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Radiosynthesis of [^{18}F]Lu29-024: A Potential PET Ligand for Brain Imaging of the Serotonergic 5-HT$_2$ Receptor

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Abstract—In a previous work, Lu29-024 (2,5-dimethyl-3-(4-fluorophenyl)-1-(1-methyl-4-piperidinyl)-1*H*-indole), a selective 5-HT$_{2A}$ receptor antagonist with nanomolar affinity and high selectivity, was labeled with carbon-11 to evaluate its behavior as a potential PET ligand for the serotonergic 5-HT$_{2A}$ receptor in the central nervous system. Administration of this tracer to rats was followed by a good brain uptake, no brain labeled metabolites but no specific, regio-selective, binding at 20 and 40 min post injection. Despite this, the data noted at 20 and 40 min suggest that this tracer, if associated with a radioactive emitter with a longer half-life than that of carbon-11, could be useful for the quantification of 5HT$_{2A}$ receptors. For these reasons, we chose to label this compound, bearing a fluorine atom, with [^{18}F]fluoride, in order to perform rat studies over a more prolonged time-scale. The precursor for the radiosynthesis of [^{18}F]Lu29-024 was obtained in an overall yield of 20% by a multi-step synthesis including an acetonylation reaction followed by a Fisher indole reaction. The radiotracer was prepared by an aromatic substitution with activated [^{18}F]fluoride followed by a decarbonylation reaction that employed Wilkinson's catalyst. The radiosynthesis of [^{18}F]Lu29-024 required approximatively 110 min with an overall radiochemical yield of 20–35% and specific activities of 37GBq/μmol. Fluorine-labeled Lu29-024 may thus be envisaged as a potentially useful PET tracer that can be applied to a wide range of neurological and psychiatric diseases.

Introduction

Significant advances are in the development of radioligands for the study of serotonin (5-HT) receptors in human brain in vivo with positron emission tomography (PET). Such radioligands offer unique opportunities for characterizing changes in 5-HT receptor populations in relation to neuropsychiatric disorders (e.g., anxiety, depression, Alzheimer's disease, schizophrenia)[1] and for characterizing and quantifying 5-HT receptor occupancy by new drugs.

In a recent paper, we have reported the labeling with carbon-11 of Lu29-024 (2,5-dimethyl-3-(4-fluorophenyl)-1-(1-methyl-4-piperidinyl)-1*H*-indole) for positron emission tomography studies.[2] This compound was described as a potent and selective 5-HT$_2$ antagonist (IC$_{50}$: 5.6 nM (5-HT$_2$), 2600 nM (D$_2$), 2700 nM (α_1)).[3] The rodent experiments performed with [^{11}C]Lu29-024 demonstrated a good brain uptake suggesting a favorable penetration of the blood–brain barrier. Moreover the labeled metabolites present in blood were not detected in brain tissue as late as 40 min after iv injection. Based on these encouraging characteristics, we further report in the present paper the results of the biological evaluation of [^{11}C]Lu29-024. In brief, the imaging technique undertaken (ex vivo phosphorplate autoradiography) failed to clearly show a specific binding of the tracer in selected cortical and sub-cortical brain areas 20 or 40 min after tracer injection. However, a tendency towards a presaturation effect could be detected over post-injection time suggesting that the nonspecific uptake of [^{11}C]-Lu29-024 may further decrease for times longer than 40 min while total uptake of the tracer remains stable (i.e., putatively receptor-bound tracer); this should consequently be reflected by the appearance of a specific binding of Lu29-024 that unfortunately cannot be detected with the very short half-life of carbon-11. These results, associated with the presence of fluorine atom in this compound, incited us to develop the labeling with fluorine-18 with the aim to study the biological behavior of this radiotracer at post-injection times over 40 min.

The synthetic route used for the preparation of [^{18}F]Lu29-024 is shown in Scheme 1. The strategy was

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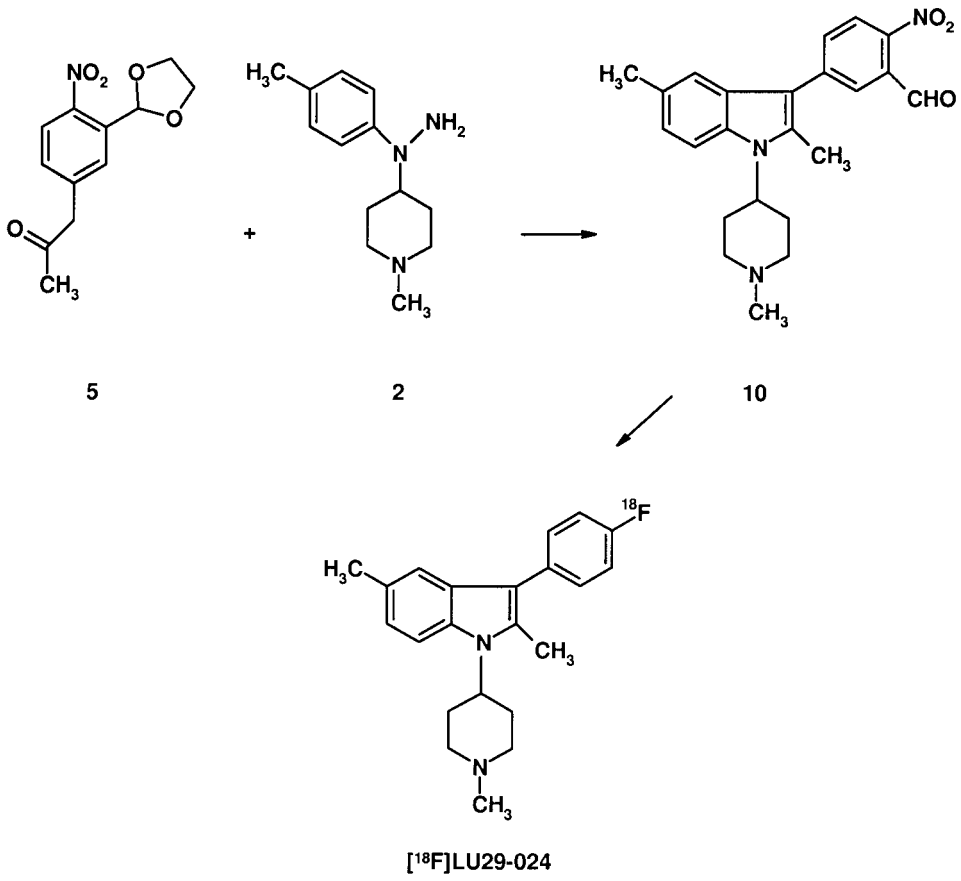


Scheme 1. Strategic route of [^{18}F]LU29-024.

to develop a nucleophilic substitution with [^{18}F]F$^-$ on activated nitro aromatic aldehyde compound **10** followed by decarbonylation using tris(triphenylphosphine) rhodium(I) chloride.

