Synthesis of [^{125}I]Iodoclorgyline, a Selective Monoamine Oxidase A Inhibitor, and Its Biodistribution in Mice

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A new radioiodinated monoamine oxidase A (MAO-A) specific inhibitor, [^{125}I]iodoclorgyline, was synthesized from its tin precursor by iododestannylation reaction using sodium [^{125}I]iodide and hydrogen peroxide with high yield and site specificity. The product possessed a high radiochemical purity as well as high specific activity. The method can be readily applicable for labeling with ^{123}I, a very suitable radioisotope for *in vivo* imaging with single photon emission computer tomography (SPECT). Biodistribution studies of the [^{125}I]iodoclorgyline in mice showed high initial uptake in the brain, and brain radioactivity reached a constant level at 60 min after intravenous injection. The results suggested that [^{125}I]iodoclorgyline might have potential as a radiopharmaceutical for MAO-A studies in the brain with SPECT.

Keywords monoamine oxidase inhibitor; iodoclorgyline; radioiodination; iododestannylation; biodistribution

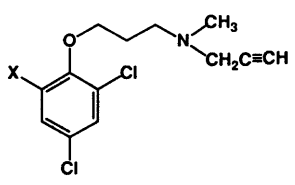
Monoamine oxidase (MAO) [E.C. 1.4.3.4] catalyzes the oxidative deamination of endogenous neurotransmitter amines as well as exogenous amines. It has been divided into two subtypes, MAO-A and MAO-B on the basis of substrate and inhibitor selectivity.[1] Clorgyline and *l*-deprenyl inhibit irreversibly and selectively MAO-A and MAO-B, respectively, by binding covalently to the enzyme itself.[2] Both subtypes, MAO-A and MAO-B, appear to be important for neurotransmitter regulation, and fluctuations in functional MAO activity may be associated with human diseases such as Parkinson's disease, depression and certain psychiatric disorders.[3]

The development of positron emission tomography (PET) and single photon emission computer tomography (SPECT) has made possible the studies of metabolism and physiological processes in the living human body utilizing organic molecules labeled with a positron emitter or a single photon emitter. For the direct and non-invasive mapping and functional studies of MAO activity in the living brain, the carbon-11 labeled suicide inhibitors, pargyline, clorgyline and *l*-deprenyl, have been investigated as positron ligands for PET studies.[4]

Despite attractive features associated with PET techniques, PET studies are still limited, since they usually require on-site cyclotrons. On the other hand, SPECT studies are more commonly used in nuclear medicine clinics. For SPECT imaging, iodine-123 possesses very suitable radiation properties, half-life of 13 h and gamma ray energy of 159 keV.

We have explored the feasibility of [^{123}I]radioiodinated MAO inhibitors as alternatives to clorgyline and *l*-deprenyl for functional MAO studies in the brain with SPECT. Previously, a series of novel iodinated clorgyline derivatives were prepared and evaluated as selective inhibitors for MAO-A.[5] *N*-[3-(2,4-Dichloro-6-iodophenoxy)propyl]-*N*-methyl-2-propynylamine (iodoclorgyline, **2**, Chart 1) was found to be relatively potent and selective toward MAO-A, comparable to clorgyline examined under the same conditions. We report here the synthesis of the radioiodinated counterpart, [^{125}I]iodoclorgyline (**3**, Chart 1). Preliminary biological studies on the *in vivo* inhibitory potency and selectivity for MAO-A and biodistribution in mice of this compound were also performed in order to evaluate it as a new ligand for *in vivo* MAO-A studies with SPECT.

Electrophilic iododestannylation reaction offers several distinct advantages for synthesis of radiopharmaceuticals.[6] The labeling procedure may be performed in the last step under very mild conditions and with very high site



