

## Research Article

# Synthesis of 3-[[4-(4-[<sup>18</sup>F]fluorophenyl)piperazin-1-yl]methyl]-1*H*-pyrrolo[2,3-*b*]pyridine

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## Summary

3-[[4-(4-[<sup>18</sup>F]fluorophenyl)piperazin-1-yl]methyl]-1*H*-pyrrolo[2,3-*b*]pyridine, a candidate to image dopamine D<sub>4</sub> receptors, was synthesised via electrophilic fluorination of a trimethylstannyl precursor with high specific radioactivity [<sup>18</sup>F]F<sub>2</sub>. The precursor was obtained by a facile four-step synthetic approach; the trimethylstannyl leaving group was introduced by displacement of iodine utilising palladium catalysis and hexamethyldistannane in an inert solvent. The total radiosynthesis time was 50 min, including purification and formulation for injection. Decay corrected radiochemical yield was <1% as calculated from the amount of [<sup>18</sup>F]F<sup>−</sup> produced. Specific radioactivity at the end of synthesis was 12.8–16.4 GBq/μmol. Radiochemical purity was 88–92%. *Ex vivo* studies in rats showed homogeneous distribution of radioactivity within rat brain. Copyright © 2002 John Wiley & Sons, Ltd.

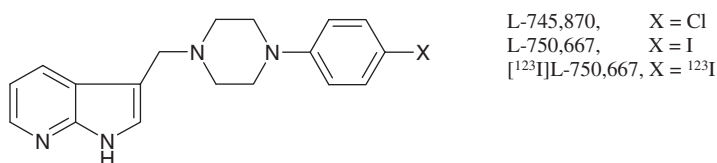
**Key Words:** <sup>18</sup>F; schizophrenia; dopamine D<sub>4</sub> receptor; stannylation; electrophilic fluorination

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## Introduction

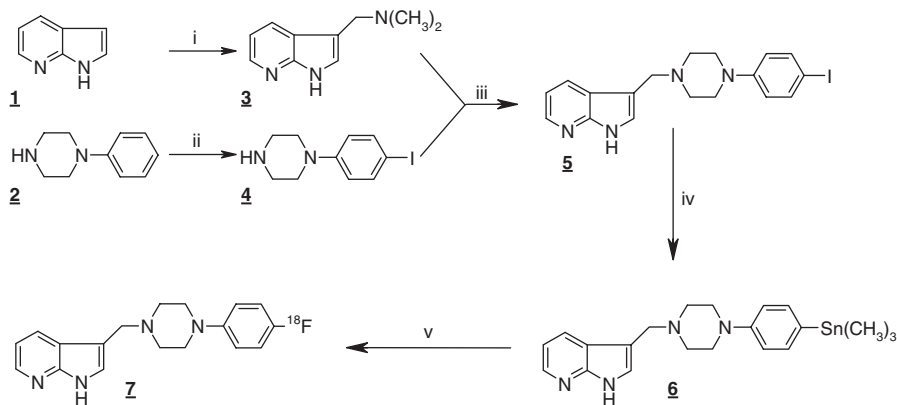
Schizophrenia is a complex psychotic disorder whose origin and etiology as yet are not fully understood. An association between the dopamine neuronal hyperactivity and schizophrenia has been studied intensively over the past few years. An increased concentration of dopamine D<sub>4</sub> receptors has been demonstrated in the post-mortem brains of schizophrenic patients,<sup>1</sup> even though these findings are controversial.<sup>2</sup> It is evident that selective ligands with a high affinity to dopamine D<sub>4</sub> receptors have to be developed in order to measure the dopamine D<sub>4</sub> receptor density in brain and to highlight their pharmacological role in schizophrenia.

Several ligands of different chemical classes have been reported to bind selectively and with high affinity to the dopamine D<sub>4</sub> receptor. A few of these ligands contain arylpiperazine<sup>3–14</sup> or azaindole moieties.<sup>15–17</sup> L-745,870 (Figure 1) is a structural combination of the



**Figure 1. L-745, 870 and L-750, 667: Selective dopamine D<sub>4</sub> receptor antagonists**

1*H*-pyrrolo[2,3-*b*]pyridine (7-azaindole) unit and 4-chlorophenylpiperazine unit linked together with a methylene spacer.<sup>18</sup> L-745,870 is a selective (>2000-fold as compared to other dopamine receptor subtypes) antagonist with high affinity to the D<sub>4</sub> receptor ( $K_i = 0.43$  nM). The 4-iodophenyl compound L-750,667<sup>19</sup> (Figure 1) has similar selectivity but slightly lower affinity ( $K_i = 0.51$  nM) than L-745,870. L-750,667 has also been radioiodinated with <sup>123</sup>I.<sup>20</sup> As a candidate to image dopamine D<sub>4</sub> receptors, we have synthesised molecule **7**, 3-[[4-(4-[<sup>18</sup>F]fluorophenyl)piperazin-1-yl]methyl]-1*H*-pyrrolo[2,3-*b*]pyridine ([<sup>18</sup>F]F5P) (Figure 2), a fluorinated analogue of the molecules L-745,870 and L-750,667. Since molecule **7** contains an <sup>18</sup>F isotope, a positron emitting nucleus, it can be used as a radiotracer with positron emission tomography (PET).