Materials and Methods

Biological studies

[^{11}C]Lu29-024 was synthesized from [^{11}C]methyl iodide as previously reported.[2] Biological studies were performed on male Sprague–Dawley rats (~250 g, $n=23$, obtained from CERJ, France) in compliance with the European Directives and the French law on animal experimentation (personal authorization: 6106 for F.D.). Anesthesia was induced and maintained by inhalation of halothane (5% for induction, then 1.0–1.5% until euthanasia). The femoral arteries and veins were catheterized for rapid blood sampling and injection of drugs. Anesthetized, thermo-regulated rats were injected with trace amounts of [^{11}C]Lu29-024 (2.6±0.3 mCi; 13±2 nmols; mean±SEM) alone (control), or 15 min after pretreatment with the selective 5-HT$_{2A}$-receptor antagonist ritanserin (presaturation; 2.5 mg/kg iv; Research Biochemical International).[13] To assess serum pharmacokinetics, arterial blood samples were collected and centrifuged (10,000 rpm; 4 min; 4 °C); radioactivity was determined on 5 μL serum aliquots with the gamma counter (Cobra autogamma, Packard). Animals were subjected to euthanasia by decapitation at pre-established time-points (20, 40 min); brains were rapidly removed, frozen in cold isopentane (−45 °C) and cryostat-cut into horizontal 25 μm thick sections. Brain sections mounted onto coverslips were apposed to phosphorimaging plates (Molecular Dynamics) for 12 h. Autoradiograms were quantified by means of a phosphorimaging analysis system (Phosphorimager SI, Molecular Dynamics) according to appropriate [^{11}C]-standards prepared and exposed in parallel with brain sections.[14] Biological data were statistically analyzed through the use of a repeated-measures ANOVA with time and condition as independent factors and brain region as an interdependent factor; a p value less than 0.05 was considered to be significant.

Chemistry and radiochemistry

Lu29-024 was provided by the H. Lündbeck A/S (Denmark). Reagents were purchased from Sigma-Aldrich or Jansen. 5-Bromo-2-nitrobenzaldehyde (**3**) was prepared in 72% yield by nitration of 3-bromobenzaldehyde using sulfuric acid–potassium nitrate.[4] Solvents were freshly distilled under nitrogen prior to use: 1,4-dioxan from sodium, *N*,*N*-dimethylformamide (DMF) and acetonitrile from calcium hydride, tetrahydrofuran (THF) from sodium benzophenone ketyl. All reactions were run under a nitrogen atmosphere. Melting points were recorded on an Reichert microscope and are uncorrected. ^{1}H and ^{13}C NMR spectra of samples in

deuteriochloroform were run on a Brucker AC instrument (250 MHz and 62.89 MHz, respectively) using tetramethylsilane (TMS) as an internal standard. The chemical shifts are recorded in parts per million (ppm) from TMS. Splitting patterns are designated as s (singlet), d (doublet), t (triplet) and m (multiplet). Flash chromatography was performed on 70–230 mesh silica gel 60 (Merck) column. The Sep-Paks C_{18} cartridges were conditioned by methanol (5 mL) then water (5 mL) prior to use. Thin layer chromatography (TLC) and radio-TLC were run on Merck 60F254/0.25 mm silica gel plates. Compounds were visualized using ultraviolet (UV) light at 254 nm, and the labeled compound was detected using an automatic TLC-linear scanner Berthold Model 20. The labeled compound was co-spotted with the authentic unlabeled compound prior to development. TLC conditions were varied and are specified individually, with R_f values, for each compound below. High performance liquid chromatography (HPLC) was carried out by means of a Waters 501 pump and Valco valve injector. The purification of the labeled product was performed on a Waters μ-porasil normal phase column (10 μm particle size, 300×7.8 mm ID) eluted with a mixture of dichloromethane:methanol:solB (97:2:1 by vol) (solB: ethanol:water:ethylamine, 94:2:4 by vol). The UV absorbance (254 nm) and radioactivity were monitored by an in-line Knauer variable-wavelength monitor and a scintillation radiodetector, respectively. With a flow rate of 3 mL/min, the retention time of [^{18}F]**11** and [^{18}F]Lu29-024 were 23 and 12 min, respectively. The radio-HPLC analyses for radiochemical purity were conducted using an analytical C_{18} reversed-phase column Waters (5 μm particle size, 250×4 mm ID) eluted with a mixture of acetonitrile: water:diethylamine (70:29:0.5 by vol). The retention time of [^{18}F]Lu29-024 was 11.5 min, with a flow rate of 1 mL/min. To determine the specific radioactivity, the peak area corresponding to the radiolabeled product was quantified by UV absorbance using a previously calibrated curve obtained from unlabeled Lu29-024 with different concentrations and measured by a Waters integrator. An aliquot of the final solution of known volume and radioactivity measured by a Capintec CRC15R dose calibrator was applied to the analytical C_{18} reversed phase column described above. The area of the UV absorbance peak corresponding to [^{18}F]Lu29-024 was compared to the standard curve.

