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Radiosynthesis of Bromine-76 and Iodine-123 Labelled Enantiomers of A-69024: Radioligands for Dopamine D1 Receptor Studies Using PET and SPECT

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Summary

The racemic dopamine D1 receptor antagonist A-69024 was labelled with bromine-76 for studies using PET and with iodine-123 for SPECT. [^{76}Br]A-69024 was prepared via electrophilic bromodestannylation with $NH_4{}^{76}Br$. The use of chloramine-T in acid media resulted in radiochemical yields of 70-80%. The racemic radioligand was purified by semi-preparative C-18 reverse-phase HPLC while the (+) and (-) enantiomers were separated and isolated using chiral HPLC. Average specific activities of 11 GBq/μmol were obtained. In an analogous manner, 2-[^{123}I]A-69024 was prepared via electrophilic iododestannylation with $Na^{123}I$ resulting in radiochemical yields of 65-70%. Chiral HPLC gave the (+) and (-) enantiomers with specific activities of >74 GBq/μmol. The chemical and enantiomeric purity of each enantiomer of both radioligands assessed by chiral HPLC was >98%. Radiochemical purities measured by radio-TLC were >98%. The average time of synthesis for both preparations was 70 minutes.

Key Words: A-69024, dopamine D1 receptors, bromine-76, PET, iodine-123, SPECT

Introduction

Changes in dopaminergic neurotransmission have been linked to several neurological disorders, such as schizophrenia, Parkinson's disease and Huntington's disease (1). Based on pharmacological work in the 1970's, dopamine receptors were initially classified into two subtypes, D1 and D2, whereas recent molecular cloning studies have demonstrated that there are at least five

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distinct genes for dopamine receptors (2). Positron Emission Tomography (PET) and Single Photon Emission Computed Tomography (SPECT) studies of dopamine D1 receptors have been limited due to the lack of radioligands that display *in vivo* stability, high affinity and selectivity for D1 receptors.

The benzazepine SCH 23390 [R-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine] was the first high affinity selective dopamine D1 receptor ligand reported (Figure 1). This benzazepine has been labelled with carbon-11 for use as a radioligand in PET studies (3-4). Although SCH 23390 has been the most widely used radioligand for dopamine D1 receptor studies, it has been shown to interact with 5-HT_2 (Ki = 20 nM) and 5-HT_{2C} (Ki = 30 nM) receptors (5-6) as well as having a low striatum-to-cerebellum ratio and rapid metabolism *in vivo*. The iodinated analogue [^{125}I]SCH 23982 [R-(+)-hydroxy-7-iodo-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine] displayed a pharmacological profile matching that of [^{11}C]SCH 23390, indicating similar binding characteristics (7). Other iodinated analogues of SCH 23390 have been introduced for SPECT studies including [^{123}I]FISCH (7-chloro-8-hydroxy-1-[4'-iodophenyl]-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine) and [^{123}I]TISCH (7-chloro-8-hydroxy-1-[3'-iodophenyl]-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine) (8-9). Only initial SPECT studies have so far been performed with these radioligands.

The benzazepine [^{11}C]SCH 39166 [(-)-*trans*-6,7,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-N-methyl-5H-benzo(*d*)naptho-(2,1-*b*)azepine] displayed lower affinity for the 5-HT_2 and 5-HT_{2C} receptors and a slower metabolism than SCH 23390 when compared in initial PET studies (10-11). The first of a new series of benzofuran substituted benzazepines NNC 756, [(+)-8-chloro-5-(2,3-dihydrobenzofuran-7-yl)-7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine], with high affinity and selectivity for D1 receptors has been labelled with carbon-11 and demonstrated a high degree of specific binding in PET studies of monkey and human brain (12-13). The bromine analogue, NNC 22-0010, has also been labelled with carbon-11 and bromine-76 (14).

