Synthesis and Characterization of ^{11}C-Labeled Fluoroclorgyline: A Monoamine Oxidase A Specific Inhibitor for Positron Emission Tomography

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A new radioligand for monoamine oxidase type A (MAO-A), [^{11}C]fluoroclorgyline, was synthesized from its desmethyl precursor by *N*-methylation reaction using [^{11}C]methyl iodide with a radiochemical yield of 75—85%. The radiochemical purity of the product was more than 99% and the specific radioactivity was 7.4—18.5 GBq/μmol. The *in vivo* tissue distribution studies of [^{11}C]fluoroclorgyline in mice demonstrated its high initial uptake and prolonged retention in the brain, comparable to those of [^{11}C]clorgyline. A selective interaction with MAO-A in the accumulation of [^{11}C]fluoroclorgyline was confirmed by a competition experiment performed with the MAO-A specific inhibitor, clorgyline, and MAO-B specific inhibitor, *l*-deprenyl. These very desirable characteristics of [^{11}C]fluoroclorgyline suggested that its ^{18}F labeled counterpart, [^{18}F]fluoroclorgyline, would have great potential as a longer-lived alternative to ^{11}C labeled clorgyline for *in vivo* studies of MAO-A in the human brain with positron emission tomography (PET).

Keywords monoamine oxidase inhibitor; fluoroclorgyline; positron emission tomography; carbon-11; fluorine-18

Monoamine oxidase (MAO) [EC 1.4.3.4] is a flavin-containing enzyme that catalyzes the oxidative deamination of endogenous neurotransmitter amines as well as exogenous amines. It has been divided into two subtypes, MAO-A and MAO-B on the basis of their different specificities toward substrates and inhibitors.[1–3] Clorgyline and *l*-deprenyl irreversibly and selectively inhibit MAO-A and MAO-B, respectively, by binding covalently to the flavin coenzyme of MAO.[4–6]

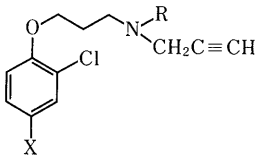
Positron emission tomography (PET) and single photon emission computed tomography (SPECT) provide the capability of noninvasively examining biochemical transformations in the intact living system utilizing organic molecules labeled with a position emitter or a single photon emitter. For the direct and noninvasive mapping and functional studies of MAO activity in the living brain, the ^{11}C labeled suicide inhibitors, pargyline,[7] clorgyline[8,9] and *l*-deprenyl,[8–12] have been investigated as positron ligands for PET.

These ^{11}C labeled inhibitors appear to be suitable as the ligands of first approach for PET studies because of their relatively easy preparation. However, the short 20 min half-life of ^{11}C, among the positron emitting radionuclides, has limited the ability to obtain an understanding of relatively slow ligand kinetics. Fluorine-18 with its half-life of 110 min may be favored as a longer-lived alternative to ^{11}C for studies of the kinetic analysis with PET.

Previously, a series of novel fluorine substituted clorgyline derivatives were prepared and evaluated as selective inhibitors for MAO-A.[13] *N*-[3-(2-Chloro-4-fluorophenoxy)-propyl]-*N*-methyl-2-propynylamine (fluoroclorgyline, **1**, Chart 1) was found to be relatively potent and selective for MAO-A *in vitro*, being comparable to clorgyline examined under the same conditions. To elucidate further the effect of fluorine substitution on the *in vivo* characteristics, we report here the synthesis and preliminary biological evaluation of ^{11}C labeled fluoroclorgyline, [^{11}C]fluoroclorgyline (**3**, Chart 1), and a comparison with [^{11}C]clorgyline.

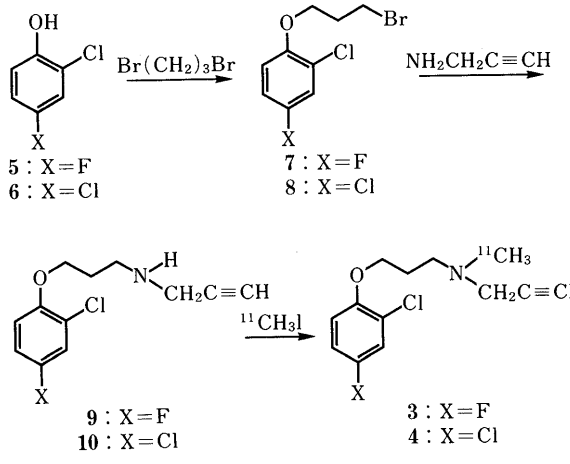
Synthesis

[^{11}C]Fluoroclorgyline and [^{11}C]clorgyline were synthesized by the reactions outlined in Chart 2, based on the published procedure for [^{11}C]clorgyline[14] with a slight modification. Phenol derivatives (**5**, **6**) were converted to the corresponding phenoxypropyl bromides (**7**, **8**).[13] Reaction of the phenoxypropyl bromides with *N*-propargylamine in the presence of potassium carbonate in acetonitrile gave



