Positron-Emitting \(^{18}\text{F}\)Fluoroalkyl and \(^{18}\text{F}\)Fluoropyrpyridinyl Analouges of Etocloride as Potential in Vivo Radioligands for Dopamine D2 Receptors

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\(^{18}\text{F}\)Fluoroalkyl and 4-fluoropyrpyridinyl eticlopride analogues with high affinity toward central nervous system dopamine D2 receptors in vitro were labelled with positron emitting fluorine-18 \((t_{1/2} = 110\text{ min})\), and their in vivo biodistribution was investigated in rats. \(^{18}\text{F}\)Fluoro-ethyl and -propyl eticlopride derivatives showed poor in vivo selectivity in the rat brain. On the other hand, 4-\(^{18}\text{F}\)fluoropyrpyridinyl 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-\(^{18}\text{F}\)fluoropyrpyridinyl eticlopride in striatal tissue appears to be due to the specific binding to dopamine D2 receptors.

Keywords: \(^{18}\text{F}\)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 \((t_{1/2} = 110\text{ min})\) compared to carbon-11 \((t_{1/2} = 20.4\text{ min})\) and it is accepted as a desirable radioisotope for PET studies of slow ligand–receptor binding.6 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 pyrpyridine 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 \(^{18}\text{F}\)-fluoroalkyl (2, 3)\(^{7}\) and 4-fluoropyrpyridinyl (4)\(^{10}\) eticlopride analogues (1), 5-(S)-5-chloro-3-ethyl-N-[\((1\text{-ethyl-2-pyrpyrpyridinyl})\]-methyl]-6-methoxysalcyclamide, which retain significant affinities toward dopamine D2 receptors in in vitro binding experiments (summarized in Table 1). In this paper, we describe the synthesis of \(^{18}\text{F}\)-labelled eticlopride analogues corresponding to 2, 3, and 4, and their in vivo biodistribution in rats. In these studies 4-\(^{18}\text{F}\)fluoropyrpyridinyl eticlopride \([\text{18}^{18}\text{F}]\text{4}\) has been found to display specific binding to dopamine D2 receptors in vivo.

Table 1. In Vitro Receptor Binding\(^{a,b}\)

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (nM)(^{b})</th>
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<tr>
<td>1</td>
<td>2.9(^{7})</td>
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<tr>
<td>2</td>
<td>23(^{7})</td>
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<tr>
<td>3</td>
<td>17(^{7})</td>
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<tr>
<td>4</td>
<td>1.9(^{8})</td>
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| a | Data taken from the literature, in which bovine\(^{5}\) or rat\(^{6}\) striatal tissue homogenates were used in vitro.  b | IC50 represents the concentration required to inhibit specific binding of \[^3\text{H}\]spiperone by 50%. |

![Image](https://example.com/image.png)

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 \[^{18}\text{F}\]2, \[^{18}\text{F}\]3, \[^{18}\text{F}\]4, the corresponding mesylates were chosen as precursors to introduce an \[^{18}\text{F}\]fluorine atom through nucleophilic substitution using the \[^{18}\text{F}\]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 \[^{18}\text{F}\]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-tetrahydropyranoyloxyethane to give 5, which was reduced with LiAlH\(_4\) to produce the corresponding diamine. It was then condensed with 5-chloro-3-ethyl-6-methoxysaliclyic acid (8) using dicyclohexylcarbodiimide (DCC) to afford 9. The

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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 via (S)-N-(3'-tetrahydroxypropylxyloxypropyl)-2-prolinamide 6, which was prepared from 1-bromo-3-tetrahydroxypropyl-2-oxo-2-propanone as an alkylating reagent and (S)-proline.

Radiochemical syntheses of $^{18}$F[2], $^{18}$F[3], and $^{18}$F[4] are summarized in Chart 2. The desired radioiodide 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$_2$H$_2$O enriched target which was used to prepare K$^{18}$F/Kryptofix2229 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 Partisil 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—19 TBq (30—50 Ci)/mmol at the end of the 90 min synthetic period. The $^{18}$F[2] derivative 3 was prepared from 14 by a similar procedure involving successive purification by normal phase HPLC (Whatman Partisil 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—19 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 $^{18}$F[2] and K$^{18}$F/Kryptofix222 were heated without solvent. Reflux condition with several solvents did not result in appreciable reproducible radiofluorination. When smaller amounts of the mesylate precursor 13 and K$_2$CO$_3$-Kryptofix222 were used in radiofluorination and normal phase HPLC column (Whatman Partisil 5 PAC RAC) was equipped with a guard column (Waters HPLC 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).
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 [18F]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 fluorene-18 labelled N-fluoroalkyl raclopride and eticlopride derivatives. Although N-fluorokylation of the pyrrolidine nitrogen of benzamide neuroleptics decreases their in vitro affinity toward CNS D2 receptors, detailed biological investigations have revealed that it may actually increase their affinity or that of [18F]-labelled metabolites for plasma proteins and result in nonspecific in vivo binding. Our research, which was initiated independently from the above reports, confirms these findings. Thus, [18F]2 and [18F]3 are not useful in vivo D2 receptor radioligands.