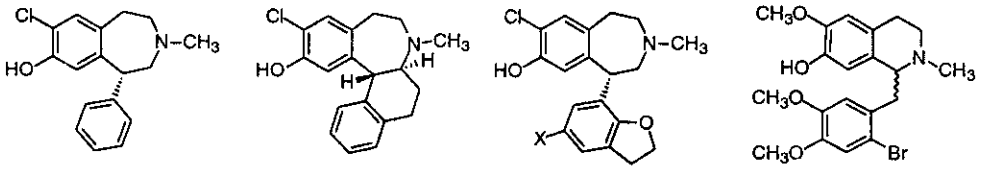


Figure 1. Dopamine D1 receptor ligands

In vitro binding experiments with the racemic non-benzazepine A-69024, [(±)-1-(2-bromo-4,5-dimethoxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline] have demonstrated that it is a selective D1 antagonist with nanomolar affinity (Ki = 12.6 nM) for the D1 receptor and micromolar affinity for D2 and 5-HT_{2C} receptors (15). For use in dopamine D1 receptor studies using PET, A-69024 has been labelled with carbon-11 (16). *In vivo*, [^{11}C]A-69024 was shown to possess the prerequisite pharmacological properties to be considered a potentially useful ligand for PET studies of dopamine D1 receptors (17). Based on these results we have chosen to label A-69024 with the β^+-emitter bromine-76 (16.2 h) and resolve the enantiomers for use in PET studies.

For use in SPECT, the iodo analogue was also labelled with the γ–emitter iodine-123 (13.2 h). A-69024 contains bromine in the 2-position of the phenyl ring thus preparation of the 2-iodo congener is a rational approach in preparation of a SPECT radioligand. In this paper, we report the radiosynthesis, purification, resolution and quality control of the (+) and (-) enantiomers of [^{76}Br]A-69024 and 2-[^{123}I]A-69024.

Materials and Methods

Chemicals were used as purchased without further purification. A-69024, was prepared as previously described (18). ^{1}H NMR spectra were obtained on a Joel FX400 NMR spectrometer. Mass spectra were performed on a VG Quatttro Triple Quadrupole in Electrospray mode in acetonitrile. Bromine-76 was produced by irradiation of arsenic with a beam of 30 MeV [^{3}He] ions (19). The target was cooled and subsequently dissolved in sulphuric acid followed by oxidation with chromic acid. The radioactive bromine was carried over with nitrogen and trapped in ammonia which was evaporated to dryness, reconstituted in water and used as the $NH_4{}^{76}Br$ salt Iodine-123 was produced by the National Medical Cyclotron, Sydney, Australia using the ^{124}Xe(p,2n) reaction and delivered as $Na^{123}I$ in 0.1 M NaOH solution.

Preparation of 2-Iodo-A-69024, [(±)-1-(2-iodo-4,5-dimethoxybenzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline]

The starting material 2-iodo-4,5-dimethoxy phenylacetic acid was prepared by treating 4,5-dimethoxy acetic acid with iodine in the presence of silver trifluoroacetate (20). This was subsequently used in the synthesis of 2-iodo-A-69024 according to methodology previously described (18). Recrystallisation from isopropanol gave 2-iodo-A-69024 as a white solid m.p. 162-164°C. ^{1}H NMR ($CDCl_3$) : δ 2.77 (3H, d J 1.1 Hz, NCH_3), 2.88-3.58 (5H, m) 3.80-3.90 (1H, m), 3.87 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 3.91 (3H, s, OCH_3), 4.40-4.50 (1H, m), 6.25 (1H, s, Ar), 6.65 (1H, s, Ar), 7.05 (1H, s, Ar), 7.30 (1H, s, Ar). Mass spectrum (ES^+) (m/z) 470 (M+1, 2%), 422 (100%). Calc for $C_{20}H_{25}NO_4I$ m/z 470.0828 found m/z 470.0829.

Resolution of A-69024 and 2-Iodo-A-69024

A-69024 (1.0 mg, 2.3 μmol) was dissolved in isopropanol (0.2 mL) followed by the addition of hexane (0.8 mL). The solution was injected onto a semi-preparative chiral HPLC column (Daicel Chiracel OD, 250 mm x 10 mm) and eluted with 85:15 hexane:isopropanol at a flow rate of 3 mL/min. The effluent from the chiral column was monitored with a UV detector at 254 nm. The retention times of A-69024 enantiomers were 17.0 and 22.2 minutes. This procedure was repeated to collected sufficient quantities of each enantiomer. The solvents were rotary evaporated to dryness under reduced pressure. Methanol (0.2 mL) was added to each enantiomer (2.0 mg) resulting in a 1% W/V solution. The specific rotation of each solution was measured in a glass micro cell (3.5 mm x10 mm, ID x l) using a polarimeter (Jasco model DIP-370) at the D line of sodium at 20 °C. Aliquots of each enantiomer was injected separately onto an analytical chiral HPLC column (Daicel Chiracel OD, 250 mm x 4.6 mm) and eluted with a mobile phase consisting of 80:20:0.01 hexane:isopropanol:diethylamine at a flow rate of 1 mL/min to determine enantiomeric purity.