1 : X=H clorgyline
2 : X=I iodoclorgyline
3 : X=^{125}I [^{125}I] iodoclorgyline

Chart 1

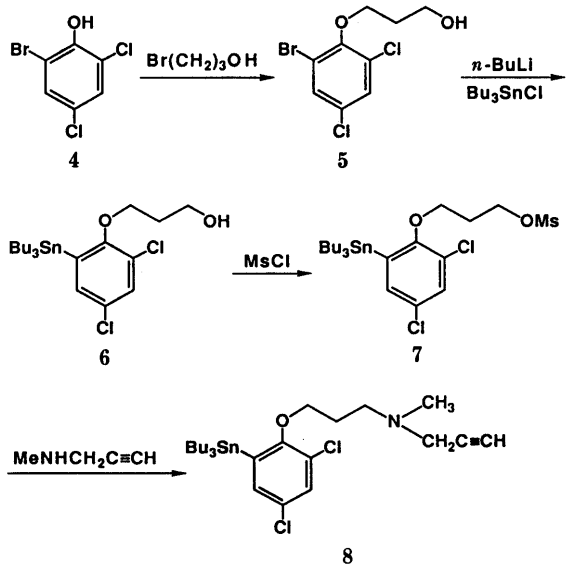


Chart 2

selectivity as well as high specific radioactivity. The requisite tin atom may be appended at any step in the synthesis. Moreover, the approach appears to be quite general with respect to the substrate. This paper describes a convenient method for the introduction of ^{125}I into clorgyline.

[^{125}I]Iodoclorgyline and its key tin precursor (**8**) were synthesized by the reactions outlined in Chart 2. The bromophenol derivative (**4**) was converted to its corresponding phenoxypropanol (**5**). Reaction of **5** with *n*-butyllithium and tri-*n*-butyltin chloride in tetrahydrofuran at $-78\,^{\circ}C$ yielded the stannyl phenoxypropanol (**6**), which was converted into a reactive mesylate (**7**) by treatment with methanesulfonyl chloride in dichloromethane. Alkylation of *N*-methylpropargylamine with **7** in the presence of potassium carbonate in acetonitrile gave the desired key intermediate (**8**).

Iodination of **8** was very easily accomplished in high yield (88%) by treatment with iodine in chloroform. Radioiodination of **8** was achieved using hydrogen peroxide[7] as an oxidant and sodium [^{125}I]iodide (specific activity 7.4 TBq/mmol) in 0.1 N HCl aqueous solution, followed by high performance liquid chromatography (HPLC) purification. The radiochemical purity of the product was higher than 99% as assessed by HPLC analysis, and the product comigrated on HPLC with authentic unlabeled material (Fig. 1). The radiochemical yield based on Na-^{125}I was 80%. The specific activity of the product was approximately 7.4 TBq/mmol.

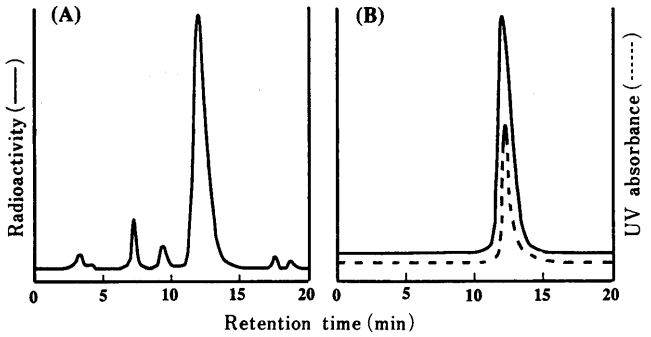


Fig. 1. HPLC Chromatograms of (A) Crude Radioiodination Reaction Mixture (Radioactivity), and (B) Purified [^{125}I]Iodoclorgyline (Radioactivity) and Authentic Unlabeled Iodoclorgyline (UV Absorbance)

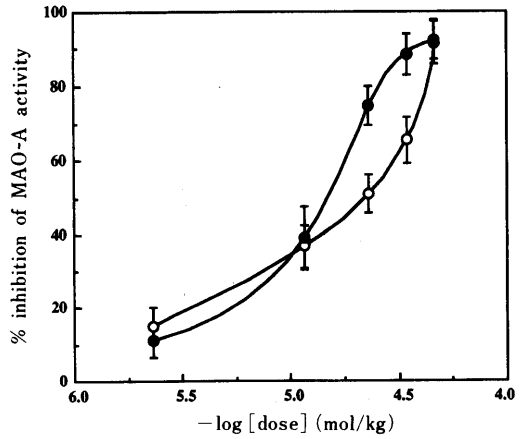


Fig. 2. Dose Response Curves for the Inhibition of Rat Brain (●) and Liver (○) MAO-A Activity by Iodoclorgyline Treatment *in Vivo*

Importantly, the tin precursor **8** is stable over six months when stored at $-20\,^{\circ}C$, and [^{125}I]iodoclorgyline can be readily generated.

