m), 3.03 (1H, ddd, $J=5.0$, 9.6, 12.4 Hz), 2.74—2.82 (1H, m), 2.61 (2H, q, $J=7.5$ Hz), 2.44 (1H, dt, $J=3.3$, 12.5 Hz), 2.28 (1H, q, $J=7.6$ Hz), 2.05—1.60 (4H, m), 1.19 (3H, t, $J=7.5$ Hz). EIMS m/z: 357 (M^+). *Anal.* Calcd for $C_{17}H_{25}ClN_2O_4$: C, 57.22; H, 7.06; N, 7.85. Found: C, 56.94; H, 7.10; N, 7.57.

(*S*)-(−)-5-Chloro-3-ethyl-*N*-{[1-(3′-hydroxypropyl)-2-pyrrolidinyl]methyl}-6-methoxysalicylamide (12) **10** (200 mg, 0.44 mmol) was hydrolyzed by the same procedure as described above, and the crude product was purified by silica gel column ($CHCl_3$) to give **12** as a colorless oil (143 mg, 88%). $[\alpha]_D^{25}$ −63.3° ($c=2.0$, $CHCl_3$). IR (neat) 3260, $1630\,cm^{-1}$. ^{1}H-NMR ($CDCl_3$) (100 MHz) δ: 13.87 (1H, br), 8.69 (1H, br), 7.22 (1H, s), 3.88 (3H, s), 3.67—3.84 (3H, m), 2.92—3.44 (3H, m), 1.61—2.73 (11H, m), 1.19 (3H, t, $J=7.6$ Hz). EIMS m/z: 370 (M^+). *Anal.* Calcd for $C_{18}H_{27}ClN_2O_4$: C, 58.29; H, 7.34; N, 7.55. Found: C, 58.12; H, 7.16; N, 7.34.

(*S*)-(−)-5-Chloro-3-ethyl-*N*-{[1-(2′-methanesulfonyloxyethyl)-2-pyrrolidinyl]methyl}-6-methoxysalicylamide (13) Methanesulfonyl chloride (40 μl, 0.52 mmol) was added dropwise to a solution of **11** (30 mg, 0.088 mmol) and triethylamine (70 μl) in anhydrous ether (1 ml) at 0 °C. After being stirred for 15 min at 0 °C, the reaction mixture was diluted with ether (50 ml), and washed successively with aqueous saturated $NaHCO_3$ (10 ml × 2), brine (10 ml × 2), and water (10 ml), then dried over anhydrous Na_2SO_4. The solvent was removed to give a crude oil, which was purified by silica gel column chromatography (AcOEt : hexane = 2 : 1—1 : 1) to give **13** as a pure oil (24 mg, 63%). $[\alpha]_D^{30}$ −82.4° ($c=1.1$, AcOEt). IR (neat) 3360, $1630\,cm^{-1}$. ^{1}H-NMR ($CDCl_3$) (270 MHz) δ: 13.776 (1H, s), 8.823 (1H, br), 7.221 (1H, s), 4.332 (1H, dd, $J=4.3$, 6.6 Hz), 3.891 (3H, s), 3.813 (1H, ddd, $J=2.3$, 7.6, 14.2 Hz), 3.298 (1H, ddd, $J=3.0$, 4.6, 14.2 Hz), 3.186 (1H, dt, $J=6.6$, 13.9 Hz), 3.055 (3H, s), 2.74—2.83 (1H, m), 2.613 (2H, dq, $J=7.3$, 0.7 Hz), 2.629 (1H, ddd, $J=4.3$, 6.6, 13.9 Hz), 2.335 (1H, q, $J=8.9$ Hz), 1.6—2.05 (4H, m), 1.196 (3H, t, $J=7.3$ Hz). FDMS m/z: 434 (M^+). HRFABMS m/z: 435.1356 calcd for $C_{18}H_{28}ClN_2O_6S$. Found: 435.1329.