2-Iodo-A-69024 was resolved using the same method as above. Using the same semi-preparative HPLC conditions the retention times of each enantiomer were 20.2 and 23.2 minutes.

Preparation of 2-(tributylstannyl)A-69024, [(±)-1-(2-tributylstannyl-4,5-dimethoxy-benzyl)-7-hydroxy-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline]

To a solution of A-69024 (0.47 g, 1.1 mmol) in freshly distilled THF (15 mL) at -78 °C under nitrogen was added a solution of butyllithium in hexanes (1.2 mL, 2.0 M, 2.4 mmol). After 15 minutes of stirring the reaction mixture was treated with a solution of tributyltin chloride (0.33 mL, 1.2 mmol) in THF (5 mL) and the reaction mixture was stirred for 40 minutes with warming to -10 °C. The reaction mixture was quenched with saturated ammonium chloride and extracted with chloroform (3 x 40 mL). The combined organic layers were washed with water, dried over sodium sulphate, and evaporated to give a light brown oil. Purification by flash chromatography (ethyl acetate:petroleum spirit, 40:60) yielded 2-(tributylstannyl)A-69024 as a pale yellow oil (0.29 g, 47%). ^{1}H NMR ($CDCl_3$) : δ 0.81-1.65 (27H, m, $SnBu_3$), 2.49 (3H, s), 2.50-3.58 (6H, m), 3.75 (1H, m), 3.80 (3H, s), 3.84 (3H, s), 3.87 (3H, s), 6.11 (1H, s), 6.55 (1H, s), 6.81 (1H, s), 6.83 (1H, s). Mass spectrum (ES^+) (m/z) 633 (M+1, 2%), 628 (6), 626 (8), 624 (7), 623 (4), 346 (16), 345 (100), 341 (7), 193 (7).

Synthesis, Purification and Separation of (+) and (-)[^{76}Br]A-69024

A solution of no-carrier-added $NH_4{}^{76}Br$ (200 MBq) in water (0.1 mL) was added to a vial containing 2-(tributylstannyl)A-69024 (0.5 mg, 0.8 μmol) dissolved in methanol (20 μL). Thereafter, freshly prepared solution of chloramine-T (200 μg, 0.9 μmol) in HCl (0.1 M, 0.1 mL) was added and the reaction mixture was allowed to stand at room temperature for 5 minutes. The reaction was quenched with an aqueous solution of $Na_2S_2O_5$ (5.5 μmol, 0.1 mL) followed by 0.5 mL of HPLC solvent consisting of 50:50 acetonitrile: 0.01M H_3PO_4 pH adjusted to 7.2 with TEA. The mixture was injected onto a μ–Bondapak C-18 (10 μm, 300 mm x 7.8 mm) semi-preparative column and eluted at a flow rate of 3 mL/min using a Waters 450 pump. The effluent from the column was monitored with a UV detector (254 nm, Waters 440) and an in-line Geiger-Muller radioactivity detector. The radioactivity peak corresponding to [^{76}Br]A-69024 (t_R = 12 min) was collected and rotary evaporated to dryness. The radiochemical yield of [^{76}Br]A-69024 was 70-80%.

The racemic mixture of [^{76}Br]A-69024 was dissolved in ethanol (50 μL) followed by the addition of water (0.5 mL) and loaded onto a Sep-Pak (C-18 Waters, preloaded with 5 mL methanol and 5 mL water), washed with water (5 mL), eluted with ethanol (3 mL) and azeotroped with hexane (1 mL x 3). The residue was dissolved in isopropanol (0.1 mL) followed by the addition of hexane (0.4 mL). The solution containing the (+) and (-) enantiomers of [^{76}Br]A-69024 was injected onto a analytical chiral HPLC column (Daicel Chiracel OD, 250 mm x 4.6 mm), with a mobile phase consisting of 80:20:0.01 hexane:isopropanol:diethylamine and monitored using the UV and radioactivity detectors as above. Using a flow rate of 1 mL/min, the radioactive peaks corresponding to authentic (+)A-69024 (t_R = 13 min), and (-)A-69024 (t_R = 19 min) were collected separately and total radioactivity measured. Aliquots of the solutions were reinjected onto the chiral HPLC column to

assess the chemical and enantiomeric purities and specific activities. Chemical and enantiomeric purities were >98% with specific activities of 11 GBq/μmol.