1 : X=F, R=CH_3 fluoroclorgyline
2 : X=Cl, R=CH_3 clorgyline
3 : X=F, R=$^{11}CH_3$[^{11}C]fluoroclorgyline
4 : X=Cl, R=$^{11}CH_3$[^{11}C]clorgyline

Chart 1



5 : X=F
6 : X=Cl
7 : X=F
8 : X=Cl
9 : X=F
10 : X=Cl
3 : X=F
4 : X=Cl

Chart 2

9 and **10**. *N*-[^{11}C]Methylation of these desmethyl precursors **9** and **10** with [^{11}C]methyl iodide in dimethylformamide-dimethylsulfoxide followed by high performance liquid chromatography (HPLC) purification produced corresponding [^{11}C]fluoroclorgyline and [^{11}C]clorgyline, respectively, with the radiochemical yield of 75—85%. The total time from the end of [^{11}C]methyl iodide trapping in dimethylformamide to HPLC purification was within 30 min. The radiochemical purity of the [^{11}C]fluoroclorgyline and [^{11}C]clorgyline thus obtained was more than 99% as assessed by HPLC analysis. The specific radioactivity was approximately 7.4—18.5 GBq/μmol.

Biological Results and Discussion

The *in vivo* tissue distribution of [^{11}C]fluoroclorgyline and [^{11}C]clorgyline was examined in male ddY mice at 5, 15, 30 and 60 min after intravenous administration. As summarized in Table I, [^{11}C]fluoroclorgyline was transported well into various organs. The initial level of accumulation of [^{11}C]fluoroclorgyline in the brain was high, 3.32% dose/g at 5 min after injection, and then the brain radioactivity level decreased gradually to 2.08% dose/g at 15 min after injection. [^{11}C]Fluoroclorgyline exhibited the desired prolonged retention in the brain (1.96% dose/g at 60 min after injection). In contrast to the high brain uptake of this agent the radioactivity in the blood was low, 1.29 and 0.77% dose/g at 5 and 60 min post injection, respectively, resulting in good brain-to-blood activity ratios of 2.57 and 2.55 at 5 and 60 min after administration, respectively. Interestingly, [^{11}C]fluoroclorgyline showed high accumulation in the pancreas, 7.98 and 4.81% dose/g at 5 and 60 min after injection, respectively.

The distribution of [^{11}C]clorgyline was comparatively studied in mice and the results are summarized in Table II. The *in vivo* distribution behavior of [^{11}C]clorgyline was very similar to that of [^{11}C]fluoroclorgyline. The accumulation of radioactivity in the brain was 3.21, 2.11 and 1.97% dose/g at 5, 15 and 60 min post injection, respectively. The brain-to-blood activity ratio was in a range of 2.03—3.13 at 5—60 min post injection.

Data derived from these comparative distribution studies of [^{11}C]fluoroclorgyline and [^{11}C]clorgyline indicated that the effect of fluorine substitution on the *in vivo* distribution was negligible. Thus, ^{18}F labeled fluoroclorgyline would be a very suitable radioligand as a longer-lived alternative to ^{11}C labeled clorgyline for *in vivo* studies of MAO-A in the living brain with PET.

The effects of pretreatment with clorgyline or *l*-deprenyl on the distribution of [^{11}C]fluoroclorgyline at 60 min after administration are presented in Fig. 1. Pretreatment with clorgyline, a MAO-A specific inhibitor, significantly reduced the uptake of [^{11}C]fluoroclorgyline in the brain (56% reduction). Although the liver uptake was increased by the clorgyline treatment, no significant changes in uptake in other tissues were observed. The brain uptake of [^{11}C]fluoroclorgyline was not affected by the pretreatment with *l*-deprenyl, a MAO-B specific inhibitor. Thus, a selective interaction with MAO-A in the accumulation of [^{11}C]fluoroclorgyline was demonstrated by the competition experiment with MAO-A and MAO-B specific inhibitors.