Since [18F]4 is scarcely soluble in saline, it was dissolved in saline containing 20–40% ethanol. The in vivo tissue distribution of [18F]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 [18F]-labelled ligands, the carbon–fluoride bond of [18F]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 [11C]eticlopride biodistribution in the monkey measured by PET. 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 [3H]-labelled eticlopride in the rat brain 120 min after injection.

**Inhibition Experiments** To confirm that the striatal uptake of [18F]4 is mediated by specific binding to dopamine D2 receptors, uptake inhibition experiments were carried out with various amounts of haloperidol, a neurolleptic 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
injection of a saline solution of [18F]4 containing 40% ethanol. The animals were anesthetized with ether and were killed 90 min after an [18F]4 injection, and its tissue distribution was investigated. Figure 1 illustrates the influence of haloperidol on [18F]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 [18F]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 [3H]spiropindrol binding to dopamine receptor is inhibited by in vitro addition of ethanol.15) During the present in vivo binding experiments with [18F]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 [18F]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 [18F]4 to the dopamine D2 receptors.

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 [3H]-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 [18F]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 [18F]4 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 [4-18F]pyrrolidinyl eticlopride ([18F]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 magnetic resonance ([4H-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 mass spectra (EI-MS) were recorded on a JEOL JMS-500 apparatus. Microanalytical data and elemental analyses were performed by the microanalytical section of Kyushu University. Microanalytical data and elemental analyses were performed by the microanalytical section of Kyushu University. Microanalytical data and elemental analyses were performed by the microanalytical section of Kyushu University.

**Chemistry**

(1-2-Tetrahydropyranoxyethyl)-2-pyrrolidinencarboxamide (5)

1-Bromo-2-tetrahydropyranoxylthene (7.5 g, 36.1 mmol) was added dropwise to a solution of 1-proline trihydrochloride (3.6 g, 0.1 mmol) and triethylamine (3.2 g, 48.2 mmol) in anhydrous acetonitrile (50 ml) at room temperature. After 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. 1H-NMR (CDCl₃) (100 MHz): δ = 7.63 (1H, br), 5.94 (1H, br), 4.60 (1H, m), 3.78–3.91 (2H, m), 3.30–3.56 (2H, m), 3.09–3.31 (2H, m), 1.68–3.00 (8H, m), 1.51–1.87 (6H, m). EIMS m/z: 242 (M⁺). Anal. Calcld for C₁₉H₂₆N₂O₅: C, 63.7; H, 9.15; N, 11.56. Found: C, 59.25; H, 9.05; N, 11.34.

1-(2-Tetrahydropyranoxylpropyl)-2-pyrrolidinencarboxamide (6)

Compounds 6 was obtained as colorless crystals (1.2 g, 47%) by the same procedure as described above using 1-proline (1.59 g, 10 mmol), 1-bromo-3-tetrahydropyranoxylpropane (23.1 g, 133 mmol), and triethylamine (2.02 g, 20 mmol). mp 57–58°C. IR (Nujol) 1640 cm⁻¹. 1H-NMR (CDCl₃) (100 MHz): δ = 7.01 (1H, br), 5.50 (1H, br), 4.63 (1H, m), 3.69–3.95 (2H, m), 3.01–3.60 (3H, m), 1.52–2.96 (14H, m). EIMS m/z: 256 (M⁺). Anal. Calcld for C₂₀H₃₂N₂O₅: C, 60.91; H, 9.44; N, 10.93. Found: C, 60.78; H, 9.31; N, 10.86.