**Figure 2.** Synthesis of the stannylated precursor **6** and the radioligand [ $^{18}\text{F}$ ]F5P **7**: (i) dimethylammoniumchloride, paraformaldehyde, butanol,  $\Delta$ ; (ii)  $\text{ICl}$ ,  $\text{AcOH}/\text{H}_2\text{O}$  (**3**/**1**),  $60^\circ\text{C}$ ; (iii) xylene,  $\Delta$ ; (iv)  $\text{Sn}_2(\text{CH}_3)_6$ ,  $(\text{PPh}_3)_4\text{Pd}(0)$ , dioxane,  $\Delta$ ; and (v)  $\text{CCl}_3\text{F}$ ,  $\text{AcOH}$ , [ $^{18}\text{F}$ ]F $_2$ .

## Results and discussion

### Precursor synthesis

Figure 2 illustrates the synthesis of precursor **6** containing a trimethylstannyl functional group, a good leaving group that can be displaced with [ $^{18}\text{F}$ ]F $_2$ . The precursor synthesis started with the preparations of the building blocks **3** and **4**. 3-*N,N*-dimethylamino-methyl-1H-pyrrolo[2,3-*b*]pyridine **3** was prepared from commercially available 7-azaindole **1** according to a literature method.<sup>21</sup> Successive crystallisations from diethylether afforded compound **3** (mp  $154^\circ\text{C}$ ). The other building block, 1-(4-iodophenyl)piperazine **4**, was synthesised by direct iodination of phenylpiperazine **2** with iodine monochloride, as described by Hanson *et al.*<sup>22</sup> With this method it was possible to introduce the iodine in a fairly selective manner to the *para*-position of the phenyl ring using a mild reaction medium and a short reaction time. Furthermore, starting materials and side products were easily removed by several recrystallisations from ethanol; no signals of *ortho*- or *meta*-iodinated side products were detected from  $^1\text{H}$  NMR spectrum of the purified compound **4** (mp  $121^\circ\text{C}$ ).

Synthesis of the iodo-compound **5**, the coupling reaction of the building blocks **3** and **4**, was performed according to the method

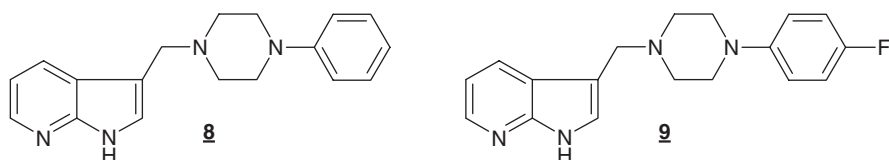
originally described by Kulagowski *et al.*<sup>23</sup> The reaction was tested in dry toluene and dry xylene at reflux. Of these two, dry xylene turned out to be a more suitable solvent for the reaction, resulting in 56% yield in comparison to the ~20% yield in dry toluene. Compound **5** (mp 179°C, accurate mass with HR-EI-MS: calc. for C<sub>18</sub>H<sub>19</sub>IN<sub>4</sub> 418.065449, found 418.065600) was purified by successive crystallisations from absolute ethanol. The chemical purity of **5** exceeded 97%, as determined with analytical HPLC (see experimental section). Recently, Staley *et al.* reported a feasible synthesis of compound **5** by Mannich reaction between 7-azaindole **1** and 4-iodophenylpiperazine **4**.<sup>20</sup> The yield of compound **5** synthesised with their method was 92%.

Organostannane **6**, the precursor for labelling, was synthesised from the iodo-derivative **5** utilising palladium catalysis and hexa-alkyldistannane, a method widely used for the preparation of functionally substituted organotin compounds.<sup>24</sup> After a 3 h reflux under an inert argon atmosphere the reaction usually proceeded to completion, as monitored by TLC, and compound **6** was purified by preparative column chromatography. However, if unreacted iodo-derivative **5** was present in the crude product, the separation of **5** and **6** with preparative column chromatography was not satisfactory. In these cases, the product was further purified with reversed-phase HPLC [ $\mu$ Bp C18 column (7.8  $\times$  300 mm), MeOH/H<sub>2</sub>O 8/2]. The traces of compound **5** were thus separated and the chemical purity of **6**, checked with analytical HPLC, exceeded 98%. Furthermore, no signals (*m/z* 418) of the iodine derivative **5** were detected from the mass spectrum of compound **6**.