The solvents were evaporated and the residues were dissolved in sterile normal saline and filtered through sterile 0.22 μm filters (Millex FG) into sterile pyrogen free evacuated vials. Radiochemical purity of each enantiomer assessed by radio-TLC, silica gel plates (90:5:0.1 CH_3Cl:CH_3OH:TEA $R_f = 0.50$) was >98%.

Synthesis, Purification and Separation of (+) and (-)2-[^{123}I]A-69024

The radioiodinated analogue of A-69024 was prepared as described above with the following modifications. No-carrier-added Na^{123}I (740 MBq, 50 μL in 0.1 M NaOH) was added to a vial containing 2-(tributylstannyl)A-69024 (0.5 mg, 0.8 μmol) dissolved in methanol (20 μL). Chloramine-T (100 μg, 0.45 μmol) in HCl (0.1 M, 0.1 mL) was added and the reaction mixture was allowed to stand at room temperature for 5 minutes. Following quenching of the reaction and addition of the same HPLC mobile phase, the mixture was injected onto a Goldpak C-18 (Activon, 10 μm, 250 mm x 10 mm) semi-preparative column and radioactivity was monitored using a NaI(Tl) crystal. At a flow rate of 3 mL/min, the radioactivity peak corresponding to 2-[^{123}I]A-69024 (t_R = 19 min) was collected. The radiochemical yield of 2-[^{123}I]A-69024 was 65-70%.

The racemic mixture of 2-[^{123}I]A-69024 was resolved using the same chiral HPLC conditions as the radiobrominated compound. The (+) and (-) enantiomers of 2-[^{123}I]A-69024 were collected at 13 and 19 minutes, respectively. Enantiomeric purities were >98% with specific activities of >74 GBq/μmol. Radiochemical purities were >98%.

Results and Discussion

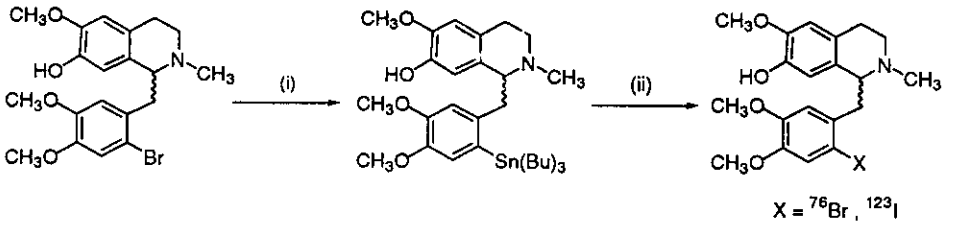
Semi-preparative chiral HPLC was used to separate the enantiomers of the racemic ligands, A-69024 and 2-iodo-A-69024. Using this chromatographic method, sufficient quantities of enantiomers from each ligand were made available for determination of specific rotation. The enantiomeric purity of each isolated enantiomer as measured by analytical chiral HPLC was >98%. The semi-preparative chiral HPLC retention time and the corresponding specific rotation of the four enantiomers are shown in Table 1. Absolute configurations could not be assigned to each of the four enantiomers since they have not been determined.

Ligand	Retention time (minutes)	Specific rotation $[\alpha]_D^{20}$ (c = 1, CH_3OH)
A-69024	17.0	+29.5°
	22.2	-43.0°
2-iodo-A-69024	20.2	+88.0°
	23.2	-99.0°

Table 1.