The *in vivo* inhibition of MAO activity by iodoclorgyline was examined in the brain and liver homogenates from iodoclorgyline administered rats. The IC_{50} values calculated from the results presented in Fig. 2 were 1.4×10^{-5} and 2.2×10^{-5} mol/kg for MAO-A in the brain and liver homogenate, respectively. On the contrary, MAO-B activity was not effected by the iodoclorgyline treatment examined under the same conditions (dose: 2.3×10^{-6}—4.6×10^{-5} mol/kg). Thus, these results showed that iodoclorgyline had a specific inhibitory effect on MAO-A *in vivo* as well as *in vitro*.[5]

The *in vivo* biodistribution behavior of [^{125}I]iodoclorgyline was examined in male ddY mice. The total amount of iodoclorgyline present in a single injection dose of [^{125}I]iodoclorgyline is significantly lower than the IC_{50} value of iodoclorgyline required for *in vivo* MAO inhibition estimated by the specific activity. Therefore, the *in vivo* behavior of this compound can be assessed without causing an inhibitory effect against MAO. As summarized the results in Table I, the [^{125}I]iodoclorgyline was transported well into various organs and disappeared slowly from the blood. Initial brain uptake was high (2.8% dose/g at 5 min post injection) and the clearance was rapid for the first 60 min and then retained thereafter. The brain to blood activity ratio was, however, in a range of 0.5—0.75 at 15—120 min post injection. It is desirable to increase this ratio to obtain high quality neuronal images. In the liver, the highest uptake and the slowest clearance were observed. The kidney uptake pattern was similar to that of the liver.

In conclusion, the new radioiodinated clorgyline derivative, [^{125}I]iodoclorgyline, was expediently synthesized from its tin precursor by iododestannylation reaction using sodium [^{125}I]iodide and hydrogen peroxide with high yield and site specificity. The product possessed a high radiochemical purity as well as high specific activity. The method can be readily applicable for the labeling with ^{123}I, a very suitable radioisotope for *in vivo* imaging with SPECT. Iodoclorgyline possessed the desirable characteristics, high inhibitory potency and selectivity for MAO-A *in vivo* as well as *in vitro*. Biodistribution studies of the [^{125}I]iodoclorgyline in mice showed high initial uptake in the brain and the brain radioactivity reached a constant level at 60 min after intravenous injection. The results

Table I. Biodistribution of Radioactivity in Mice after Intravenous Injection of [^{125}I]Iodoclorgyline

	Mean% injected dose ± s.d./g organ[a]				
	5 min	15 min	30 min	60 min	120 min
Blood	1.58±0.11	2.01±0.30	2.25±0.10	1.28±0.16	1.28±0.23
Pancreas	6.37±0.11	4.11±0.89	2.57±0.30	1.21±0.26	1.03±0.16
Spleen	2.15±0.43	1.60±0.30	1.29±0.19	0.81±0.20	0.79±0.17
Liver	10.20±0.88	10.07±1.83	9.72±0.45	5.62±1.50	5.67±2.09
Kidney	7.71±0.55	6.49±0.99	8.88±1.55	5.47±2.95	4.05±1.72
Heart	3.27±0.23	1.73±0.31	1.51±0.20	0.73±0.11	0.69±0.11
Lung	5.97±1.19	3.34±0.67	3.03±0.63	1.84±0.26	1.68±0.42
Brain	2.80±0.24	1.51±0.31	1.10±0.13	0.64±0.17	0.69±0.12

a) Four animals per each point.

suggested that [^{125}I]iodoclorgyline might have potential as a SPECT radiopharmaceutical for MAO-A studies in the brain. Further studies of this new agent are now in progress.

Experimental

All melting points are uncorrected. Infrared (IR) spectra were recorded on a JASCO IR-700 spectrometer. 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 UV detector (254 nm), a Beckman 170 NaI radioactivity detector, and a Cosmosil 5C18-AR column (10 mm × 25 cm, Nacalai tesque).

