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SYNTHESIS OF [^{11}C]LU 29-066, A 5-HT$_2$ RECEPTOR ANTAGONIST

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SUMMARY

Lu 29-066 (1-(2-{4-[2,5-dimethyl-3-(4-fluorophenyl)-1*H*-indol-1-yl]piperidin-1-yl}ethyl)-2-imidazolidinone), a selective antagonist of serotoninergic 5-HT$_2$ receptors, was labelled with carbon-11. The synthesis of the non-radioactive precursor and its reaction with [^{11}C]phosgene affording [^{11}C]Lu 29-066 are described. Approximately 1.5 to 2.2 GBq (40-60 mCi) of the radioligand were obtained with a specific activity ranging from 11 to 18.5 GBq/μmol (300-500 mCi/μmol) and analytical HPLC showed a radiochemical purity over 99%.

Key words: carbon-11, 5-HT$_2$ antagonist.

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INTRODUCTION

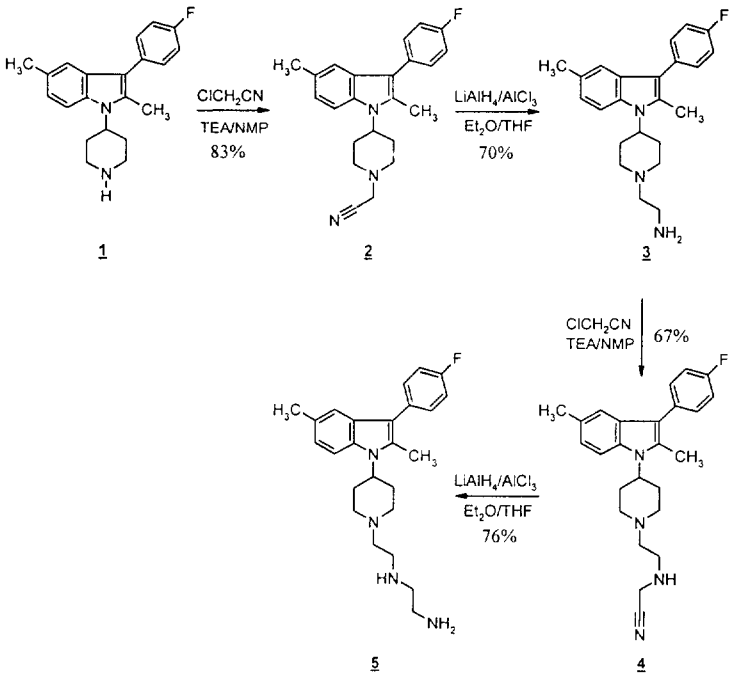
By molecular cloning techniques, up to 15 distinct serotonin receptors have been identified to date. Among them, 5-HT_2 receptors have been the object of intense research since they are supposed to be involved in neuropsychiatric disorders such as anxiety, depression (1), Alzheimer's disease (2) and schizophrenia (3), and in the action of many antipsychotic (4) and antidepressant (5) agents.

By its capacity to measure the distribution and concentration of neurotransmitter receptors *in vivo*, Positron Emission Tomography (PET) may be a useful tool for psychopathological research. To achieve this goal, effective radioligands must be available.

A selective and potent 5-HT_2 antagonist Lu 29-066 (1-(2-{4-[2,5-dimethyl-3-(4-fluorophenyl)-1*H*-indol-1-yl]piperidin-1-yl}ethyl)-2-imidazolidinone) exhibiting a high affinity (IC_{50}=3.4-15 nM) and selectivity versus both dopamine D_2 (IC_{50}=6900 nM) and α_1-adrenergic (IC_{50}=2300 nM) receptors *in vitro*, has been described (6,7). This compound presents a chemical structure compatible with carbon-11 labelling, which makes it a potentially interesting candidate for PET studies. In this paper, we describe the synthesis of the required non-radioactive precursor **5** and its radiochemical conversion into [^{11}C]Lu 29-066 using [^{11}C]phosgene.

RESULTS AND DISCUSSION

Due to the short half-life of carbon-11 (20.4 min), the most practical site to label Lu 29-066 is the carbonyl function of the imidazolidinone group using [^{11}C]phosgene. For this purpose, the synthesis of the precursor **5** from **1** was required (Scheme 1).