The carrier effect on the brain uptake of [^{11}C]fluoroclorgyline was investigated by using various doses of the cold ligand, from 0.01 to 10 mg/kg. As shown in Fig. 2, the brain uptake of the radioactivity was not significantly different at the dose range of 0.01—0.1 mg/kg. By contrast, higher doses (1—10 mg/kg) reduced the brain accumulation of [^{11}C]fluoroclorgyline. This reduction of the brain uptake

TABLE I. Tissue Distribution of Radioactivity in Mice after Intravenous Injection of [^{11}C]Fluoroclorgyline[a]

Tissue	Time after injection			
	5 min	15 min	30 min	60 min
Blood	1.29±0.07	0.97±0.08	0.75±0.26	0.77±0.29
Pancreas	7.98±0.73	6.53±0.29	5.14±0.62	4.81±0.31
Liver	4.92±0.23	4.43±0.35	2.87±0.25	2.67±0.38
Kidney	8.16±0.56	6.96±1.03	4.63±0.91	4.73±0.53
Heart	2.62±0.26	1.74±0.12	1.24±0.16	1.03±0.10
Lung	6.04±0.30	4.67±0.96	3.30±0.45	3.07±0.49
Brain	3.32±0.11	2.08±0.10	1.91±0.21	1.96±0.25

a) Mean % injected dose±S.D. per gram tissue of four animals.

TABLE II. Tissue Distribution of Radioactivity in Mice after Intravenous Injection of [^{11}C]Clorgyline[a]

Tissue	Time after injection			
	5 min	15 min	30 min	60 min
Blood	1.58±0.20	0.94±0.17	0.71±0.08	0.63±0.10
Pancreas	8.16±1.20	5.60±0.70	4.57±0.57	4.29±0.30
Liver	5.50±0.29	4.50±0.59	3.60±0.38	2.90±0.21
Kidney	11.33±4.08	6.80±1.17	5.07±0.72	4.01±0.11
Heart	2.98±0.38	1.84±0.43	1.41±0.23	1.01±0.15
Lung	8.77±3.17	5.03±0.82	4.02±0.69	3.92±0.53
Brain	3.21±0.41	2.11±0.26	2.02±0.33	1.97±0.14

a) Mean % injected dose±S.D. per gram tissue of four animals.

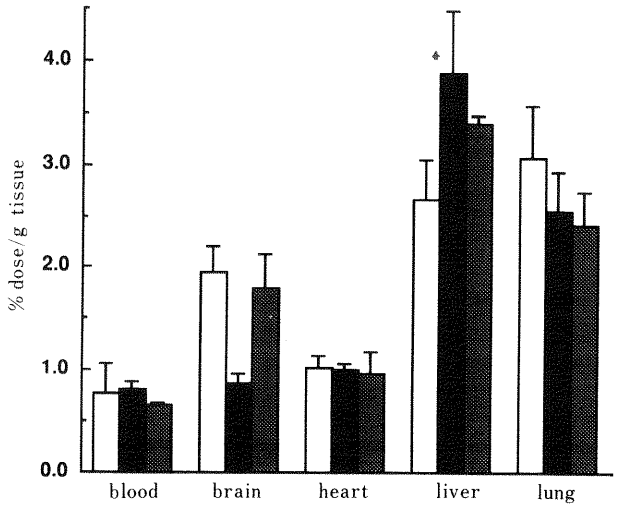


Fig. 1. Effects of Pretreatment with Clorgyline and *l*-Deprenyl on the Distribution of [^{11}C]Fluoroclorgyline in Mice

Mice were injected intraperitoneally with clorgyline or *l*-deprenyl 60 min before the intravenous injection of [^{11}C]fluoroclorgyline. The animals were sacrificed at 60 min after the radioligand administration, then the distribution of [^{11}C]fluoroclorgyline was studied as described under Experimental. Results are expressed as the mean % injected dose±S.D. per gram tissue of four animals. □, control; ■, pretreated with clorgyline; ■, pretreated with *l*-deprenyl.