3-(2-Bromo-4,6-dichlorophenoxy)-1-propanol (5) A solution of sodium hydroxide (1.2 g, 30 mmol) in water (5 ml) was added to a mixture of 2-bromo-4,6-dichlorophenol (6.0 g, 25 mmol) and 3-bromo-1-propanol (3.5 g, 25 mmol) in ethanol (20 ml), and the reaction mixture was stirred at reflux overnight. After removing the solvent *in vacuo*, the residue was taken up with chloroform (50 ml) and washed with water (30 ml × 3). The organic layer was dried over sodium sulfate and evaporated *in vacuo* to give crude crystals. Recrystallization from hexane afforded **5** (6.2 g, 83%), mp 60—62 °C. *Anal.* Calcd for $C_9H_9BrCl_2O_2$: C, 36.04; H, 3.02. Found: C, 36.18; H, 3.05. IR ($CHCl_3$): 3620, 3018, 2974, 1450, 1217, 1045 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.92 (1H, br, OH), 2.10 (2H, quintet, J=5.9 Hz, $CH_2C\underline{H}_2CH_2$), 3.97 (2H, q, J=5.9 Hz, $C\underline{H}_2OH$), 4.15 (2H, t, J=5.9 Hz, OCH_2), 7.36 (1H, d, J=2.4 Hz, aromatic), 7.48 (1H, d, J=2.4 Hz, aromatic). HRMS Calcd for $C_9H_9BrCl_2O_2$ m/z: 299.9143. Found: 299.9140.

3-(2,4-Dichloro-6-tributylstannylphenoxy)-1-propanol (6) A 2.0 M solution of *n*-butyllithium (9.0 ml, 18 mmol) in pentane was added to a solution of **5** (2.4 g, 8 mmol) in dry tetrahydrofuran (15 ml) at −78 °C under argon atmosphere. After 30 min, tributyltin chloride (5.9 g, 18 mmol) was added and the resulting solution was allowed to warm gradually to room temperature. The reaction was quenched with a 10% aqueous ammonium chloride solution (10 ml). The reaction mixture was extracted with ether (20 ml × 3). The combined organic extracts were washed with water (30 ml), dried over sodium sulfate and the solvent was removed *in vacuo* to give an oil. Column chromatography on silica gel eluting with chloroform afforded **6** (2.3 g, 56%) as a colorless liquid. IR ($CHCl_3$): 3016, 2958, 2926, 1421, 1375, 1217, 1045 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.86—1.57 (27H, m, $SnBu_3$), 1.94 (1H, t, J=5.9 Hz, OH), 2.06 (2H, quintet, J=5.9 Hz, $CH_2C\underline{H}_2CH_2$), 3.93 (2H, q, J=5.9 Hz, $C\underline{H}_2OH$), 4.06 (2H, t, J=5.9 Hz, OCH_2), 7.19 (1H, d, J=2.4 Hz, aromatic), 7.33 (1H, d, J=2.4 Hz, aromatic). CI-HRMS Calcd for $C_{21}H_{37}Cl_2O_2Sn$ (MH^+) m/z: 511.1192. Found: 511.1196.

3-(2,4-Dichloro-6-tributylstannylphenoxy)-1-(methanesulfonyloxy)-propane (7) A solution of methanesulfonyl chloride (0.27 g, 2.4 mmol) in dichloromethane (2 ml) was added slowly at 0 °C to a mixture of **6** (1.02 g, 2.0 mmol) and triethylamine (0.24 g, 2.4 mmol) in dichloromethane (20 ml). After stirring at 0 °C for 2 h, the reaction mixture was washed with water (10 ml × 3), dried over sodium sulfate and the solvent was removed *in vacuo* to give crude **7** (1.06 g, 90%) as an oil, which was used without further purification.

***N*-[3-(2,4-Dichloro-6-tributylstannylphenoxy)propyl]-*N*-methyl-2-propynylamine (8)** A solution of potassium carbonate (0.7 g, 5 mmol) in water (2 ml) was added to a solution of **7** (1.06 g, 1.8 mmol) in acetonitrile (5 ml) followed by *N*-methylpropargylamine (0.5 ml, 5.9 mmol) and the resulting solution was stirred at room temperature for 3 d. After dilution with ether (30 ml), the reaction mixture was washed with water (10 ml × 3) and dried over sodium sulfate. Removal of the solvent *in vacuo* gave an oil. Purification by column chromatography on silica gel using 10% ethyl acetate in chloroform as an eluent afforded **8** (0.60 g, 59%) as a colorless liquid. IR ($CHCl_3$): 2956, 2926, 2852, 1462, 1421, 1374, 1071 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.86—1.55 (27H, m, $SnBu_3$), 1.98 (2H, quintet, J=6.8 Hz, $CH_2C\underline{H}_2CH_2$), 2.22 (1H, t, J=2.3 Hz, C≡CH), 2.35 (3H, s, NCH_3), 2.64 (2H, t, J=6.8 Hz, $CH_2C\underline{H}_2N$), 3.38 (2H, d, J=2.3 Hz, $C\underline{H}_2C{\equiv}CH$), 3.96 (2H, t, J=6.8 Hz, OCH_2), 7.17 (1H, d, J=2.6 Hz, aromatic), 7.32 (1H, d, J=2.6 Hz, aromatic). CI-HRMS Calcd for $C_{25}H_{42}Cl_2NOSn$ (MH^+) m/z: 562.1665. Found: 562.1663.