5-[(4S)-5-Chloro-3-ethyl-N-[1-(2-tetrahydropyranoxyl)-2-pyrrolidinyl][methyl]-6-methoxysalicilic acid (9) A solution of 5 (2.72 g, 11 mmol) in anhydrous tetrahydrofuran (THF, 25 ml) was added dropwise to a suspension of LiAlH₄ (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 LiAlH₄ was quenched by the addition of aqueous saturated Na₂SO₄. The precipitate was filtered off through a celite pad. The filtrate was dried over anhydrous Na₂SO₄, and evaporated to give 2-aminoethyl-1-(2-tetrahydropyranoxyl)pyrrolidin as an oily product (2.2 g, 82%). 1H-NMR (CDCl₃) (100 MHz): δ = 4.61 (1H, m), 3.79–3.93 (2H, m), 3.47–3.65 (3H, m), 2.93–3.22 (2H, m), 2.70–2.71 (2H, m), 2.23–2.24 (3H, m), 1.83–1.92 (2H, m), 1.60–1.82 (4H, m), 1.48–1.68 (6H, m). FABMS m/z: 229 (MH⁺). HRMS m/z: 229.1941. Halad. Calcld for C₁₉H₂₃N₂O₅: C, 59.95; H, 7.54; N, 6.26. Found: C, 59.72; H, 7.49; N, 6.26.

2-(4S)-5-Chloro-3-ethyl-N-[1-(3-tetrahydropyranoxylpropyl)-2-pyrrolidinyl][methyl]-6-methoxysalicilic acid (10) Compound 1 (1 g, 3.9 mmol) was reduced with LiAlH₄ (0.30 g, 11.3 mmol) by the same procedure
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)6) and the amine obtained above (500 mg, 2.06 mmol) were condensed with N,N'-dicyclohexylcarbodiimide (DCC)6) under anhydrous conditions for the synthesis of 9. Purification by column chromatography (silica gel, CHCl₃) afforded 10 as a colorless oil (563 mg, 62%). IR (neat at 1630 cm⁻¹). ¹H-NMR (CDCl₃) (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 (2H, m), 1.54-3.09 (17H, m), 1.19 (3H, t, J = 7.6 Hz). EIMS m/z: 454 (M⁺). Anal. Calc. for C₂₂H₂₂Cl₂N₂O₂: C, 60.7; H, 7.75; N, 6.16. Found: C, 60.73; H, 7.7; N, 6.07.

(5S)-5-Chloro-3-ethyl-N-[[(2'-hydroxyethyl)-2-phenylidinyl]-methyl]-6-methoxysalicylamide (11) A solution of 9 (50 mg, 0.11 mmol) in 1N HCl was stirred at room temperature for 1 h, and extracted with ether. The layer was separated, and alkalized to pH 10 with aqueous NH₄OH, then extracted with CH₂Cl₂. The extract was dried over anhydrous Na₂SO₄ and evaporated to give a crude oil, which was chromatographed on a silica gel column (CHCl₃) to afford a colorless oil (374 mg, 94%). 

(5S)-5-Chloro-3-ethyl-N-[[(2'-hydroxyethyl)-2-phenylidinyl]-methyl]-6-methoxysalicylamide (12) 10 (100 mg, 0.24 mmol) was purified by the same procedure as described above, and the crude product was purified by gel silica column (CHCl₃) to give 12 as a colorless oil (143 mg, 88%). 

(5S)-5-Chloro-3-ethyl-N-[[(2'-methanesulfonyloxyethyl)-2-phenylidinyl]-methyl]-6-methoxysalicylamide (13) Methanesulfonyl chloride (40 µl, 0.52 mmol) was added dropwise to a solution of 11 (50 mg, 0.10 mmol) and triethylamine (70 µl) in anhydrous ether (1 ml) at 0℃. After being stirred for 15 min at 0℃, the reaction mixture was diluted with ether (50 ml), and washed successively with aqueous saturated NaHCO₃ (10 ml x 2), brine (10 ml x 2), and water (10 ml), then dried over anhydrous Na₂SO₄. The solvent was removed to give a crude oil, which was purified by silica gel column chromatography (AcOEt:hexane = 2:1) to afford the staring material (98 mg) for the next reaction (95% yield). IR (neat) 3360, 1630 cm⁻¹. ¹H-NMR (CDCl₃) (270 MHz) δ: 13.76 (1H, br), 8.823 (1H, br), 7.221 (1H, s), 3.89 (3H, s), 3.813 (1H, dd, J=2.3, 7.6, 14.2 Hz), 3.298 (1H, dd, J=3.0, 4.6, 14.2 Hz), 3.186 (1H, dt, J=6.0, 13.9 Hz), 3.055 (3H, s), 2.74-2.83 (1H, m), 2.613 (2H, dq, J=7.5, 5.7 Hz), 2.629 (1H, dd, J=4.3, 6.6, 13.9 Hz), 2.355 (1H, s, J=8.9, 15.6 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 calculated for C₂₇H₂₄Cl₂N₂O₄S. Found: 435.1329.