***N*-(1-Methyl-4-piperidinyl)-4-toluidine (1).** A solution of *p*-toluidine (5.71 g; 0.053 mol) and 1-methyl-piperidin-4-one (4.6 g; 0.041 mol) in dry methanol (80 mL) was stirred 6 h at room temperature in presence of molecular sieves (3 Å, 4 g; 5 Å, 4 g). Then, acetic acid (30 mL) was added and the reaction was stirred for 14 h. $NaBH_3CN$ (1.70 g; 0.027 mol) was added and the resulting solution was stirred for 3 h more. After filtration, the molecular sieves were washed with methanol. The solvent was evaporated and the crude material was then dissolved in ethyl acetate (200 mL) and washed with brine (2×30 mL) and water (30 mL). The organic layer was dried on anhydrous Na_2SO_4, filtered and the solvent evaporated. After chromatography on silica a gel column (eluent: CH_2Cl_2:CH_3OH:Et_3N; 96:3:1) 6 g of **1** was obtained (72%) as a white solid. Melting point = 50.5–51.5 °C. R_f = 0.32 (CH_2Cl_2:MeOH (90:10)). Elemental analysis $C_{13}H_{20}N_2$: required C, 76.42%; H, 9.87%; N, 13.71%; found C, 76.28%; H, 9.91%; N, 13.65%. ^{1}H NMR ($CDCl_3$): 6.95 (d, 2H, J = 8 Hz), 6.5 (d, 2H, J = 8 Hz), 3.41 (s, 1H), 3.22 (m, 1H), 2.76 (dm, 2H, J = 12 Hz), 2.27 (s, 3H), 2.21 (s, 3H), 2.1 (m, 4H), 1.47 (qd, 2H, J = 12 Hz, 3.5 Hz).^{13}C NMR ($CDCl_3$): 144.8, 129.9, 126.7, 113.8, 54.6, 49.6, 46.1, 32.4, 20.4.

***N*^{1}-(1-Methyl-4-piperidinyl)-4-toluylhydrazine (2).** Compound **1** (4.80 g; 23.51 mmol) in absolute ethanol (60 mL) was cooled at 0 °C and stirred with a mechanical stirrer. HCl_{conc} (50 mL) was added slowly. A solution of $NaNO_2$ (1.792 g; 30.4 mmol) in H_2O (12 mL) was added dropwise so as to keep the reaction mixture below 0 °C. The addition completed, the mixture was stirred at this temperature for 3 h. Water (600 mL) was added, and the mixture was made alkaline with NaOH (6 N). The solution was extracted with ethyl acetate (3×150 mL), washed with water, dried on anhydrous Na_2SO_4, filtered and the solvent evaporated. The crude nitroso compound was used without further purification. A suspension of $LiAlH_4$ (1.86 g) in tetrahydrofuran (10 mL) was heated to reflux. The crude nitroso compound (4.82 g) in THF (40 mL) was added dropwise. The addition completed, the reaction mixture was stirred at reflux for 1 h and then cooled to room temperature. Successively, water (1.5 mL), aqueous NaOH (3 N; 1.5 mL) and water (1.5 mL) were added dropwise. The mixture was filtered on Celite and the residue washed several times with dichloromethane. The solvent was removed, and the product was recrystallized in ethyl acetate: 4.8 g of **2** was obtained (93%) as a white solid. Melting point = 75–76 °C. R_f = 0.2 (CH_2Cl_2:MeOH (90:10)). Elemental analysis $C_{13}H_{21}N_3$: required C, 71.19%; H, 9.65%; N, 19.16%; found C, 71.09%; H, 9.71%; N, 19.31%. ^{1}H NMR ($CDCl_3$): 7.05 (d, 2H, J = 8.6 Hz), 6.95 (d, 2H, J = 8.5 Hz), 3.47 (m, 1H), 3.28 (s, 2H), 2.9 (d, 2H, J = 9.6 Hz), 2.3 (s, 3H), 2.25 (s, 3H), 2.05 (td, 2H, J = 12 Hz, 2.1 Hz), 1.88 (qd, 2H, J = 12.1 Hz, 3.6 Hz), 1.7 (m, 2H). ^{13}C NMR ($CDCl_3$): 149.3, 129.7, 128.1, 114.65, 58.45, 55.5, 46.3, 27.2, 20.4.

2-(5-Bromo-2-nitrophenyl)-[1,3]dioxolane (4). 5-Bromo-2-nitrobenzaldehyde **3** (5.1 g; 22.2 mmol) in toluene (90 mL), ethylene glycol (2.15 mL; 38.5 mmol) and *p*-toluenesulfonic acid (90 mg; 0.52 mmol) were stirred and heated. Water formed during the reaction was removed azeotropically (Dean–Stark trap) during 24 h. After evaporation of the solvent and a purification by flash column chromatography on silica gel (eluent: CH_2Cl_2), compound **4** was isolated as a white solid. Yield: 5.88 g (96%). Melting point: 68–68.5 °C. R_f = 0.6 (CH_2Cl_2). Elemental analysis $C_9H_8BrNO_4$: required C, 39.44%; N, 5.11%; Br, 29.18%; found C, 39.63%; N, 4.93%; Br, 28.97%. ^{1}H NMR ($CDCl_3$): 7.95 (d, 1H, J = 2.1 Hz), 7.80 (d, 1H, J = 8.6 Hz), 7.63 (dd, 1H, J = 8.6 Hz, 2.1 Hz), 6.47 (s, 1H), 4.04 (m, 4H). ^{13}C NMR ($CDCl_3$): 147.9, 135.7, 133.1, 131.2, 128.2, 126.5, 99.3, 65.8.

1-(3-[1,3]Dioxolan-2-yl-4-nitrophenyl)-propan-2-one (5). Sodium acetylacetonate was previously prepared from

the reaction of 2,4-pentadione (10 g, 0.1 mol) with an equimolar amount of NaOH (4 g, 0.1 mol) in ethanol (50 mL). Compound **4** and sodium acetylacetonate were freshly prepared and dried under reduced pressure (10^{-2} mbar) at 60 °C for 4 h before use. Under a nitrogen current, **4** (2.92 g; 10.65 mmol) was dissolved in dry dimethylformamide (18 mL) (DMF was bubbled by nitrogen for 5 min before use) then acetylacetonate (6.42 g; 52.6 mmol) and cuprous iodide (2.23 g; 11.7 mmol) were added, the reaction mixture was stirred and heated for 6 h at 100 °C. Aqueous NaOH (10 mL, 1 N) was added at 100 °C and the reaction stirred for 16 h more at room temperature. The solution was extracted with ethyl acetate, washed with brine, dried on anhydrous Na_2SO_4, filtered, and the solvent evaporated. After chromatography on a silica gel column (eluent: heptane: ethyl acetate 50:50), 1.9 g of **5** as a pale yellow oil (71%) were isolated. $R_f = 0.25$ (heptane:ethyl acetate (50:50)). ^{1}H NMR ($CDCl_3$): 7.89 (d, 1H, $J = 8.2$ Hz), 7.61 (d, 1H, $J = 1.9$ Hz), 7.33 (dd, 1H, $J = 8.2$ Hz, 1.9 Hz), 6.47 (s, 1H), 4.05 (m, 4H), 3.82 (s, 2H), 2.22 (s, 3H).^{13}C NMR ($CDCl_3$): 204.3, 147.6, 139.8, 133.6, 130.7, 128.8, 125, 99.5, 65.3, 50.1, 29.9.