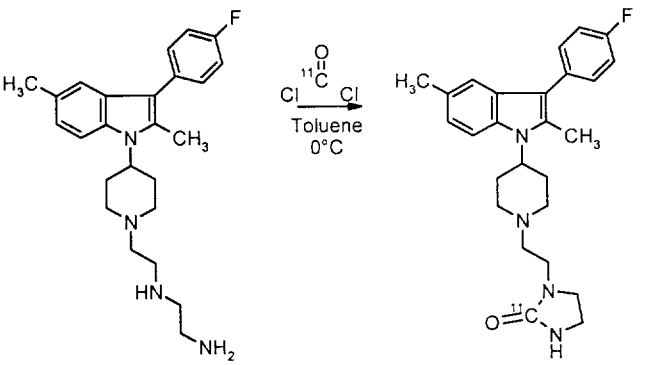


Scheme 1: Synthesis of the precursor 5

The starting material **1** was obtained, in 53% yield by a five-step synthesis, via the Fischer indole synthesis as described in a recent paper (8). The introduction of the amino side chain was achieved by two successive alkylation - reduction steps affording the compound **5**. Alkylation with chloroacetonitrile was most conveniently performed in N-methyl-2-pyrrolidinone (NMP) and triethylamine (TEA) to avoid precipitation of the starting piperidine. The cyano group was reduced with AlH_3, formed in situ from $LiAlH_4$ by addition of $AlCl_3$ (9). The compound **5** was obtained with an overall yield of 30%.

The radiochemical synthesis of [^{11}C]Lu 29-066 was realised through cyclisation reaction of the amino derivative **5** and [^{11}C]phosgene (Scheme 2). No carrier-added (n.c.a) [^{11}C]phosgene was synthesised from cyclotron-produced [^{11}C]methane, according to the method described by Landais *et al.* (10) and improved by the modification recently reported by Link *et al.* (11). The [^{11}C]methane previously mixed with chlorine was passed into an empty quartz tube at 560°C

resulting in the formation of [^{11}C]carbon tetrachloride. [^{11}C]Phosgene was produced by passing the latter through a second tube filled with iron chips at 320°C. The precursor **5** reacted smoothly in an immediate condensation with [^{11}C]phosgene in toluene, at 0°C. Finally, after purification and formulation in an injectable solution, 1.5-2.2 GBq (40-60 mCi) of [^{11}C]Lu 29-066 was obtained within 45-50 min of the end of bombardment with a specific activity ranging from 11 to 18.5 GBq/μmol (300-500 mCi/μmol). The quality control analyses (radio-TLC and analytical HPLC) showed high radiochemical purity (>99%).



5 **[^{11}C]Lu 29-066**

Scheme 2: Radiosynthesis

In order to determine the lipophilicity of the compound, the octanol-water partition coefficient was measured according to the literature method (12). The experimental values of $logP_{7,4}$ obtained (2.23±0.12 (n=8)) demonstrated that the lipophilicity of [^{11}C]Lu 29-066 was moderate.

EXPERIMENTAL

Materials and Methods

Lu 29-066 was provided by H. Lundbeck A/S, Denmark. Reagents were purchased from Aldrich Chemical Company or Jansen. Solvents were obtained from Merck and used without further purification unless otherwise specified. Flash chromatography was performed using Merck silica gel 60 (70-230 mesh). Melting points were determined on a Büchi SMP-20 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Brücker AC250 spectrometer; deuterated chloroform (99.8% D) was used as a solvent with tetramethylsilane (TMS) as internal standard. Chemical shift values are expressed in ppm values and the following abbreviations are used for multiplicity of ^{1}H NMR signals: s = singlet, d = doublet, t = triplet, q = quartet , dd = double doublet , m = multiplet.

Thin layer chromatography (TLC) and radio-TLC were run on Merck 60F254/0.25mm silica gel plates. Compounds were visualised using UV light at 254 nm, and the labelled compound was detected using an automatic TLC-linear scanner Berthold Model 20. The labelled compound was co-spotted with the authentic unlabelled compound prior to development performed with dichloromethane/ methanol mixture. The Rf values for [^{11}C]Lu 29-066 were 0.5 and 0.7 for the ratio 90/10 and 70/30 respectively.

