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Research Article

Radiosynthesis and *in vivo* evaluation of [^{11}C]Ro-647312: a novel NR1/2B subtype selective NMDA receptor radioligand

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Summary

[2-(3,4-Dihydro-1H-isoquinolin-2-yl)-pyridin-4-yl]-dimethylamine, Ro-647312 (**1**) represents a new novel class of NR1/2B subtype selective NMDA ligand. Ro-647312 has been radiolabelled with carbon-11 using [^{11}C]methyl triflate from the *nor*-methyl compound **2**. The reaction was performed in acetone as solvent using aqueous NaOH as base. Following HPLC purification [^{11}C]Ro-647312 ([^{11}C]-**1**) was obtained in 6.9–9.2% ($n = 3$) radiochemical yield decay-corrected based on starting [^{11}C]CO_2, with specific radioactivity measured at the end of the radiosynthesis ranging from 1.0 to 3.5 Ci/μmol (37–129 GBq/μmol). Radiochemical and chemical purities were assessed as >99 and $>95\%$, respectively. Following *i.v.* injection of [^{11}C]-**1** in rat, the distribution of radioactivity was homogeneous in all brain structures and did not correlate with the known distribution of NR2B subunits. The radioactivity observed in plasma was also higher than any brain structure throughout the time course of the experiment. [^{11}C]-**1** does not possess the required properties for imaging NMDA receptors using positron emission tomography. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: Ro-647312; NMDA receptor; carbon-11; positron emission tomography; methyl triflate

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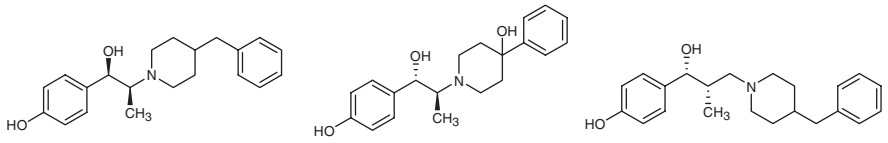
Introduction

Glutamate is the major excitatory neurotransmitter in the mammalian CNS. Glutamate receptors are divided into ionotropic and metabotropic receptors. In addition, ionotropic glutamate receptors are further divided into *N*-methyl-D-aspartate (NMDA) receptors and non-NMDA receptors such as AMPA and Kainate receptors.[1] An overactivity of glutamertergic systems has been implicated in the neuropathology of many neurological and neuropsychiatric disorders, such as Parkinson's disease, Huntington's chorea, schizophrenia, alcoholism and stroke.[2,3] Neuronal cell death which is caused by this overactivity is mainly found to be mediated by ionotropic glutamate receptors, namely NMDA and also non-NMDA (AMPA and Kainate) receptors.[4,5]

Functional NMDA receptors are heteromers composed of both members of the two subunit families, namely NR1 (eight different splice variants) and NR2 (A-D) subunits.[6] The subunit composition of the NMDA receptor confers diverse properties with the NR1 subunits responsible for forming a functional NMDA channel while the role of the NR2 subunits are thought to modulate the functions of the ion channel.[7] Blockage of ionotropic glutamate receptors, in particular the NMDA receptors, is believed to have therapeutic potential in treatment of many neurological and neuropsychiatric disorders. The clinical usefulness of first-generation non-subtype selective NMDA antagonists were limited by severe side effects. However, second generation NR1/2B subtype selective antagonists such as ifenprodil, CP-101,606 and Ro-25-6981 displayed improved neuroprotective potential with reduced side-effects (Figure 1).[8,9]

Introduction of non-invasive imaging techniques such as positron emission tomography (PET) has made possible the study of neuroreceptors in the living human brain. Such studies have proven useful in the localisation and quantification of neuroreceptors and offer insight into the relationship of these receptors in normal and pathological states.[10] Attempts to image NMDA receptors using PET have mainly focused on development of carbon-11-labelled radioligands for the glycine binding site[11–14] and the ion channel[15–19] which have proved unsuccessful. Recently, ifenprodil analogues selective for the NR2B subunit have also been radiolabelled and evaluated, which include [^{11}C]CP-101,606 (IC_{50} = 10 nM for protection against glutamate toxicity),[20] 5-[3-(4-benzylpiperidin-1-yl)prop-1-ynyl]-1,3-dihydrobenzoimidazol-2-[^{11}C]one



Ifenprodil CP-101,606 Ro-25-6981

Figure 1. NR1/2B NMDA receptor subtype selective ligands

($IC_{50} = 5.3$ nM for inhibition of NMDA responses)[21] and [^{11}C]EMD-95885 ($IC_{50} = 3.9$ nM for the NR2B subunit labelled with [^{3}H]ifenprodil).[22] Even though these radioligands displayed *in vitro* selectivity for the NR2B subunit, this selectivity was not observed *in vivo*.