3-(3-[1,3]Dioxolan-2-yl-4-nitrophenyl)-pentane-2,4-dione (6). 0.19 g (yield 6%). $R_f = 0.55$ (heptane:ethyl acetate (50:50)). ^{1}H NMR ($CDCl_3$): 7.95 (d, 1H, $J = 8.2$ Hz), 7.60 (d, 1H, $J = 1.9$ Hz), 7.34 (dd, 1H, $J = 8.2$ Hz; 1.9 Hz), 6.47 (s, 1H), 4.03 (m, 4H), 3.4 (s, 1H), 1.90 (s, 6H). ^{13}C NMR ($CDCl_3$): 190.6, 147.95, 142.3, 133.75, 132.4, 130.5, 125.1, 113.7, 99.4, 65.45, 24.3.

2 - (2 - Nitrophényl) - [1,3]dioxolane (7). 0.33 g (yield 16%) $R_f = 0.6$ (heptane:ethyl acetate (50:50)). ^{1}H NMR ($CDCl_3$): 7.88 (dd, 1H, $J = 7.9$ Hz, 1.2 Hz), 7.80 (dd, 1H, $J = 7.7$ Hz, 1.4 Hz), 7.60 (td, 1H, $J = 7.6$ Hz, 1.3 Hz), 7.48 (td, 1H, $J = 7.8$ Hz, 1.5 Hz), 6.47 (s, 1H), 4.03 (m, 4H). ^{13}C NMR ($CDCl_3$): 147.85, 132.2, 131.8, 128.6, 126.6, 123.4, 98.56, 64.3.

2-Nitro-5-(2-oxo-propyl)-benzaldehyde (8). To a solution of **5** (0.5 g, 2 mmol) in THF (20 mL) were added HCl 3 M (5 mL) and water (8 mL). The reaction mixture was heated at reflux for 2 h. The solvent was removed and the solution extracted with dichloromethane, washed with water, dried on anhydrous Na_2SO_4, filtered and the solvent evaporated. The product was recrystallized in ethyl acetate: 0.35 g of **8** was obtained (85%) as a yellow solid product. Melting point = 72.5–73 °C. $R_f = 0.7$ (ethyl acetate). Elemental analysis $C_{10}H_9NO_4$: required C, 57.97%, H, 4.38%; N, 6.76%; found C, 58.03%, H 4.43%, N, 6.67%. ^{1}H NMR ($CDCl_3$): 10.45 (s, 1H), 8.09 (d, 1H, $J = 8.3$ Hz), 7.73 (d, 1H, $J = 1.9$ Hz), 7.56 (dd, 1H, $J = 8.3$ Hz, 1.9 Hz), 3.89 (s, 2H), 2.26 (s, 3H). ^{13}C NMR ($CDCl_3$): 202.5, 187.2, 147.2, 140.35, 133.85, 130.45, 129.7, 123.8, 48.45, 29.1.

3-(3-Diethoxymethyl-4-nitrophenyl)-2,5-dimethyl-1-(1-methyl-4-piperidinyl)-1*H*-indole (9). A solution of the hydrazine **2** (0.873 g; 3.98 mmol) and compound **5** (1 g; 3.98 mmol) in absolute ethanol (20 mL) was stirred and heated to reflux for 18 h. After evaporation of the solvent, ethyl acetate (100 mL) was added. The organic layer was washed with brine, dried on anhydrous $MgSO_4$, filtered and the solvent evaporated. To a residue dissolved in absolute ethanol, sulfuric acid (96%, 0.2 mL) was added and the reaction mixture heated to reflux for 18 h. Then the solution was made alkaline with NaOH (1 N). Ethanol was removed and the extraction performed with ethyl acetate (3×100 mL); the organic layers were washed with brine, dried on anhydrous $MgSO_4$, filtered and the solvent evaporated. After chromatography on a silica gel column (eluent: CH_2Cl_2:CH_3OH:Et_3N, 98.5:1:0.5), 1.25 g of **9** was obtained as orange crystals (67%). Melting point: 81–82 °C. $R_f = 0.7$ (CH_2Cl_2:MeOH (90:10)). Elemental analysis $C_{27}H_{35}N_3O_4$: required C, 69.65%; H, 7.58%; N, 9.02%, found C, 69.71%; H, 7.72%; N, 8.87%. ^{1}H NMR ($CDCl_3$): 7.98 (d, 1H, $J = 8.4$ Hz), 7.92 (s, 1H), 7.55–7.50 (m, 2H), 7.4 (s, 1H), 7.0 (d, 1H, $J = 9.5$ Hz), 6.15 (s, 1H), 4.20–4.40 (m, 1H), 3.85–3.55 (m, 4H), 3.1 (dm, 2H, $J = 11.6$ Hz), 2.75 (qd, 2H, $J = 12.6$, 3.2 Hz), 2.5 (s, 3H), 2.41 (s, 3H), 2.38 (s, 3H), 2.15 (t, 2H, $J = 10.2$ Hz), 1.9 (d, 2H, $J = 12.2$ Hz), 1.25 (t, 6H, 7 Hz). ^{13}C NMR ($CDCl_3$): 141.6, 129.9, 129.5, 129.1, 126.5, 125, 123, 118.3, 111.6, 98.9, 63.75, 56, 54.1, 46.35, 30.5, 21.4, 15.2, 12.25.