The reference compound **9** was prepared from 7-azagamine **3** and commercially available 4-fluorophenylpiperazine. The synthesis resembled that of the iodo-compound **5** resulting in a 51% yield. The chemical purity of **9**, determined with analytical HPLC, exceeded 99.5%.

### Radiolabelling

Radioligand **7**, [<sup>18</sup>F]F5P, was synthesised by electrophilic fluorodestannylation of **6** (see Figure 2). The average synthesis time was 50 min. The electrophilic labelling agent [<sup>18</sup>F]F<sub>2</sub>, produced from [<sup>18</sup>F]F<sup>−</sup> according to literature methods,<sup>25,26</sup> was bubbled at room temperature through a CCl<sub>3</sub>F/CH<sub>3</sub>COOH mixture containing precursor **6**. The major chemical impurity that was formed using the method in question was found to be the phenylpiperazine analogue **8** (Figure 3). Reversed-phase HPLC was



**Figure 3.** Structural formulae of compounds **8**, the major chemical impurity generated in labelling synthesis, and **9**, the reference compound

used to separate this and other side products from compound **7**. The radiofluorination proceeded at relatively low radiochemical yield, in average  $0.7 \pm 0.1\%$  (decay corrected to the end of bombardment) as calculated from the amount of [ $^{18}\text{F}$ ]F $^-$  produced. This was due to the formation of several radiofluorinated side products, more polar (shorter retention time in the reversed-phase HPLC column) than compound **7**. Most likely these were a result of fragmentation, a phenomenon common with highly reactive F $_2$ . Furthermore, the pyrrolic nitrogen of precursor **6** was unprotected, which undoubtedly increased the amount of radiolabelled side products. Since the absolute yield of **7**, in average  $183 \pm 32$  MBq at the end of synthesis, was high enough to perform the preclinical animal studies, no attempts were made to optimise the labelling conditions.

HPLC analysis showed the presence of an unidentified  $^{18}\text{F}$ -labelled contaminant in the formulated final solution of **7**. This contaminant, eluting as a bulky broad peak from the semi-preparative HPLC column before compound **7**, decreased the radiochemical purity of **7**, which was in average  $90.3 \pm 1.7\%$ . The chemical purity, however, exceeded 95%, with respect to the phenylpiperazine analogue **8**. The specific radioactivity of **7** (at the end of synthesis) was in average  $14.6 \pm 1.8$  GBq/ $\mu\text{mol}$ .

### *Animal studies*

In peripheral tissues in mice a high level of expression of the D $_4$  mRNA has been detected in the eye, adrenal gland and testis.<sup>27</sup> In our biodistribution studies in peripheral organs compound **7** showed high uptake (%ID/g) in adrenal gland (7.2), pancreas (2.0), lung (2.0) and liver (1.1) as well as in urine (5.2) at 5 min *post-injection*. The uptake in liver tended to increase as a function of time.

In rat brain the dopamine D<sub>4</sub> receptors are mainly localised in entorhinal cortex, hippocampus and hypothalamus.<sup>28</sup> As measured with digital autoradiography, the radioactivity uptake in brain at 5 min was about 1%ID/g in cortex, hippocampus, thalamus and striatum as well as in various other brain regions, including cerebellum. At 2 h *post-injection* the radioactivity was uniformly distributed in whole brain.

Based on these results it was concluded the **7** is not a suitable compound for visualising the D<sub>4</sub> receptor *in vivo* in rat. Even though the compound showed relatively high uptake in brain, the radioactivity distribution in brain was uniform and not markedly concentrated into known dopamine D<sub>4</sub>-rich areas. Recently, Staley *et al.* reported the synthesis of [<sup>123</sup>I]L-750,667, the radioiodinated analogue of compound **7**.<sup>20</sup> Their conclusion was that [<sup>123</sup>I]L-750,667 was not suited as a radiotracer for the *in vivo* imaging of the D<sub>4</sub> receptor but, however, the ligand is selective for D<sub>4</sub> receptor *in vitro*. The specific radioactivity of tracer **7** was 12.8–16.4 GBq/μmol. However, the D<sub>4</sub> receptor density in rodent brain is low, in the order of 30 fmol/mg protein.<sup>28</sup> Thus the possibility that the amount of tracer mass (~1 μg/kg per rat) injected in this study saturated the receptor binding sites cannot be excluded.