More recently a series of novel 2-(3,4-dihydro-1H-isoquinolin-2yl)-pyridines have been reported as a new class of selective NR1/2B NMDA receptor antagonists.[23] From this series, [2-(3,4-dihydro-1H-isoquinolin-2-yl)-pyridin-4-yl]-dimethylamine, Ro-647312 (**1**), displayed high affinity for NMDA receptors ($Ki = 8$ nM labelled with [^{3}H]Ro-256981) when compared to adrenergic α1 and muscarinic M1 receptors ($Ki = 490$ and 150 nM, respectively).[23] Based on these *in vitro* observations and the fact that **1** could be labelled with carbon-11 using [^{11}C]methyl triflate at its methyl tertiary-amine function, it was considered an appropriate candidate for potential imaging of NMDA receptors with PET.

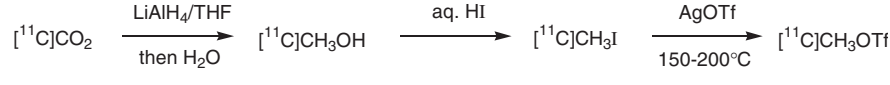
In this paper we report the radiosynthesis of [^{11}C]Ro-647312 and its initial biodistribution study in rats.

Results and discussion

Radiochemistry

Ro-647312 (**1**) was labelled with carbon-11 at its methyl tertiary-amine function from the corresponding *N-nor*-methyl precursor **2** and the highly efficient methylation reagent [^{11}C]methyl triflate.

[^{11}C]Methyl triflate was prepared according to a literature procedure from [^{11}C]methyl iodide using silver triflate.[24] [^{11}C]Methyl iodide was prepared from [^{11}C]carbon dioxide using the well-known two step, one pot protocol, consisting of the trapping of [^{11}C]CO_2 and conversion into [^{11}C]methanol ($LiAlH_4$) followed by iodination using aqueous HI giving [^{11}C]methyl iodide (Scheme 1).[25]

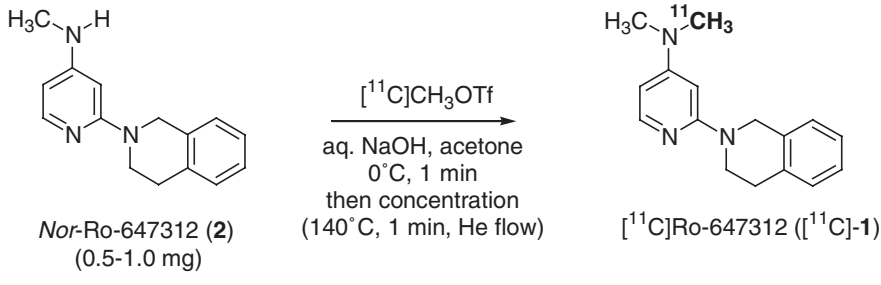


Scheme 1. [^{11}C]Methyl iodide and [^{11}C]methyl triflate preparation from [^{11}C]carbon dioxide

On average, about 650 mCi (24.1 GBq) of [^{11}C]CH_3OTf was routinely obtained in 7–8 min after end of bombardment (EOB) in 70% decay-corrected yield, based on starting [^{11}C]CO_2.

Reaction of the *nor*-methyl compound **2** (1.0 mg) with [^{11}C]methyl triflate as the alkylating agent, employing the standard conditions that have so far been used in our laboratory for the routine radiosynthesis of several