The racemates [^{76}Br]A-69024 and 2-[^{123}I]A-69024 were prepared by electrophilic halogeno-destannylation using $NH_4{}^{76}Br$ and Na^{123}I respectively. A stannane precursor was chosen for

radiosynthesis of both radioligands as it allows rapid regioselective labelling of compounds. 2-(Tributylstannyl)A-69024 was prepared as shown in Scheme 1. Chloramine-T in acidic media was the optimum oxidising agent, which gave the most reproducible and highest radiochemical yields (Scheme 1). Reverse-phase semi-preparative HPLC was used to purify and isolate the two radioligands. The average radiochemical yield for both [^{76}Br]A-69024 and 2-[^{123}I]A-69024 was 70%.



Scheme 1. Synthesis and radiolabelling of 2-(tributylstannyl)A-69024. (i) n-BuLi / -78°C, $(Bu)_3SnCl$ (ii) CAT / 0.1M HCl, $NH_4^{76}Br$ or $Na^{123}I$, RP-HPLC.

Using the racemic tributylstannane precursor, both (+) and (-) radiolabelled enantiomers of the brominated and iodinated ligands are made available in one synthesis following chiral resolution. Before the purified racemates were resolved, they were passed through a solid phase C18 extraction cartridge to remove salts and water from the reverse phase HPLC buffer. The (+) and (-) enantiomers of the racemic ligands, [^{76}Br]A-69024 and of 2-[^{123}I]A-69024, were then separated using an analytical chiral HPLC column. An alternate method for preparing enantiomerically pure radioligands could be accomplished using enantiomerically pure precursor. The resolution of the tributylstannane compound using chiral HPLC was not attempted.

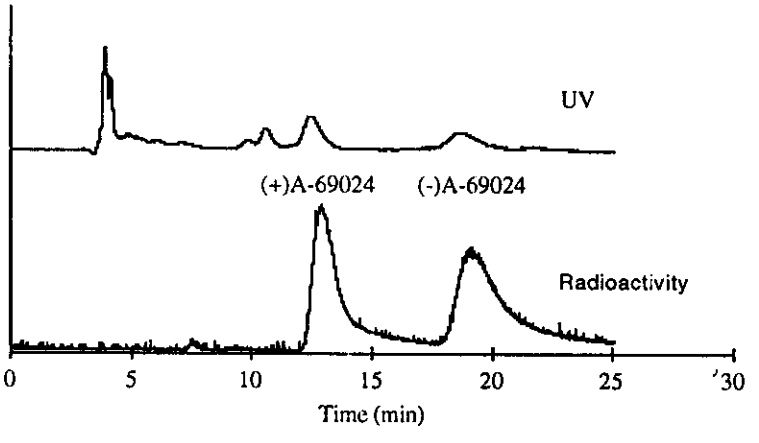


Figure 2. Chiral HPLC chromatogram of [^{76}Br]A-69024

For the (+) and (-) enantiomers of each radioligand, the chemical and enantiomeric purity assessed by chiral HPLC was found to be >98%. The resolution of [^{76}Br]A-69024 using the analytical chiral HPLC column is illustrated in Figure 2. The (+) and (-) enantiomers eluted at 13 and 19 minutes respectively, and displayed good separation and baseline resolution. The specific activity of the (+) and (-) enantiomers of [^{76}Br]A-69024 was 11 GBq/μmol, and >74 GBq/μmol for 2-[^{123}I]A-69024. Radiochemical purities assessed by radio-TLC were >98% for each enantiomer. The total time of preparation for both radioligands was 70 minutes.

In vitro binding experiments have demonstrated that the (+) enantiomers of [^{76}Br]A-69024 and for 2-[^{123}I]A-69024 bind dopamine D1 receptors with high affinity (Kd = 0.6 nM and 1.3 nM respectively) while the (-) enantiomers are devoid of D1 affinity. Full details of *in vitro* and *in vivo* evaluation of all enantiomers will be published elsewhere.

Conclusion

The racemates [^{76}Br]A-69024 and 2-[^{123}I]A-69024 were prepared by bromination and iodination, respectively, via electrophilic halogenodestannylation of 2-(tributylstannyl)A-69024 with no carrier added radiohalogens. The (+) and (-) enantiomers of [^{76}Br]A-69024 and of 2-[^{123}I]A-69024 were separated using chiral HPLC. The radiosyntheses produced radioligands of high enantiomeric, chemical, and radiochemical purity. These radioligands can be routinely produced in sufficient quantities for *in vitro* pharmacological characterisation and for *in vivo* studies of dopamine D1 receptors in the brain using PET and SPECT.

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