5-[2,5-Dimethyl-1-(1-methyl-4-piperidinyl)-1*H*-indol-3-yl]-2-nitrobenzaldehyde (10). Compound **9** (300 mg; 0.64 mmol) in acetone (20 mL) and in presence of HCl_{conc} (100 μL) was stirred at room temperature for 1 h. The solution was made alkaline with NaOH (5 N). After extraction with ethyl acetate, the organic layer was dried on $MgSO_4$, filtered and the solvent evaporated. After chromatography on silica gel column (eluent: CH_2Cl_2: CH_3OH:Et_3N, 98.5:1:0.5), compound **10** (220 mg) was isolated as red crystals (87%). Melting point: 130–131 °C. $R_f = 0.35$ (CH_2Cl_2:MeOH (90:10)). Elemental analysis $C_{23}H_{25}N_3O_3$: required C, 70.57%; H, 6.44%; N, 10.73%; found C, 70.83%; H, 6.61%; N, 10.47%. ^{1}H NMR ($CDCl_3$): 10.5 (s, 1H), 8.2 (d, 1H, $J = 8.5$ Hz), 7.95 (s, 1H), 7.8 (d, 1H, $J = 8.45$ Hz), 7.6 (d, 1H, $J = 8.5$ Hz), 7.4 (s, 1H), 7.05 (d, 1H, $J = 8.5$ Hz), 4.20–4,40 (m, 1H), 3.1 (dm, 2H, $J = 11.6$ Hz), 2.75 (qd, 2H, $J = 12.5$ Hz, 3.3 Hz), 2.5 (s, 3H), 2.4 (s, 6H), 2.15 (m, 2H), 1.9 (dm, 2H, $J = 12$ Hz). ^{13}C NMR ($CDCl_3$): 189, 146.25, 143.4, 133.6, 132.7, 131.25, 129, 126.2, 124, 123.3, 116.8, 118, 111.6, 54.8, 53.1, 45.25, 29.4, 20.4, 12.3.

Preparation of nucleophilic [^{18}F]fluoride ion. No-carrier-added aqueous [^{18}F]fluoride was produced via ^{18}O$(p,n)^{18}$F nuclear reaction by irradiation of 1.5 mL ^{18}O-enriched water (95%) on a baby cyclotron (CGR MeV 325). $^{18}F^-$ was separated from ^{18}O-enriched water on an anion exchange resin (Bio-Rad, AG1-X8, 100–200 mesh, chloride form) and recovered by elution with 0.5 mL of aqueous potassium carbonate (5 mg/mL). The aqueous [^{18}F]fluoride solution was collected into a conical Reactivial® containing K_2CO_3 (4.5 mg; 0.032 mmol) and aminopolyether Kyptofix 222 [(4,7,13,16,21,24)hexaoxa-1,10-diazabicyclo(8,8,8)hexacosane] (22 mg; 0.052 mmol) dissolved in water:acetonitrile mixture (0.1 mL:1 mL). The water was removed azeotropically with acetonitrile under a stream of nitrogen and a dry residue of $[K/K_{222}]^+{}^{18}F^-$ was obtained.

5-[2,5-Dimethyl-1-(1-methyl-4-piperidinyl)-1*H*-indol-3yl]-2-[^{18}F]fluorobenzaldehyde (11). A solution of compound **10** (10 mg; 0.025 mmol) in DMSO (1 mL) was added to the above-prepared $[K/K_{222}]^{+}{}^{18}F^{-}$ complex and heated at 140 °C for 10 min. After cooling, the solution was diluted with water and passed through the conditioned SepPak C_{18}. The SepPak C_{18} was additionally washed with water (10 mL) and aqueous eluate containing DMSO and unreacted [^{18}F]fluoride was discarded. Residual water on the SepPak cartridge was partly removed by nitrogen stream. Compound **11** was eluted with CH_2Cl_2 (3 mL) and passed through a column packed with $MgSO_4$:K_2CO_3 (1 g, 50:50, w:w). After partial evaporation of CH_2Cl_2 (residual volume 1 mL), the reaction mixture was applied to HPLC separation.

2,5-Dimethyl-3-(4-[^{18}F]fluorophenyl)-1-(1-methyl-4-piperidinyl)-1*H*-indole [^{18}F]Lu29-024. After evaporation of the HPLC solvent, compound **11** was dissolved in 1,4-dioxan (1 mL) under nitrogen stream and chlorotris(triphenylphosphine)rhodium (Wilkinson's catalyst, 20 mg) was added. The reaction mixture was heated at 150 °C for 10 min. After cooling, the solution was diluted with water (8 mL) and the solution was passed through a conditioned SepPak C_{18} (10 mL of methanol followed by 10 mL of water). The SepPak was washed with water (10 mL) and the organic product was eluted with CH_2Cl_2 (3 mL). The organic phase was dried by passage through a small column of $MgSO_4$:K_2CO_3 (1 g, 50:50, w:w) and the solvent was evaporated to a residual volume of 0.5 mL. The residue was applied to HPLC purification; HPLC fraction was collected and evaporated to dryness. The residue was taken up in a physiological saline solution (2 mL) containing 10% ethanol. An aliquot of [^{18}F]Lu29-024 was injected on an analytical reversed phase column and the chemical and radiochemical purity was then greater than 95%.

Results and Discussion

Biological evaluation of [^{11}C]Lu29-024 in the rat

Intravenous injection of [^{11}C]Lu29-024 was followed by the rapid appearance of a plasmatic peak of radioactivity (~20 s after administration; 1.38±0.71% ID/mL); then radioactivity decreased with a bi-exponential curve with respective distribution and elimination half-lives of 7 s and 226 min). At the brain level, phosphorimaging autoradiography allowed the determination of radioactivity profiles in selected brain regions (Table 1), 20 min and 40 min after radiotracer administration in both control and presaturation conditions. If the global statistical analysis revealed an overall presaturation effect ($p = 0.0003$) and a difference in the distribution of radioactivity within cerebral structures ($p < 0.0001$), it failed to clearly identify the between-condition differences at individual post-injection times and for each brain region (i.e., a difference between total and nonspecific conditions at 20 or 40 min and for each region of interest). However, it appears that, from 20 to 40 min, the amount of radioactivity in the selected regions of interest is slightly increased, while the radioactive concentrations obtained in animals in which $5HT_{2A}$ receptors have been presaturated with ritanserin moderately decreased (Table 1). Such a situation could suggest the putative appearance of a clear presaturation effect (i.e., a pharmacological specific binding of [^{11}C]Lu29-024) for post-injection times over 40 min. Further experiments are needed to test such a hypothesis; however, the use of carbon-11 as the radioactive emitter is not appropriate given its too rapid decay ($t_{\frac{1}{2}} = 20$ min) and the higher variability obtained at 40 min when compared to 20 min data (Table 1). Such experiments could be undertaken with the same tracer labeled with another positron emitter with a longer half-life such as [^{18}F] ($t_{\frac{1}{2}} = 110$ min).