(*S*)-(−)-5-Chloro-3-ethyl-*N*-{[1-(3′-methanesulfonyloxypropyl)-2-pyrrolidinyl]methyl}-6-methoxysalicylamide (14) Methanesulfonyl chloride (9.2 mg, 0.081 mmol) was added dropwise into a solution of **12** (20 mg, 0.054 mmol) and triethylamine (10 mg, 0.3 mmol) in CH_2Cl_2 (2 ml), and the reaction mixture was stirred for 15 min at 0 °C. The solvent was removed, and the residue was purified by a silica gel column chromatography ($CHCl_3$) to give a colorless oil (7 mg, 28%). $[\alpha]_D^{25}$ −63.8° ($c=1.6$, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) (100 MHz) δ: 13.80 (1H, s), 8.74 (1H, br), 7.21 (1H, s), 4.32 (2H, ddd, $J=6.6$, 6.6, 3.9 Hz), 3.88 (3H, s), 3.70 (1H, dd, $J=7.1$, 2.4 Hz), 3.22—3.41 (3H, m), 2.97 (3H, s), 1.59—2.90 (12H, m), 1.19 (3H, t, $J=7.4$ Hz). EIMS m/z: 447 $(M-1)^+$. *Anal.* Calcd for $C_{19}H_{29}ClN_2O_6S$: C, 50.83; H, 6.51; N, 6.24. Found: C, 50.63; H, 6.68; N, 6.33.

Radiochemistry. Preparation of [^{18}F]Fluorinating Reagent, K^{18}F/Kryptofix222 Complex Radiosynthesis was performed in a TPX vessel unless otherwise noted. Kryptofix2.2.2 (11 mg) and $K_2CO_3 \cdot 1.5H_2O$ (2.2 mg) were added to a solution of [^{18}F]fluoride from the irradiated water target, and the solution was evaporated to dryness under an argon stream at 100 °C. Three portions of acetonitrile (150—500 μl) were added and evaporated under argon stream at 100 °C.[9]

***N*-2-[^{18}F]Fluoroethyl Eticlopride ([^{18}F]2): (*S*)-(−)-5-Chloro-3-ethyl-*N*-{[1-(2-[^{18}F]fluoroethyl)-2-pyrrolidinyl]methyl}-6-methoxysalicylamide** A solution of **13** (3—10 mg) in acetonitrile (500 μl) was added to the above residue, and the mixture was heated at 80 °C for 10 min. The solvent was evaporated, and the residue was dissolved in n-hexane : CH_2Cl_2 = 1 : 1, then purified successively with normal phase HPLC ($t_R=18$ min, column: Whatman Partsil 5 PAC RAC 9.4 × 100 mm; eluent: n-hexane : CH_2Cl_2 = 85 : 15; flow rate: 4.0 ml/min) and reverse HPLC ($t_R=18$ min, column: YMC YMC-Pack ODS-AQ 10 × 300 mm; eluent: $CH_3CN:H_2O:HCl$ = 40 : 60 : 0.1; flow rate: 4.0 ml/min) to give [^{18}F]**2** in 3—10% radiochemical yields (total synthetic time: 90 min, uncorrected for decay) with specific radioactivities of 1.1—1.9 TBq (30—50 Ci)/mmol at the end of synthesis. The isolated [^{18}F]**2** showed an identical t_R to that of unlabelled **2**, and was radiochemically as well as chemically pure by analytical radio-HPLC with a reverse phase HPLC column (YMC YMC-Pack ODS-AQ, $CH_3CN:H_2O:HCl$ = 40 : 60 : 0.1; flow rate: 4.0 ml/min). The solvent was removed, and the residue was dissolved in saline and used for the *in vivo* studies.

***N*-3-[^{18}F]Fluoropropyl Eticlopride ([^{18}F]3): (*S*)-(−)-5-Chloro-3-ethyl-*N*-{[1-(3-[^{18}F]fluoropropyl)-2-pyrrolidinyl]methyl}-6-methoxysalicylamide** Compound [^{18}F]**3** was synthesized by the same procedure as described above. Successive purification by normal phase HPLC ($t_R=18$ min, column: Whatman Partsil 5 PAC RAC 9.4 × 100 mm; eluent: n-hexane : CH_2Cl_2 = 115 : 85; flow rate: 4.0 ml/min) and by reverse HPLC ($t_R=21$ min, column: YMC YMC-Pack ODS-AQ 10 × 300 mm; eluent: $CH_3CN:H_2O:HCl$ = 40 : 60 : 0.1; flow rate: 4.0 ml/min) gave [^{18}F]**3** in 13—18% radiochemical yields (total synthetic time: 120 min, uncorrected for decay) with specific radioactivities of 1.1—1.9 TBq (30—50 Ci)/mmol at the end of synthesis. The isolated [^{18}F]**3** showed an identical t_R to that of unlabelled **3**, and was radiochemically as well as chemically pure by analytical radio-HPLC with a reverse phase HPLC column (YMC YMC-Pack ODS-AQ, $CH_3CN:H_2O:HCl$ = 40 : 60 : 0.1; flow rate: 4.0 ml/min). The solvent was removed, and the residue was dissolved in saline and used for *in vivo* studies.