radiotracers,[26–28] gave the best results for the preparation of [^{11}C]Ro-647312 ([^{11}C]-**1**) (Scheme 2). The conditions used were the following: (1) trapping at room temperature of [^{11}C]methyl triflate in 300 µl of acetone containing 1.0 mg of precursor **2** (4.2 µmol) and 4 µl of a 3 M solution of NaOH in water (about 3 eq.); (2) concentration to dryness of the reaction mixture (at 110°C, using an helium stream for 2 min); (3) taking up the residue with 0.5 ml of the HPLC mobile phase and (4) purification using semi-preparative HPLC. Typically, starting from a 1.2 Ci (44.4 GBq) [^{11}C]CO_2 production batch, 30–40 mCi (1.11–1.48 GBq) of [^{11}C]-**1** was obtained within 30 min of radiosynthesis, including HPLC purification. The total decay-corrected radiochemical yield of [^{11}C]-**1**, based on starting [^{11}C]CO_2, was 6.9–9.2% ($n = 3$). The specific radioactivity measured at the end of the radiosynthesis ranged from 1.0 to 3.5 Ci/µmol (37–129 GBq/µmol). The HPLC-purification chromatogram was systematically complex showing at least five additional peaks, representing radioactive side-products. [^{11}C]Ro-647312 ([^{11}C]-**1**) was nevertheless the major product of the reaction and was efficiently isolated.



Scheme 2. Preparation of [^{11}C]Ro-647312 ([^{11}C]-1) from [^{11}C]methyl triflate

The use of another base, trimethylbenzylammonium hydroxide (TMBA), as well as the use of slightly more (4 eq.) or less (1–2 eq.) of the base did not improve the yield. Tributylphosphate or acetonitrile as the solvent gave lower yields. No further efforts were made in order to increase the observed low yields.

Formulation and quality control

Formulation of [^{11}C]Ro-647312 ([^{11}C]-**1**) for *i.v.* injection was carried out as follows: The HPLC-collected fraction containing [^{11}C]-**1** was diluted with water and the resulting solution was passed through a C18 Sep-pak® cartridge. The cartridge was then washed twice with water, partially dried with nitrogen and finally eluted with ethanol. The solution was then sterile-filtered and diluted with physiological saline.

The radiopharmaceutical preparation was a clear and colourless solution and its pH was between 5 and 7. As demonstrated by HPLC analysis, the radiopharmaceutical preparation was found to be >95% chemically and >99% radiochemically pure and was radiochemically stable for at least 30 min. Administration to animals was performed within 10 min following end of synthesis.

Biodistribution studies

Uptake of [^{11}C]Ro-647312 ([^{11}C]-**1**) in the rat brain was determined in biodistribution experiments (Figure 2). Following *i.v.* injection of [^{11}C]Ro-647312 ([^{11}C]-**1**, 15–30 μCi with specific radioactivity greater than 1 Ci/μmol), the distribution of the radioactivity was homogeneous in all brain structures studied. The uptake in the cerebellum and striatum at 5 min was 0.2 and 0.1% ID/g tissue, respectively. NR2B subunits are primarily localised in forebrain regions, hippocampus > cortex > striatum > thalamus > cerebellum.[29] When specific binding was defined as the ratio of the radioactivity concentration in hippocampus and cerebellum, a value of 1.0–0.7 was obtained throughout the time course of the experiment. Therefore, the *in vivo* regional brain distribution of [^{11}C]Ro-647312 ([^{11}C]-**1**) did not correlate with the known distribution of NR2B subunits. The radioactivity observed in plasma was higher than any brain structure at all time points, again indicative of non-specific binding. In the peripheral organs, the kidney and the lung showed the highest radioactivity (Figure 3).