The metabolism of the tracer was not studied, although the increasing uptake in liver as a function of time does indicate metabolic degradation of the tracer. Unchanged uptake in bone as a function of time indicated that the molecule **7**, or its metabolites were not defluorinated to any larger extent.

The number of animals in this study was kept small. This was warranted as early results demonstrated that the tracer was clearly less than optimal as a dopamine D<sub>4</sub> tracer.

## Experimental

### *General methods*

All the chemicals used in this study were bought, unless otherwise noted, from commercial suppliers. NMR spectra were recorded on JEOL JNM-GX400 spectrometer with chemical shifts reported in δ values (parts per million) relative to tetramethylsilane (δ = 0). Melting points (mp) were detected with Mettler FP80 apparatus equipped with a Mettler FP81 MBC cell and they are uncorrected. High-resolution mass spectrometry was done with VG ZabSpec spectrometer operated in

electron ionisation (EI) mode. Merck silica gel 60 (230–400 mesh ASTM) was used for preparative column chromatography separations. Thin layer chromatography was done with Merck TLC aluminium sheets (silica gel 60 F<sub>254</sub>). A Merck Hitachi L-6200 pump was used for semi-preparative HPLC. The semi-preparative column was Waters  $\mu$ Bondapak C18 HPLC column (7.8  $\times$  300 mm, 10  $\mu$ m) connected in series with a Merck Hitachi L-4000A UV absorption detector ( $\lambda$  = 280 nm) and a 2  $\times$  2" sodium iodide crystal for radioactivity detection. The chromatograms were registered with a Goerz Metra Watt SE 120 two-channel recorder. The column was eluted isocratically with 0.1 M ammonium formate solution for 2 min with a flow rate of 4 ml/min and then isocratically with a mobile phase of 0.1 M ammonium formate/MeOH (45:55) maintaining the same flow rate.

A Merck Hitachi L-6200 pump was used for analytical HPLC. A Waters  $\mu$ Bondapak C18 HPLC column (3.9  $\times$  300 mm, 10  $\mu$ m) was eluted isocratically (flow rate 1.5 ml/min) using 0.1 M ammonium formate/MeOH (40:60) as a mobile phase. The column was connected in series with a Shimadzu (model SPD-M6A) UV/VIS photodiode array detector ( $\lambda$  = 210–250 nm), 2  $\times$  2" NaI crystal radioactivity detector and Waters 740 integrator. This analytical HPLC set-up ( $\lambda$  = 254 nm) was also used in determination of the chemical purities of the compounds **5** ( $R_t$  12.3 min), **6** ( $R_t$  33 min) and **9** ( $R_t$  5.6 min). Radioactivity was measured with a radioisotope calibrator (CRC-15R, Capintec Inc., Pittsburgh, USA).

Radiochemical yields were calculated based on the amount of  $^{18}\text{F}$  produced at the end of bombardment. Determinations of chemical and radiochemical purity and specific radioactivity were done by comparisons of HPLC retention times and peak intensities with the reference compound **9** (Figure 3) of known concentration.

### Chemistry

Compounds **3**,<sup>21</sup> **4**<sup>22</sup> and **5**<sup>23</sup> were prepared as described in the literature.

3-[[4-(4-trimethylstannylphenyl)piperazin-1-yl]methyl]-1H-pyrrolo[2,3-b]pyridine **6**. The iodo-derivative **5** (1.0 g, 2.39 mmol) was refluxed with hexamethylditin (1.3 g, 4.0 mmol) and tetrakis(triphenylphosphine)palladium(0) in dry dioxane (40 ml) under argon atmosphere for 3 h. The mixture was allowed to cool down and the solvent was removed by a rotary evaporator. The crude product was purified by column

chromatography ( $\text{CH}_2\text{Cl}_2\text{:EtOAc:Et}_3\text{N}$  40:60:10;  $R_f$  0.12). This afforded the stannyl derivative **6** (620 mg, 57%) as a white solid (mp  $180^\circ\text{C}$ ).  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) 10.8 (1 H; s), 8.33 (1 H; d;  $J=6\text{ Hz}$ ), 8.11 (1 H; d;  $J=9\text{ Hz}$ ), 7.36 (2 H; d;  $J=9\text{ Hz}$ ), 7.33 (1 H; s), 7.10 (1 H; dd;  $J=4.5$  and  $12.5\text{ Hz}$ ), 6.90 (2 H; d;  $J=9\text{ Hz}$ ), 3.77 (2 H; s), 3.21 (4 H; m; piperazinyl  $\text{CH}_2$ ), 2.66 (4 H; m; piperazinyl  $\text{CH}_2$ ), 0.28 (9 H; s;  $\text{Sn}(\text{CH}_3)_3$ ). Accurate mass with HR-EI-MS: calculated for  $\text{C}_{21}\text{H}_{28}\text{N}_4\text{Sn}$  454.133004, found 454.133800.