Preparative HPLC work was carried out using a Waters 501 pump and Valco valve injector. The purification of [^{11}C]Lu 29-066 was performed on a Waters μPorasil normal phase column (10 μm particle size, 250x10 mm I.D.) eluted with the following solvent system: dichloromethane/methanol/sol B (97/2.5/0.5 by vol.) with sol B: ethanol/water/ ethylamine (94/2/4 by vol.). Detection was achieved by UV absorbance at 254 nm with LC Spectrophotometer, and radiodetection was carried out with a Geiger-Müller radiodetector. With a flow rate of 4 mL/min, the retention time of [^{11}C]Lu 29-066 was 11 min.

Analytical work for radiochemical purity was carried out using an analytical Nucleosil C-18 reversed phase column (5 μm particle size, 250x4 mm I.D.) eluted

with a mixture of methanol/water/diethylamine (80/19.5/0.5 by vol.). With a flow rate of 1 mL/min, the retention time of [^{11}C]Lu 29-066 was 8.5 min. To determine the specific radioactivity, the peak area corresponding to the labelled product was quantified by UV absorbance using a previously generated calibration curve obtained from unlabelled Lu 29-066 at different concentrations.

2,5-dimethyl-3-(4-fluorophenyl)-1-[1-(cyanomethyl)piperidin-4-yl]-1*H*- indole **2**

To a solution of 2,5-dimethyl-3-(4-fluorophenyl)-1-(piperidin-4-yl)-1*H*- indole **1** (7.5 g, 23.2 mmol) in NMP (50 mL) and TEA (5 mL), was added dropwise chloroacetonitrile (1.9 g, 25.1 mmol). The mixture was heated at 60°C for 30 min and subsequently cooled to room temperature before addition of H_2O (200 mL) and ethyl acetate (200 mL). The organic layer was washed with brine, dried (Na_2SO_4), filtered and the solvent was evaporated *in vacuo*, to obtain 6.9 g of pure white solid (83%), after crystallisation in methanol/ethyl acetate mixture.

melting point: 181.0-181.3°C.

^{1}H NMR ($CDCl_3$) δ 7.45 (d, J=8.6Hz, 1H) 7.35 (m, 3H) 7.15 (t, J=8.8Hz, 2H) 6.95 (dd J= 8.8Hz;1.4Hz, 1H) 4.25 (m, 1H) 3.6 (s, 2H) 2.95 (d, J=10.3Hz, 2H) 2.65 (m, 4H) 2.41 (s,3H) 2.39 (s,3H) 1.90 (d, J=14.3Hz, 2H).

^{13}C NMR ($CDCl_3$) δ 161.8 (d, J_{C-F}=245Hz); 133.45; 133,2; 132,15; 132,1; 131,9 (d, J_{C-F} =8 Hz); 129,25; 128,85; 122,95; 119,0; 115,75 (d, J_{C-F} =21Hz); 115,05; 113,95; 111,4; 53.6; 52.85; 46.7; 30.55; 21.8; 12.35.

Elemental analysis $C_{23}H_{24}FN_3$: theoretical C 76.41%, H 6.70%, N 11.62%; found C 76.56%, H 6.80%, N 11.61%.

2,5-dimethyl-3-(4-fluorophenyl)-1-[1-(2-aminoethyl)piperidin-4-yl]-1*H*- indole **3**

To a suspension of $LiAlH_4$ (1.34g, 35.3 mmol) in dry diethyl ether (30 mL), a solution of $AlCl_3$ (1.34g, 10 mmol) in dry diethyl ether (30mL) was added dropwise, at 0°C. To the resulting mixture, a solution of **2** (8.5g, 23.5 mmol) in THF (100mL) was added dropwise whilst keeping the temperature at 10-15°C. After the addition was completed the mixture was heated under reflux for 2 hours. Successively, water

(1.4 mL), aqueous NaOH (3.5M, 1.4 mL) and water (1.4 mL) were added dropwise. The mixture was filtered on celite and the precipitate was washed several times with dichloromethane. The organic layer was washed with brine (2x30mL), dried (Na_2SO_4), filtered and the solvent was evaporated *in vacuo* to obtain 6 g of a yellow-pale powder (70%). This product was used in the next step without further purification. For analytical purposes, a small amount was purified by flash chromatography (eluent CH_2Cl_2/MeOH/NH_4OH (89/10/1 by vol.)).