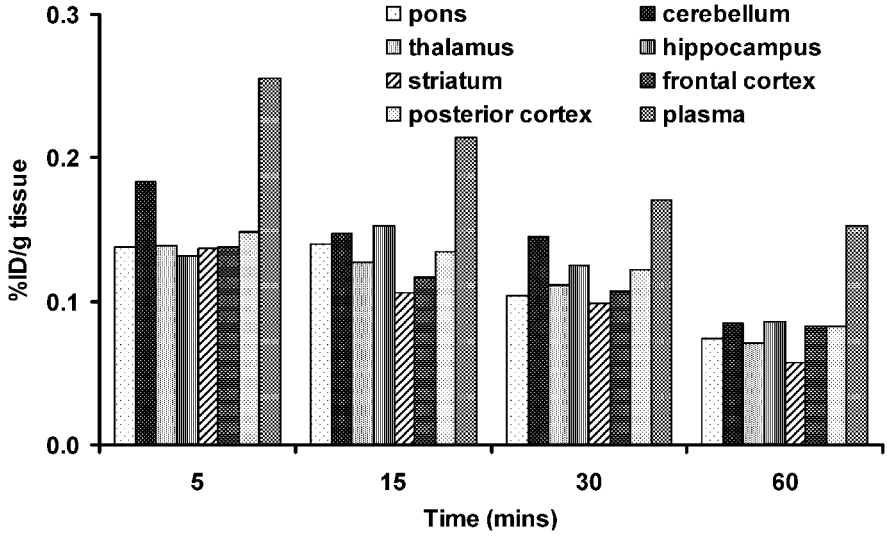


Figure 2. Biodistribution of [^{11}C]Ro-647312 ([^{11}C]-1) in rat brain

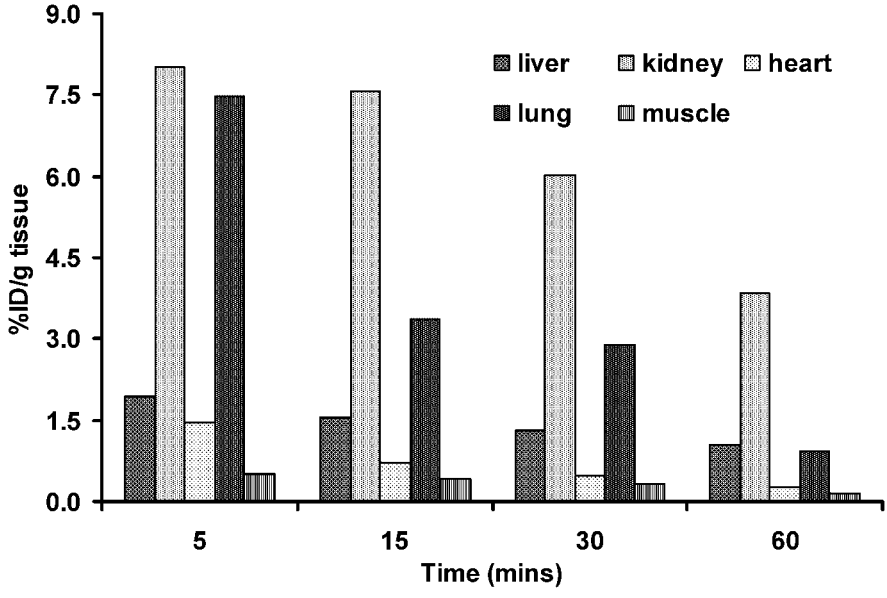


Figure 3. Biodistribution of [^{11}C]Ro-647312 ([^{11}C]-1) in rat peripheral organs

Experimental

General

Chemicals were purchased from standard commercial sources (Aldrich, Fluka or Sigma France) and were used without further purification unless stated otherwise. Ro-647312 (**1**, fumarate salt, 50 mg) as well as its *N-nor*-methyl derivative (**2**, fumarate salt, 30 mg) as precursor for labelling with carbon-11 were kindly donated by Hoffman-La Roche (Dr Bernd Buttelman, Basel Switzerland). HPLCs: HPLC A: Equipment: Waters or Shimadzu systems. For example, Waters systems equipped with a 510 pump, 440 UV detector or 481/486 UV-multiwavelength detectors; column: semipreparative Symmetry-Prep® C-18, Waters (300 × 7.8 mm); porosity: 7 μm; conditions: isocratic elution with: H_2O/CH_3CN/TFA: 75/25/0.1 (v:v:v); flow rate:7.0 ml/min; temperature: RT; UV detection at λ: 243 nm; HPLC B: Equipment: Waters Alliance 2690 equipped with a UV spectrophotometer (Photodiode Array Detector, Waters 996) and a Berthold LB509 radioactivity detector; column: analytical Symmetry-M® C-18, Waters (4.6 × 50 mm, microcolumn); porosity: 5 μm; conditions: isocratic elution with solvA/solvB: 40/60 (v:v) [solvA: H_2O containing Low-UV PIC® B7 reagent (Waters), 20 ml for 1000 ml; solvB: H_2O/CH_3CN: 50/50 (v:v) containing Low-UV PIC® B7 reagent (% by weight: methanol (18–22%), heptane sulfonic acid - sodium salts (4–6%), phosphate buffer solution (3–7%), water (65–75%), pH 3, Waters), 20 ml for 1000 ml];

flow rate: 2.0 ml/min; temperature: 30°C; UV detection at λ: 214 nm. Radiosyntheses were performed in a 5-cm-lead shielded hot cell.