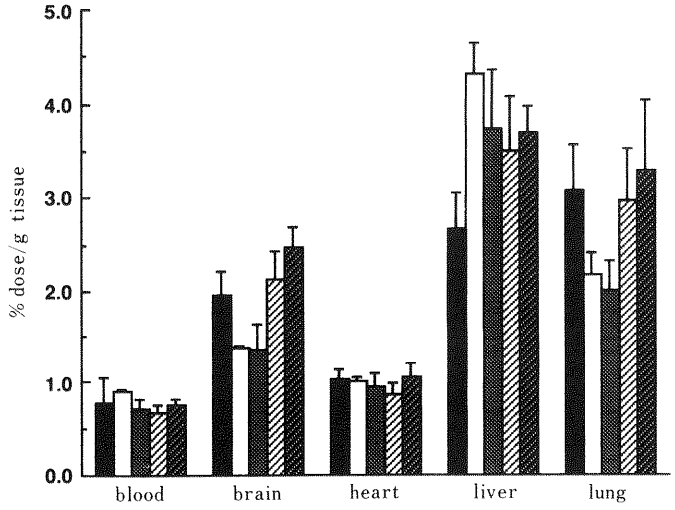


Fig. 2. Dose Effect on the Distribution of [^{11}C]Fluoroclorgyline in Mice

[^{11}C]Fluoroclorgyline was injected intravenously into mice simultaneously with various doses of cold fluoroclorgyline (0.01—10 mg/kg). The animals were sacrificed at 60 min after administration, then the distribution of [^{11}C]fluoroclorgyline was studied as described under Experimental. Results are expressed as the mean % injected dose ± S.D. per gram tissue of four animals. ■, control; □, 10 mg/kg; ■, 1 mg/kg; ▨, 0.1 mg/kg; ■, 0.01 mg/kg.

of [^{11}C]fluoroclorgyline by the high dose of the cold ligand seems to be a similar effect to that obtained by the pretreatment with clorgyline (Fig. 1). The total amount of fluoroclorgyline present in a single injection dose of [^{11}C]fluoroclorgyline, less than 0.6 μg/kg estimated from the specific radioactivity, is significantly lower than the value of fluoroclorgyline required for *in vivo* MAO inhibition. Therefore, the *in vivo* behavior of this radioligand can be assessed without causing an inhibitory effect on MAO.

In conclusion, the new radioligand for MAO-A, [^{11}C]fluoroclorgyline, was synthesized from its desmethyl precursor by *N*-[^{11}C]methylation with high yield. The product possessed a high radiochemical purity as well as high specific radioactivity. The *in vivo* tissue distribution studies of [^{11}C]fluoroclorgyline demonstrated its high initial uptake and prolonged retention in the brain, comparable to those of [^{11}C]clorgyline. A selective interaction with MAO-A in the accumulation of [^{11}C]fluoroclorgyline was confirmed by the competition experiment with the two MAO specific inhibitors, clorgyline and *l*-deprenyl. These very desirable characteristics of [^{11}C]-fluoroclorgyline suggested that ^{18}F labeled counterpart, [^{18}F]fluoroclorgyline, would have great potential as a longer-lived alternative to ^{11}C labeled clorgyline for *in vivo* studies of MAO-A in the human brain with PET. Further studies of this new radiopharmaceutical, including radiofluorination with ^{18}F, are in progress.

Experimental

All melting points are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian Gemini-200 (200 MHz) spectrometer and the chemical shifts are reported in ppm downfield from an internal tetramethylsilane standard. High resolution mass spectra (HRMS) were obtained on a Hitachi M-80 instrument. The HPLC system used included a Waters M 600 pump, a Lambda-Max 481 ultraviolet detector, a Beckman 170 NaI radioactivity detector, and a Cosmosil 5C18-AR column (10 × 250 mm, Nacalai Tesque).

Materials 2-Chloro-4-fluorophenol (**5**) and 2,4-dichlorophenol (**6**) were obtained commercially and used without further purification. Fluoroclorgyline (**1**), clorgyline (**2**), 3-(2-chloro-4-fluorophenoxy)propyl bromide (**7**), and 3-(2,4-dichlorophenoxy)propyl bromide (**8**) were synthesized by the reported methods.[13] *l*-Deprenyl was purchased from Research Biochemicals Inc. The other chemicals used were of reagent grade. Male ddY mice weighing 20—25 g were supplied by Japan SLC Co., Ltd.