(5S)-5-Chloro-3-ethyl-N-[[(2'-methanesulfonyloxypropyl)-2-phenylidinyl]-methyl]-6-methoxysalicylamide (14) Methanesulfonyl chloride (9.2 mg, 0.081 mmol) was added dropwise into a solution of 12 (208 µg, 0.054 mmol) and triethylamine (10 µg, 0.33 mmol) in CH₂Cl₂ (2ml), and the reaction mixture was stirred for 15 min at 0℃. The solvent was removed, and the residue was purified by a silica gel column chromatography (CHCl₃:MeOH = 90:1) to give a colorless oil (7 mg, 28%). 

(5S)-5-Chloro-3-ethyl-N-[[(2'-methanesulfonyloxypropyl)-2-phenylidinyl]-methyl]-6-methoxysalicylamide (15) 14 (10 mg, 0.02 mmol) was added to a solution of 11 (50 mg, 0.10 mmol) and triethylamine (70 µl) in anhydrous ether (1 ml) at 0℃. After being stirred for 15 min at 0℃, the reaction mixture was diluted with ether (50 ml), and washed successively with aqueous saturated NaHCO₃ (10 ml x 2), brine (10 ml x 2), and water (10 ml), then dried over anhydrous Na₂SO₄. The solvent was removed to give a crude oil, which was purified by silica gel column chromatography (AcOEt:hexane = 2:1) to afford the staring material (40 mg) for the next reaction (95% yield). IR (neat) 3360, 1630 cm⁻¹. ¹H-NMR (CDCl₃) (270 MHz) δ: 13.76 (1H, br), 8.823 (1H, br), 7.221 (1H, s), 3.89 (3H, s), 3.813 (1H, dd, J=2.3, 7.6, 14.2 Hz), 3.298 (1H, dd, J=3.0, 4.6, 14.2 Hz), 3.186 (1H, dt, J=6.0, 13.9 Hz), 3.055 (3H, s), 2.74-2.83 (1H, m), 2.613 (2H, dq, J=7.5, 5.7 Hz), 2.629 (1H, dd, J=4.3, 6.6, 13.9 Hz), 2.355 (1H, s, J=8.9, 15.6 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 calculated for C₂₇H₂₄Cl₂N₂O₄S. Found: 435.1329.

(5S)-5-Chloro-3-ethyl-N-[[(2'-hydroxyethyl)-2-phenylidinyl]-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℃ for 10 min. The solvent was evaporated, and the residue was dissolved in water (50 µl). IR (neat at 1630 cm⁻¹). ¹H-NMR (CDCl₃) (100 MHz) δ: 13.80 (1H, s), 8.74 (1H, br), 7.21 (1H, s), 4.32 (2H, dd, J=6.6, 6.6, 3.98 Hz, 3.85 (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. Calc. for C₂₂H₂₂Cl₂N₂O₄S: C, 60.83; H, 6.51; N, 6.24. Found: C, 60.63; H, 6.88; N, 6.33.

Chemistry. Preparation of [¹⁸F]Fluorinating Reagent, K[¹⁸F]Kryptofix222 Complex Radiosynthesis was performed in a TPX vessel unless otherwise noted. Kryptofix222 (11.2 mg) and K₂CO₃ (1.53H₂O (2.2mg) were added to a solution of [¹⁸F]fluoride from the irradiated water target, and the solution was evaporated to dryness under an argon stream at 100℃. Three portions of acetone (150-500 µl) were added and evaporated under an argon stream at 100℃. The mixture was filtered through a Sep Pak C₁₈ cartridge.}

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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% NaHCO₃, affording 0.5—1.0 ml of the injection preparation including hydrochloric acid. The injection containing DMSO was prepared by dissolving [¹⁸F]F⁻ in a saline containing 1% DMSO (0.5—1.0 ml), 10.0—18.0 μCi of [¹⁸F]F⁻ 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/kg, are compared in Fig. 2.

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