4-[^{18}F]Fluoropyrrolidinyl Eticlopride ([^{18}F]4): (2*S*,4*R*)-(−)-5-Chloro-3-ethyl-*N*-{[1-ethyl-2-(4-[^{18}F]fluoropyrrolidinyl)]methyl}-6-methoxysalicylamide K^{18}F/Kryptofix222 was prepared using Kryptofix2.2.2 (3.6 mg) and K_2CO_3 (0.8 mg), and the reagent was dried under vacuum for 15 min. A solution of **14** (1 mg) in acetonitrile was added to the above reagent, and the solvent was evaporated under an argon stream. The residue was then heated at 110—120 °C for 5 min. The crude residue was dissolved in AcOEt (0.5—0.8 ml) and filtered (Millipore FH 0.5 μ), then purified by normal phase HPLC ($t_R=20$ min, column: Whatman Partsil 5 PAC RAC 9.4 × 100 mm; eluent: n-hexane : AcOEt : EtOH = 190 : 10 : 0.8; flow rate: 4.0 ml/min) equipped with a guard column (Waters HP CN) to give [^{18}F]**4** in 4.3—6.2% radiochemical yields (total synthetic time: 90 min, uncorrected for decay) with specific radioactivities of 0.6—5.6 TBq (15—150 Ci)/mmol at the end of synthesis. The isolated [^{18}F]**4** showed an identical t_R to that of unlabelled **4**, and was radiochemically as well as chemically pure by analytical radio-HPLC with a reverse phase HPLC column (YMC YMC-Pack ODS-AQ, $CH_3CN:H_2O:HCl$ = 40 : 60 : 0.1; flow rate: 4.0 ml/min). The solvent was removed, and the residue was dissolved in saline containing either 20—40% ethanol, HCl, or 1% DMSO. A solution containing HCl was adjusted to pH 3.3 with the addition of a saline solution of 0.035% $NaHCO_3$. Each injection preparation was used for the *in vivo* experiments.

***In Vivo* Biodistribution** Male Wistar rats, weighing 160—230 g used for *in vivo* studies were allowed access to food and water *ad libitum*. A saline solution of radioligand was injected in rat tail veins. The animals were anesthetized with ether, and were killed at indicated times after injection. Organs were first dissected, and brains were regionally dissected to cerebellum, striatum, and cerebral cortex. Tissue radioactivity was measured, corrected for both decay and the tissue weight, and expressed as %dose/g in Tables II—IV. The radioactivity used for each animal experiment ranged from 13.6—44.8 μCi for [^{18}F]**2** and [^{18}F]**3** in a 0.2—0.5 ml saline solution, and 5—21.5 μCi for [^{18}F]**4** in a 0.2—1 ml of saline solution.

Inhibition Experiments A saline solution containing haloperidol at doses of 0.08, 0.3, and 0.8 μmol/kg was injected into rat tail veins. Thirty mimutes later, a solution of [^{18}F]**4** (11.4—16.1 μCi) in 0.5 ml saline containing 40% ethanol was injected in rat tail veins. The animals were anesthetized with ether, and killed 90 min after the injection. Tissue radioactivity was determined as described above. Control rats were treated with a saline solution under identical conditions. Uptakes of selected tissues are compared in Fig. 1.

***In Vivo* Distribution *via* Different Injections** Compound [^{18}F]**4** was

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dissolved in ethanol–HCl, and the solvent was evaporated. The residue was dissolved in saline and the solution was adjusted to pH 3.3 by the addition of a saline solution containing 0.035% $NaHCO_3$, affording 0.5—1.0 ml of the injection preparation including hydrochloric acid. The injection containing DMSO was prepared by dissolving [^{18}F]**4** in a saline containing 1% DMSO (0.5—1.0 ml), 10.0—18.0 μCi of [^{18}F]**4** in 0.5—1.0 ml of saline solution containing either hydrochloric acid or DMSO was injected into the tail vein of rats. The animals were anesthetized with ether, and killed 90 min after the injection. Tissue radioactivity was then determined. Uptakes of selected tissues, expressed as %dose/g, are compared in Fig. 2.

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