***N*-[3-(2-Chloro-4-fluorophenoxy)propyl]-2-propynylamine (9)** To a solution of 3-(2-chloro-4-fluorophenoxy)propyl bromide (**7**) (1.96 g, 10 mmol) in acetonitrile (30 ml) was added a solution of potassium carbonate (1.52 g, 11 mmol) in water (3 ml), followed by propargylamine (1.10 g, 20 mmol). The resultant mixture was stirred at ambient temperature for 5 d. After removal of the volatile components *in vacuo*, the residue was partitioned between ether (30 ml) and 1 N hydrochloric acid (30 ml). The aqueous phase was separated and basified with 5 N sodium hydroxide solution, then extracted with ether (30 ml × 3). The combined ether layers were washed with water, dried over sodium sulfate and evaporated *in vacuo*. The crude amine obtained was converted to its hydrochloride salt, which was recrystallized from methanol–ether to give pure **9** as the hydrochloride salt (2.06 g, 74%). mp 140—142 °C. *Anal.* Calcd for $C_{12}H_{13}ClFNO \cdot HCl$: C, 51.81; H, 5.07; N, 5.04. Found: C, 51.70; H, 5.06; N, 5.09. ^{1}H-NMR (free base, $CDCl_3$) δ: 1.54 (1H, br, NH), 2.02 (2H, quintet, J=6.3 Hz, $CH_2\underline{CH_2}CH_2$), 2.22 (1H, t, J=2.4 Hz, C≡CH), 2.94 (2H, t, J=6.3 Hz, $CH_2\underline{CH_2}N$), 3.46 (2H, d, J=2.4 Hz, $NC\underline{H_2}C{\equiv}CH$), 4.09 (2H, t, J=6.3 Hz, OCH_2, 6.86—7.15 (3H, m, aromatics). HRMS Calcd for $C_{12}H_{13}ClFNO$ (free base) *m/z*: 241.0670. Found: 241.0674.

***N*-[3-(2,4-Dichlorophenoxy)propyl]-2-propynylamine (10)** *N*-[3-(2,4-Dichlorophenoxy)propyl]-2-propynylamine (**10**) was synthesized in a similar manner to that of **9** from 3-(2,4-dichlorophenoxy)propyl bromide (**8**). mp (hydrochloride salt) 145—147 °C (lit.[14] 145—147 °C).

***N*-[3-(2-Chloro-4-fluorophenoxy)propyl]-*N*-[^{11}C]methyl-2-propynylamine ([^{11}C]Fluoroclorgyline, 3)** [^{11}C]Fluoroclorgyline (**3**) was synthesized according to the method of MacGregor *et al.*[14] with a slight modification.

[^{11}C]Carbon dioxide was produced by ^{14}N(p, α) ^{11}C reaction using an ultracompact cyclotron (Sumitomo model-325), then trapped in a solution of lithium aluminum hydride in tetrahydrofuran and reduced to [^{11}C]methanol as previously described.[15] After removal of the solvent, 54% hydriodic acid was added, and the [^{11}C]methyl iodide obtained was trapped in dimethylformamide under a stream of nitrogen gas. The solution of [^{11}C]methyl iodide (7.5—20 GBq) in dimethylformamide (160 μl) was added to a solution of *N*-[3-(2-chloro-4-fluorophenoxy)propyl]-2-propynylamine (**9**) (2 μl of the free base) in dimethylsulfoxide (400 μl) in a sealed reaction vial. The vial was heated at 125 °C for 5 min. After cooling, the reaction mixture was purified by HPLC using 0.05 N ammonium formate–methanol (10 : 90, v/v) as an eluent at a flow rate of 3.0 ml/min. The fraction corresponding to fluoroclorgyline (t_R = 5.2 min) was collected and the solvent was removed *in vacuo*. The final product, [^{11}C]fluoroclorgyline, was taken up in an isotopic saline solution and passed through a 0.22 μm filter. The radiochemical yield was 75—85%. The radiochemical purity was more than 99% as determined by HPLC using the same elution conditions as described above. The specific radioactivity was about 7.4—18.5 GBq/μmol as estimated from the ultraviolet absorbance at 254 nm and radioactivity.

***N*-[3-(2,4-Dichlorophenoxy)propyl]-*N*-[^{11}C]methyl-2-propynylamine ([^{11}C]Clorgyline, 4)** [^{11}C]Clorgyline (**4**) was prepared in a similar manner to [^{11}C]fluoroclorgyline (**3**), from *N*-[3-(2,4-dichlorophenoxy)-propyl]-2-propynylamine (**10**) and [^{11}C]methyl iodide. The radiochemical yield was 75—85%. The radiochemical purity and specific radioactivity were more than 99% and about 11.1—18.5 GBq/μmol, respectively.