Table 1. Distribution of [^{11}C]Lu29-024 in the rat brain[a]

Brain region	20 min		40 min	
	Control	Presaturation	Control	Presaturation
Frontal Cx	46±7	21±5	51±21	17±11
Parietal Cx	48±7	23±5	49±21	19±13
Temporal Cx	41±7	20±5	46±20	18±11
Occipital Cx	41±11	18±7	35±16	11±7
Caudate-putamen	26±2	14±5	33±16	8±4
Hippocampus	35±11	24±9	41±18	26±7
Thalamus	42±11	26±9	28±18	15±2
Inferior colliculus	51±11	32±13	22±13	15±9
Cerebellum	35±7	21±9	4±2	2±2
Pons	44±11	24±9	23±11	8±4

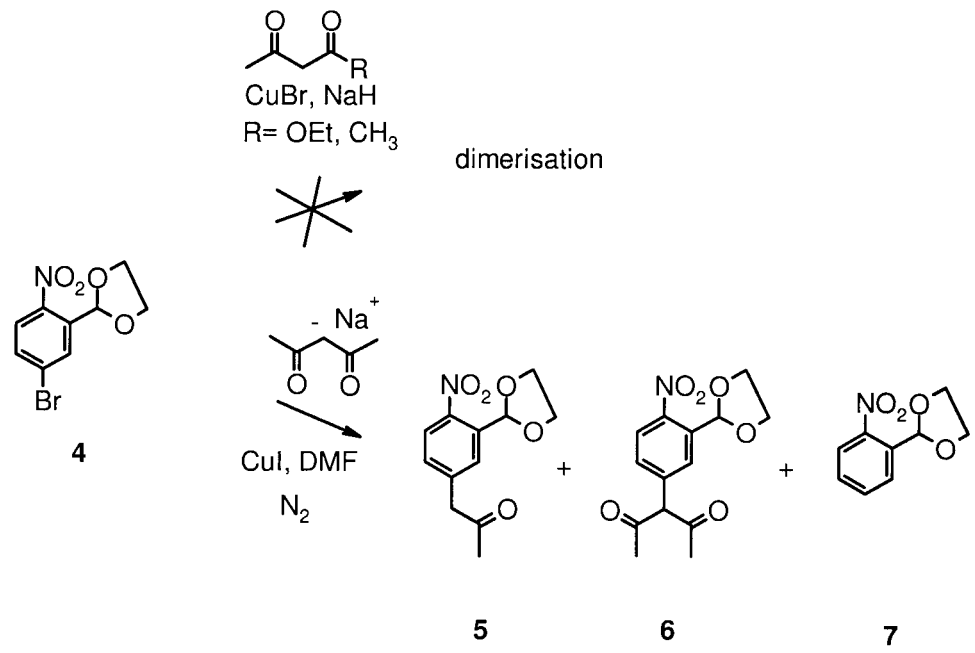
[a]Radioactive concentrations (expressed as 10^{-3}% ID/mg protein; mean±SEM; $n = 3$–6 independent determinations) were measured autoradiographically through the use of a phosphorimaging plate system. Radioactivity values, obtained in each region of interest were corrected for radioactivity measured in white matter (*corpus callosum*). For statistical analysis, see text.

Radiosynthesis of [^{18}F]Lu29-024

The strategy for the labelling of Lu29-024 consisted of a nucleophilic substitution with $[^{18}F]F^{-}$ on activated nitro aromatic aldehyde precursor followed by decarbonylation using the Wilkinson's catalyst. For this purpose we have developed a Fisher indole reaction from compounds **2** and **5** which allowed us to obtain compound **10** (Scheme 1).

The synthesis of arylacetone **5** was performed from protected 5-bromo-2-nitrobenzaldehyde **4** by an acetonylation reaction. Our initial approach to **5** used a copper-catalyzed condensation of β-dicarbonyl compound with **4**. This reaction was carried out either with ethyl acetoacetate or acetylacetone in the presence of sodium hydride as base and Cu(I) bromide as catalyst[5] without solvent at 80–85 °C (Scheme 2); variation of the reaction time and relative stoichiometries of CuBr were unsuccessful; using 0.1 or 1 equivalent of CuBr and 2.5 equivalents of NaH with either acetylacetone or ethyl ester for 5 or 6 hours resulted only in the dimerization of the dicarbonyl compounds identified by ^{1}H NMR.

A versatile synthesis[6] of **5** was envisaged (Scheme 2) in the presence of sodium acetylacetonate previously prepared from the reaction of acetylacetone with an equimolar amount of NaOH dissolved in ethanol followed by drying the isolated salt under reduced pressure. In this reaction, the differences of conditions in changes of



Scheme 2. Acetonylation reaction.

temperature and deacetylation conditions have been investigated. We have observed a conversion rate of **4** ranging from 80 to 90%; the yields of **5**, **6** and **7** were expressed after purification and in relative to the conversion rate of **4** (Table 2). The results showed two competitive reactions involving oxidative addition and reductive elimination which led to **5** and **6** in competition with a reductive dehalogenation which led to **7**. In run 1, arylation and deacetylation reactions were performed separately; after isolation and purification of **6** the alkaline hydrolysis with NaOH (3 N) gave tarry products, presumably owing to displacement or condensation reactions of the nitro-group.[7] In the other experiments, the deacetylation was performed 'one pot'; the addition of water at room temperature was not sufficient (run 2) by comparison of the use of NaOH (1 N) heated to 100 °C[8] which led, after the mixture was left at room temperature for 16 h, to **5** in 71%.

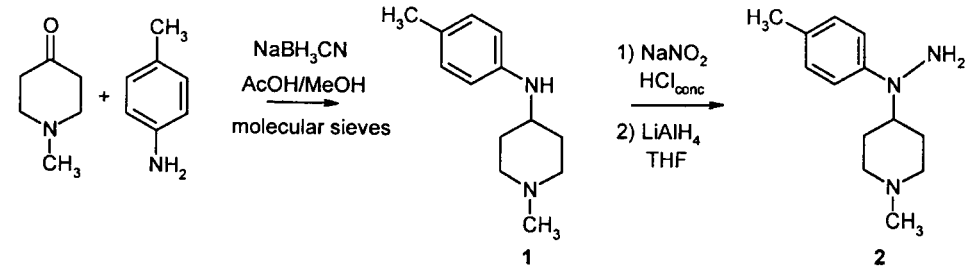
Table 2. Arylation and deacetylation conditions

Run	Arylation conditions T (°C)	Deacetylation conditions 'one pot'	Yield (%)[a]		
			5	**6**	**7**
1[b]	80		0	57	20
2	120	Water RT; 16 h RT	0	16	53
3	100	NaOH 1N RT; 30min RT	30	35	18
4	100	NaOH 1N RT; 16 h RT	41	33	20
5	100	NaOH 1N 100 °C; 16 h RT	71	6	16