***N*-[3-(2,4-Dichloro-6-iodophenoxy)propyl]-*N*-methyl-2-propynylamine (Iodoclorgyline, 2)** A 0.1 M solution of iodine in chloroform was added at room temperature to a solution of **8** (1.0 g, 1.8 mmol) in chloroform (2 ml) until a pink color remained. The reaction mixture was washed with 10% aqueous sodium thiosulfate solution (10 ml) and then with water (10 ml). The organic phase was then extracted with 1 N HCl solution (7 ml × 3). The combined aqueous layers were made basic with a 5 N NaOH solution and extracted with chloroform (10 ml × 3). The combined organic layers were washed with water (20 ml), dried over sodium sulfate and the solvent was removed *in vacuo* to give free base, which was then converted to hydrochloride salt. Recrystallization from ethanol–ether afforded iodoclorgyline hydrochloride (0.68 g, 88%), mp 177—178 °C. *Anal.* Calcd for $C_{13}H_{15}Cl_3INO$: C, 35.93; H, 3.48; N, 3.22. Found: C, 35.90; H, 3.34; N, 3.17. IR (KBr): 3208, 2934, 2628, 1467, 1442, 1375, 1244 cm^{-1}. ^{1}H-NMR (free base, $CDCl_3$) δ: 2.04 (2H, quintet, J=6.8 Hz, $CH_2C\underline{H}_2CH_2$), 2.23 (1H, t, J=2.3 Hz, C≡CH), 2.35 (3H, s, NCH_3), 2.69 (2H, t, J=6.8 Hz, $CH_2C\underline{H}_2N$), 3.39 (2H, d, J=2.3 Hz, $C\underline{H}_2C{\equiv}CH$), 4.03 (2H, t, J=6.8 Hz, OCH_2), 7.37 (1H, d, J=2.2 Hz, aromatic), 7.66 (1H, d, J=2.2 Hz, aromatic). HRMS Calcd for $C_{13}H_{14}Cl_2INO$ (free base) m/z: 396.9496. Found: 396.9498.

***N*-[3-(2,4-Dichloro-6-[^{125}I]iodophenoxy)propyl]-*N*-methyl-2-propynylamine ([^{125}I]Iodoclorgyline, 3)** Aqueous hydrogen peroxide (10 μl, 30%, w/v) was added to a mixture of **8** (10 μl, 1 mg/ml in ethanol), 0.1 N HCl (0.1 ml), and sodium [^{125}I]iodide (10 μl, 7.4 MBq, specific activity 7.4 TBq/mmol) in a sealed vial. The reaction was allowed to proceed for 30 min at room temperature, after which it was terminated by the addition of sodium bisulfite (0.1 ml, 100 mg/ml in water). The desired product was isolated by HPLC using 0.05 N ammonium formate–methanol (1 : 9, v/v) as an eluent at a flow rate of 3.0 ml/min. The product fractions were collected and the solvent was removed *in vacuo*. The final product, [^{125}I]iodoclorgyline, was taken up in an isotonic saline solution and passed through a 0.22 μm filter. The radiochemical yield was 80%. The radiochemical purity and the specific activity were higher than 99% and about 7.4 TBq/mmol, respectively, determined by HPLC.

Assay of MAO Activity in the Brain and Liver Male Wistar rats (200—250 g) were administered iodoclorgyline (2.3×10^{-6}—4.6×10^{-5} mol/kg, 0.1 ml in water) intraperitoneally. The animals were sacrificed 1 h post injection, and the brain and liver were excised and homogenized. The MAO activity was assayed fluorometrically using kynutramine as a substrate according to the previously reported method.[8]

Biodistribution of [^{125}I]Iodoclorgyline in Mice [^{125}I]Iodoclorgyline (37 kBq in 0.1 ml saline) was injected intravenously into male ddY mice (20—25 g) through a lateral tail vein. At the desired time interval after administration, the animals were sacrificed. Samples of blood and organs of interest were excised, weighed, and the radioactivity was measured with a NaI(Tl) gamma scintillation counter. The results were expressed as percent injected dose per gram of blood or organ.

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