Melting point: 130-130.5°C.

^{1}H NMR ($CDCl_3$) δ 7.5 (d, J=8.44Hz, 1H) 7.35 (m, 3H) 7.15 (t, J=8.8Hz, 2H) 6.98 (dd J= 8.45Hz;1.5Hz, 1H) 4.2 (m, 1H) 3.1 (d, J=11.6Hz, 2H) 2.85 (t, J=5.92Hz 2H) 2.65 (m, 2H) 2.5 (t, J=6.1Hz, 2H) 2.42 (s,3H) 2.40 (s,3H) 2.2(t, J=11.8Hz, 2H) 1.85 (d, J= 12.2, 2H).

^{13}C NMR ($CDCl_3$) δ 161.7 (d, J_{C-F}=244.4Hz); 133,5; 133,15; 132,2; 132,15; 131,9 (d, J_{C-F}=7.7Hz); 129,05; 128,65; 122,7; 115,8; 115,6 (d, J_{C-F}=21.2Hz); 111,5; 61.3; 54.8; 54.35; 51.0; 39.5; 31.05; 21.7; 12.35.

Elemental analysis $C_{23}H_{28}FN_3$: theoretical C 75.58%, H 7.72%, N 11.50%; found C 75.51%, H 7.60%, N 11.56%.

2,5-dimethyl-3-(4-fluorophenyl)-1-(1-{2-[(cyanomethyl)amino]ethyl}piperidin-4-yl)-1*H*-indole **4**

To a solution of **3** (3.9 g, 10.7 mmol) in NMP (35 mL) and TEA (4 mL), chloroacetonitrile (0.81 g, 10.7 mmol) was added dropwise. The mixture was heated at 60°C for 1 hour. After cooling to room temperature, water (200 mL) and ethyl acetate (200 mL) were added. After extraction, the organic layer was washed with brine, dried (Na_2SO_4), filtered, and the solvent was evaporated *in vacuo*. Purification by flash chromatography (EtOAc/EtOH/TEA (74/25/1 by vol.)) afforded 2.9 g of **4** as a pure white solid (67%).

Melting point: 141-141.5°C.

^{1}H NMR ($CDCl_3$) δ 7.47 (d, J=8.45Hz, 1H) 7.35 (m, 3H) 7.15 (t, J=8.8Hz, 2H) 6.98 (dd J= 8.4Hz;1.28Hz, 1H) 4.2 (m, 1H) 3.67 (s, 2H) 3.1 (d, J=11.55Hz, 2H) 2.85 (m,

2H) 2.6 (m, 4H) 2.42 (s,3H) 2.40 (s,3H) 2.2(t, J=11.8Hz, 2H) 1.85 (m, 3H).

^{13}C NMR ($CDCl_3$) δ 161.8 (d, J_{C-F}=244.4Hz); 133.65; 133,3; 132,35; 132,3; 132 (d, J_{C-F} =7.8 Hz); 129.25; 128,8; 122,9; 118,9; 118.5; 115,8 (d, J_{C-F} =21.1Hz); 113,9; 111,6; 57.8; 54.95; 54.45; 46.1; 38.1; 32.2; 21.9; 12.5.

Elemental analysis $C_{25}H_{29}FN_4$: theoretical C 74.21%, H 7.24%, N 13.85%; found C 74.32%, H 7.40%, N 13.62%.