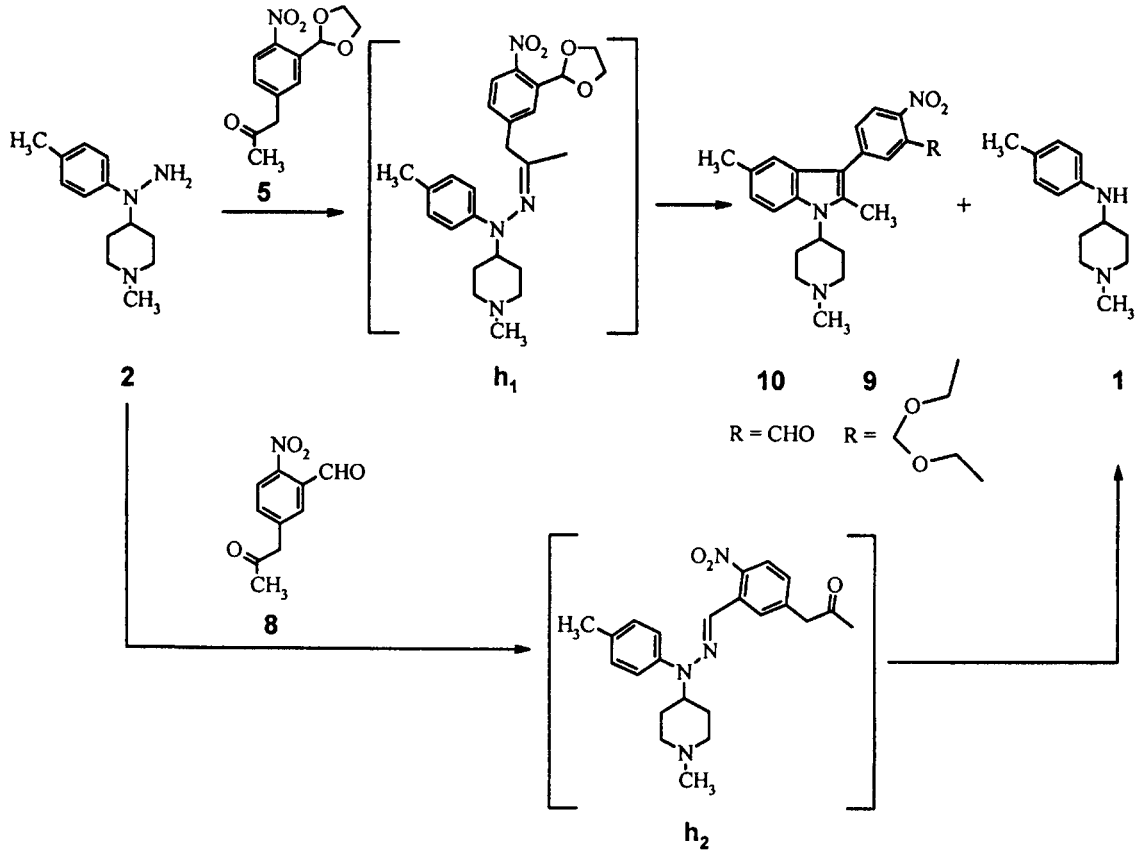
[a]Yields are relative to converted **4**.
[b]Arylation and deacetylation reactions were performed in a two step procedure.

The disubstituted aniline **1** was obtained in 72% yield by a reductive amination of *p*-toluidine with 1-methylpiperidin-4-one in the presence of sodium cyanoborohydride[9] and molecular sieves,[10] which serve simultaneously as a dehydrating agent and as a catalyst. The nitrosation followed by a reduction step with $LiAlH_4$ has afforded the hydrazine **2** in 93% yield (Scheme 3).

The Fisher indole reaction from hydrazine **2** and compound **5** was performed in different conditions. The first approach performed 'one pot' under acidic conditions afforded the formation of **10** in a poor yield (4%) along with **9** (35%) and the disubstituted aniline **1** (55%) (Scheme 4); this suggests a partial deprotection of **5** in



Scheme 3.

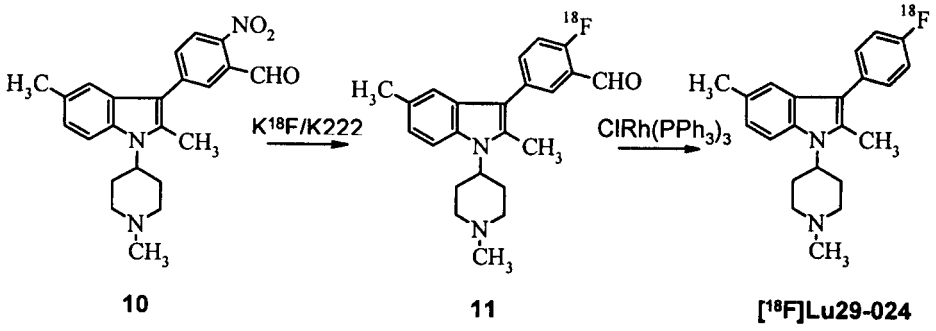


Scheme 4.

acidic conditions where aldehyde function of **8** was in competition with the ketone function leading to intermediary hydrazone h_2 then **1**. Consequently, this reaction was carried out in a two step procedure where hydrazone h_1 was obtained by condensation in neutral conditions before the Fisher cyclization in acidic conditions; this procedure permitted to obtain **9** in 67% yield, its deprotection in acidic conditions afforded **10** in 58.5% overall yield.

The no-carrier-added synthesis for the preparation of [^{18}F]Lu29-024 involved two steps (Scheme 5). The first one was the radiofluorination of **10** by nucleophilic aromatic displacement by [^{18}F]KF/K_{222}.[11] Reaction time, temperature and solvent were studied. This reaction, carried out in DMSO, allowed us to obtain **11** with, for a given temperature (110 or 140 °C), comparable radiochemical yields, irrespective of the reaction time (from 5 to 25 min) (Fig. 1). Nevertheless, an appreciable increase (20%) in yield was noted when the fluorination temperature was raised from 110 to 140 °C. This reaction failed to occur in CH_3CN.