3-[[4-(4- $^{18}\text{F}$ ]fluorophenyl)piperazin-1-yl]methyl]-1H-pyrrolo[2,3-b]pyridine **7**. The stannylated precursor **6** was radiolabelled with electrophilic  $^{18}\text{F}\text{F}_2$  gas according to methods discussed in detail in the literature.<sup>25,26</sup> Three hundred micrograms ( $0.66\text{ }\mu\text{mol}$ ) of **6** was dissolved in a solution containing freon-11 and dry acetic acid.  $^{18}\text{F}\text{F}_2$  gas (specific radioactivity  $\sim 55\text{ GBq}/\mu\text{mol}$  at EOB) was bubbled through the reaction mixture. Freon-11 was evaporated and the residue was dissolved in  $0.1\text{ M HCO}_2\text{NH}_4$  solution, which was injected on the semi-preparative HPLC column. The fraction containing compound **7**, eluting at  $\approx 18\text{ min}$ , was collected and the radioactivity was measured. This fraction was then evaporated to dryness and the dry residue was dissolved in  $0.9\%$  NaCl solution (pH 4.7). This solution was used for the *in vivo* studies with rats.

3-[[4-(4-fluorophenyl)piperazin-1-yl]methyl]-1H-pyrrolo[2,3-b]pyridine **9**. A solution of **3** ( $0.5\text{ g}$ ,  $2.8\text{ mmol}$ ) and 1-(4-fluorophenyl)piperazine ( $0.51\text{ g}$ ,  $2.8\text{ mmol}$ ) was refluxed in dry xylene in argon atmosphere for 7 h. The reaction mixture was allowed to cool down and the solids were separated by filtration and washed with ethanol. Three recrystallisations from ethanol afforded compound **9** ( $440\text{ mg}$ ,  $51\%$ ) as a light white powder (mp  $211^\circ\text{C}$ ).  $^1\text{H}$  NMR (DMSO) 8.18 (1 H; d;  $J=6\text{ Hz}$ ), 8.03 (1 H; d;  $J=9\text{ Hz}$ ), 7.38 (1 H; s), 6.98–7.05 (3 H; m), 6.90 (2 H; m), 3.66 (2 H; s), 3.03 (4 H; m; piperazinyl  $\text{CH}_2$ ), 2.49 (4 H; m; piperazinyl  $\text{CH}_2$ ). Accurate mass with HR-EI-MS: calculated for  $\text{C}_{18}\text{H}_{19}\text{FN}_4$  310.159375, found 310.159600.

### Animal studies

The biodistribution of compound **7** was determined in male Sprague-Dawley (Hsd:SD) rats (Harlan Sprague-Dawley, IN, USA). About  $22\text{ MBq}$  (about  $0.5\text{ }\mu\text{g}$ ) of compound **7** was injected intravenously



through the tail vein. After specific times (5, 15, 30 and 120 min) the rats were sacrificed in a  $\text{CO}_2$  chamber. Blood, urine and organs to be examined were rapidly removed, measured for radioactivity and weighed. The results are expressed as % injected dose per gram tissue (%ID/g). The brains were frozen and sliced with a cryomicrotome. Coronal sections (20  $\mu\text{m}$ ) were cut and thaw-mounted on microscopic slides. Brain sections were placed in an exposure cassette with imaging plate (BAS-TR2025, Fuji Photo Film Co., Japan) and exposed for 4 h. The spatial distribution of radioactivity in the brain sections was recorded with a phosphoimaging device (Fujifilm BAS-5000, Fuji Photo Film Co Ltd, Japan). Brain sections were then stained with haematoxylin/eosin and the brain regions were identified from stained sections using a rat brain atlas.<sup>29</sup>

## Conclusion

[ $^{18}\text{F}$ ]F5P **7** was synthesised from trimethylstannyl precursor **6** by electrophilic destannylation with high specific radioactivity [ $^{18}\text{F}$ ]F<sub>2</sub>. Radiochemical yields of **7** were low and the specific radioactivity was moderately high. *Ex vivo* biodistribution studies showed homogeneous distribution of radioactivity within rat brain. Thus, we conclude that **7** is probably not a suitable radiotracer to image dopamine D<sub>4</sub> receptors.

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