Tissue Distribution Studies in Mice Groups of four male ddY mice (20—25 g) were injected intravenously through a lateral tail vein with [^{11}C]fluoroclorgyline or [^{11}C]clorgyline (370 kBq) in 0.1 ml of saline solution. At the desired time interval after administration, the animals were sacificed. Samples of blood and organs of interest were excised and weighed. The radioactivity was measured using a well-type NaI(Tl) gamma scintillation counter. The results were expressed in terms of the percentage of the injected dose per gram of blood or organ.

Effect of Clorgyline and *l*-Deprenyl Pretreatment on [^{11}C]Fluoroclorgyline Distribution in Mice Groups of four mice were injected intraperitoneally with 10 mg/kg of clorgyline or *l*-deprenyl in 0.1 ml of saline solution. After 60 min, [^{11}C]fluoroclorgyline (370 kBq) in 0.1 ml of saline solution was injected intravenously through a lateral tail vein. The animals were sacrificed at 60 min after the radioligand administration, then the distribution of [^{11}C]fluoroclorgyline was studied as described above.

Dose Effect on [^{11}C]Fluoroclorgyline Distribution in Mice Unlabeled fluoroclorgyline (0.01—10 mg/kg) was injected simultaneously with the

radioligand, [^{11}C]fluoroclorgyline (370 kBq), into groups of four mice through a lateral tail vein. The animals were sacrificed at 60 min after administration, then the distribution of [^{11}C]fluoroclorgyline was studied as described above.

References

1) D. L. Murphy, *Biochem. Pharmacol.*, **27**, 1889 (1978).
2) C. J. Fowler, L. Oreland, B. A. Callingham, *J. Pharm. Pharmacol.*, **33**, 341 (1981).
3) C. J. Fowler, S. B. Ross, *Med. Res. Rev.*, **4**, 323 (1984).
4) R. R. Rando, *Science*, **185**, 320 (1974).
5) J. I. Salach, K. Detmer, *Mol. Pharmacol.*, **16**, 234 (1979).
6) J. Nagy, J. I. Salach, *Arch. Biochem. Biophys.*, **208**, 388 (1981).
7) K. Ishiwata, T. Ido, K. Yanai, K. Kawashima, Y. Miura, M. Monma, S. Watanuki, T. Takahashi, R. Iwata, *J. Nucl. Med.*, **26**, 630 (1985).
8) R. R. MacGregor, C. Halldin, J. S. Fowler, A. P. Wolf, C. D. Arnett, B. Langstrom, D. Alexoff, *Biochem. Pharmacol.*, **34**, 3207 (1985).
9) J. S. Fowler, R. R. MacGregor, A. P. Wolf, C. D. Arnett, S. L. Dewey, D. J. Schlyer, D. Christman, J. Logan, M. Smith, H. Sachs, S. M. Aquilonius, P. Bjurling, C. Halldin, P. Hartvig, K. L. Leenders, H. Lundqvist, L. Oreland, C. G. Stalnacke, B. Langstrom, *Science*, **235**, 481 (1987).
10) C. D. Arnett, J. S. Fowler, R. R. MacGregor, D. J. Schlyer, A. P. Wolf, B. Langstrom, C. Halldin, *J. Neurochem.*, **49**, 522 (1987).
11) J. S. Fowler, A. P. Wolf, R. R. MacGregor, S. L. Dewey J. Logan, D. J. Schlyer, B. Langstrom, *J. Neurochem.*, **51**, 1524 (1988).
12) S. S. Jossan, R.d'Argy, P. G. Gillberg, S. M. Aquilonius, B. Langstrom, C. Halldin, P. Bjurling, C. G. Stalnacke, J. S. Fowler, R. R. MacGregor, L. Oreland, *J. Neural Transm.*, **77**, 55 (1989).
13) Y. Ohmomo, M. Hirata, K. Murakami, Y. Magata, C. Tanaka, A. Yokoyama, *Chem. Pharm. Bull.*, **39**, 1038 (1991).
14) R. R. MacGregor, J. S. Fowler, A. P. Wolf, C. Halldin, B. Langstrom, *J. Labelled Compd. Radiopharm.*, **25**, 1 (1988).
15) H. Saji, Y. Magata, Y. Yamada, K. Tajima, Y. Yonekura, J. Konishi, Y. Ohmomo, A. Yokoyama, *Chem. Pharm. Bull.*, **40**, 734 (1992).