The crude product purification on a Sep Pak C_{18} cartridge afforded **11** in 35–60% radiochemical yield. The decarbonylation reaction[12] of **11** was done in 1,4-dioxan with Wilkinson's catalyst at 150 °C for 10 min in 85–95% radiochemical yield. The major problem of this radiosynthesis was the chemical purity of the final product. In spite of several HPLC conditions tested on normal or reversed phase, it was not possible to obtain the radiotracer [^{18}F]Lu29-024 chemically pure. To avoid



Scheme 5. Radiosynthesis of [^{18}F]Lu29-024.

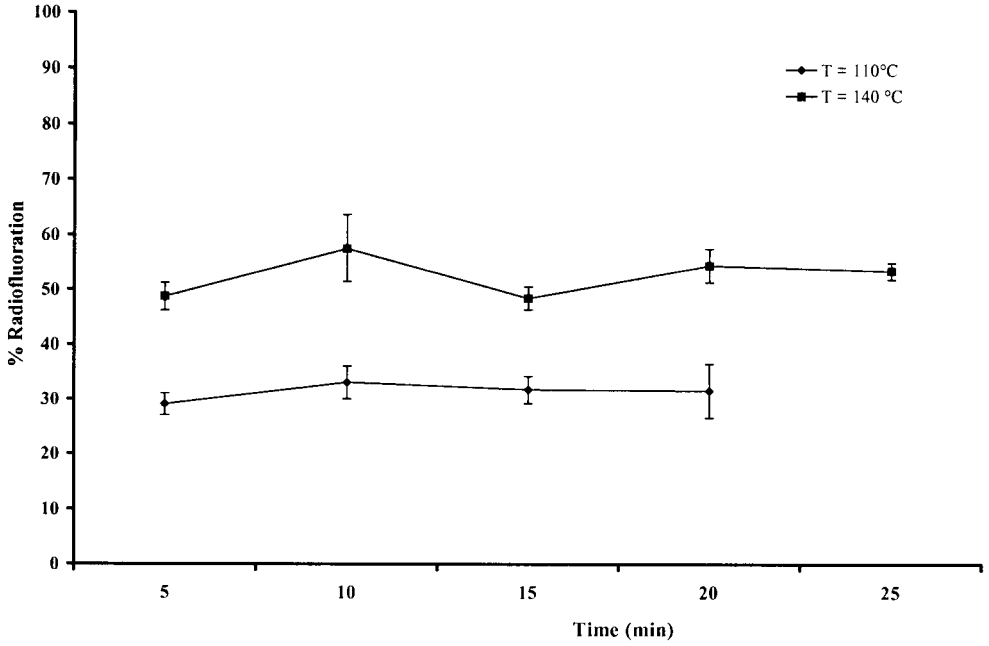


Figure 1.

this difficulty, we included an intermediary HPLC purification of [^{18}F]**11** before the decarbonylation reaction. The same HPLC conditions were used to purify [^{18}F]Lu29-024. The overall radiochemical yield was 20–35% (7–12 mCi were obtained decay corrected from [^{18}F]fluoride) with a total synthesis time of 110 min. Quality control analyses on analytical HPLC indicated a chemical and radiochemical purity of [^{18}F]Lu29-024, always greater than 95%, with specific activities greater than 37 GBq/μmol (1 Ci/μmol).

Conclusion

[^{11}C]Lu29-024, despite interesting biological features, such as a marked brain uptake (present study) and the lack of radiolabeled metabolites in the cerebral parenchyma,[2] failed to reveal ex vivo binding for $5HT_{2A}$ receptors in the rat brain for post-injection times up to 40 min. Nonetheless, the tendency for a specific binding of [^{11}C]Lu29-024 that we have shown, at 20 and 40 min, suggests that this tracer labeled with [^{18}F]fluoride could be useful for the assessment of $5HT_{2A}$ receptors. For this reason, [^{18}F]Lu29-024 was synthesized, in a two-step procedure, with reproducible yield and higher specific activities than those obtained from [^{11}C]Lu29-024 (11 to 15 GBq/μmol). The biological characteristics of such a radioligand, which are presently under determination, might open a number of avenues for future clinical investigations.

References

1. (a) Perry, E. K.; Perry, R. H.; Candy, J. M.; Fairbairn, A. F.; Blessed, G.; Dick, D. J. *Neurosci. Lett*. **1984**, *51*, 353. (b) Dean, B.; Hayes, W. *Schizophr. Res*. **1996**, *21*, 133.
2. Amokhtari, M.; Andersen, K.; Ibazizene, M.; Dhilly, M.; Dauphin, F.; Barré, L. *Nucl. Med. Biol*. **1998**, *25*, 517.
3. Andersen, K.; Perregaard, J.; Arnt, J.; Bay Nielsen, J.; Begtrup, M. *J. Med. Chem*. **1992**, *35*, 4823.
4. Pelchowicz, Z.; Kaluszyner, A.; Bentov, M. *J. Chem. Soc*. **1961**, *5418*.
5. Bruggink, A.; McKillop, A. *Tetrahedron* **1975**, *31*, 2607.
6. Sugai, S.; Ikawa, H.; Okasaki, T.; Akaboshi, S.; Ikegami, S. *Chem. Lett*. **1982**, *5*, 597.
7. Ames, D. E.; Ribeiro, O. *J. Chem. Soc., Perkin Trans. 1* **1976**, *1073*.
8. Adams, R.; Blomstrom, D. C. *J. Am. Chem. Soc*. **1953**, *3403*.
9. Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc*. **1971**, *93*, 2897.
10. Taguchi, K.; Westheimer, F. H *J. Org. Chem*. **1971**, *36*, 1570.
11. Lemaire, C.; Damhaut, P.; Plenevaux, A.; Cantineau, R.; Christiaens, L.; Guillaume, M. *Appl. Radiat. Isot*. **1992**, *43*, 485.
12. Plenevaux, A.; Lemaire, C.; Palmer, A. J.; Damhaut, P.; Comar, D. *Appl. Radiat. Isot*. **1992**, *43*, 1035.
13. Besret, L.; Dauphin, F.; Huard, C.; Lasne, M. C.; Vivet, R.; Mickala, P.; Barbelivien, A.; Baron, J. C. *Nucl. Med. Biol*. **1996**, *23*, 169.
14. Besret, L.; Dauphin, F.; Guillouet, S.; Dhilly, M.; Gourand, F.; Blaizot, X.; Young, A. R.; Petit-Taboué, M. C.; Mickala, P.; Barbelivien, A.; Rault, S.; Barré, L.; Baron, J. C. *Life Sci*. **1998**, *62*, 115.