2,5-dimethyl-3-(4-fluorophenyl)-1-(1-{2-[2-(aminoethyl)amino]ethyl}piperidin-4-yl) -1*H*- indole **5**

To a suspension of $LiAlH_4$ (70 mg, 1.85 mmol) in dry diethyl ether (1.5 mL), was added, dropwise, a solution of $AlCl_3$ (70 mg, 0.5 mmol) in dry diethyl ether (1.5 mL), at 0°C. To the resulting mixture, a solution of **4** (0.5 g, 1.23 mmol) in THF (9 mL) was added dropwise, at a temperature below 10°C. After the addition was completed, the mixture was heated under reflux for 2 hours. Successively, water (70 µL), aqueous NaOH (3.5 M, 70 µL) and water (70 µL) were added dropwise. The mixture was filtered on Celite, and the precipitate was washed several times with dichloromethane. The organic layer was washed with brine (2x10 mL), dried (Na_2SO_4), filtered and the solvent was evaporated *in vacuo*. The crude product was purified by flash chromatography (eluent CH_2Cl_2/MeOH/NH_4OH (89/10/1 by vol.)) to afford 0.42 g of **5** as a yellow-pale oil (76%).

^1H NMR ($CDCl_3$) δ 7.5 (d, J=8.44Hz, 1H) 7.35 (m, 3H) 7.15 (t, J=8.7Hz, 2H) 6.98 (dd J= 7.4Hz;1.03Hz, 1H) 4.2 (m, 1H) 3.1 (d, J=11.5Hz, 2H) 2.90 (m, 2H) 2.75 (m, 4H) 2.6 (m, 4H) 2.42 (s,3H) 2.40 (s,3H) 2.2 (t, J=12Hz, 2H) 1.85 (d, 2H).

^{13}C NMR ($CDCl_3$) δ 161. (d, J_{C-F}=244.3Hz); 133.2; 132,9; 132,0; 131.5 (d, J_{C-F} =7.8 Hz); 130,2; 128,8; 125,8; 122,4; 118,7; 118.5; 115,3 (d, J_{C-F} =21.1Hz); 113,4; 111,3; 57.9; 54.95; 54.15; 49.0; 36.5; 30.7; 21.5; 12.1.

For storage, this compound was converted to its hydrochloride form by addition of hydrogen chloride in diethyl ether (1 M).

Elemental analysis $C_{25}H_{33}FN_4$.HCl: theoretical C 67.46%, H 7.71%, N 12.59%; found C 67.39%, H 7.79%, N 12.47%.

Production of [^{11}C]phosgene

[^{11}C]Methane was produced during the irradiation of nitrogen containing 5% hydrogen by a proton beam. At the end of bombardment, the radioactive gas was condensed in two successive traps containing Porapak Q and cooled in liquid nitrogen. [^{11}C]Methane was then released to a glass homogenisation cell (20 mL) previously filled with chlorine. When all radioactivity was collected the chlorine – [^{11}C]methane mixture gas was transferred to two ovens in series by a flow of nitrogen containing 2% oxygen (10 ml / min). In the first oven containing an empty quartz tube, the chlorination of [^{11}C]methane to [^{11}C]carbon tetrachloride was performed at 560°C. [^{11}C]CCl_4 was subsequently converted into [^{11}C]phosgene in a second oven by contact with iron chips at 320°C. Excess of chlorine was removed from the resulting gas mixture by an antimony trap.

[^{11}C]1-(2-{4-[2,5-dimethyl-3-(4-fluorophenyl)-1*H*-indol-1-yl]-1-piperidinyl}ethyl)-2-imidazolidinone **6**

[^{11}C]phosgene was gently bubbled into an open conical glass vial containing a solution of the precursor **5** (6mg) in freshly distilled toluene (500 µL) at 0°C. When the accumulation of radioactivity was maximal, the glass vial was allowed to warm to room temperature.

After addition of HPLC mobile phase (300 µL), the mixture was applied to a semi-preparative HPLC, and the eluent containing the labelled compound was collected and evaporated to dryness. The residue was dissolved in a physiological saline solution (2mL) containing ethanol (10%) and filtered through a 0.22 µm filter (Waters).

Lipophilicity of [^{11}C]Lu 29-066

The lipophilicity was measured by the octanol/water distribution coefficient using a mixture of equal volumes (2 mL) of 1-octanol and 6.6 mM phosphate buffer (pH 7.4). The mixture was vortexed for 10 min before adding [^{11}C]Lu 29-066 and then

vortexed thoroughly for 60 min. After centrifugation at 3000 g for 5 min, the layers were counted separately.

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