Preparation of [^{11}C]CO_2

[^{11}C]CO_2 was produced via the ^{14}N[p,α]^{11}C nuclear reaction by irradiation of an ultrapure N60 Air Liquide N_2 target with a 20 MeV proton beam (at 30 μA) on a CGR-MeV 520 cyclotron (15 μA h in about 30 min) or by irradiation of an ultrapure Air Liquide 99.5/0.5 mixture of N_2/O_2 target with a 18 MeV proton beam (at 25 μA) on a IBA Cyclone-18/9 cyclotron (8.5 μA h in about 20 min). At the end of the bombardment, the target contents were transferred to the 5-cm-lead shielded hot cell and passed through a glass P_2O_5-guard (70 mm length, 3 mm internal diameter) in order to remove moisture. [^{11}C]CO_2 was then separated from the target gas by trapping in an empty stainless-steel coil (150 mm length, 0.51 mm internal diameter), cooled at −186°C using liquid argon. On average, about 1.20 Ci or 44.40 GBq (EOB) of [^{11}C]CO_2 is routinely obtained in our laboratory for the irradiations described above.

Preparation of [^{11}C]CH_3OTf

[^{11}C]CO_2 was released from the trap by raising the stainless-steel coil temperature to ambient, swept away by a flow of nitrogen gas (40 ml/min) and trapped at −10°C (EtOH-ice bath) in 55 μl of THF containing 5 μl of 1.0 M THF solution of lithium aluminium hydride. Concentration to dryness (evaporation of solvent at 165°C using a stream of nitrogen) followed by hydrolysis (100 μl of deionized water) of the formed aluminium complex afforded [^{11}C]CH_3OH, which was distilled using a flow of nitrogen gas into 1 ml of an aqueous 57% HI solution (heating block at 165°C). The [^{11}C]CH_3I thus synthesized was continuously swept away by a flow of nitrogen gas, passed through a combined 1/1 (v:v) soda lime/P_2O_5-guard (35 mm length each, 3 mm internal diameter) and converted into [^{11}C]CH_3OTf by passing through a glass column (33 cm length, 5 mm internal diameter), heated at 200°C and containing silver-triflate-impregnated graphitized carbon (200 mg). About 650 mCi (24.1 GBq) of [^{11}C]CH_3I or [^{11}C]CH_3OTf is routinely obtained in our laboratory in 7–8 min after EOB in 70% decay-corrected yield, based on starting [^{11}C]CO_2.

*Preparation of [^{11}C]Ro-647312 ([^{11}C]-**1**)*

[^{11}C]CH_3OTf, carried by a flow of nitrogen gas, was trapped (bubbling through) at 0°C (ice bath) in a reaction vessel containing 0.5–1.0 mg of *nor*-Ro-647312 (**2**, as free base, 2.1–4.2 μmol) dissolved in 200–400 μl of the solvent used (acetonitrile, tributylphosphate or acetone) and 0.5–4.5 eq. of the base used (for TMBA, a 0.5 M solution of TMBA in EtOH; for NaOH, a 3 M

solution of NaOH in water). Trapping of [^{11}C]CH_3OTf was monitored using an ionisation-chamber probe. When the reading had reached its maximum (2–3 min), the reaction vessel was then isolated, heated at 80–140°C using a heating block for 0.5–1 min. When acetone was used as the solvent, the reaction mixture was concentrated to dryness (at 140°C, using a helium stream for 2 min), then the residue was taken up with 0.5 ml of the HPLC mobile phase and was simply injected onto the column. When acetonitrile or tributylphosphate was used, the reaction vessel was cooled (EtOH-ice bath) and the reaction mixture was then diluted with 0.5 ml of the HPLC mobile phase and was injected onto the column. HPLC-purification (HPLC A) gave radiochemically pure [^{11}C]Ro-647312 ([^{11}C]-(**1**), Rt: (**1**): 8.5–9.0 min), well separated from the unlabelled precursor **2** (6.0–7.0 min).

Optimal conditions

[^{11}C]CH_3OTf, carried by a flow of nitrogen gas, was trapped (bubbling through) at 0°C (ice bath) in a reaction vessel containing 1.0 mg of *N-nor*-methyl compound (**2**, as free base, 4.2 μmol) dissolved in acetone (300 μl) containing 4 μl of a 3 M aq. NaOH solution (12 μmol, about 3 eq.).

*Formulation of [^{11}C]Ro-647312 ([^{11}C]-**1**)*

Formulation of labelled product for *i.v.* injection was carried out as follows: The HPLC-collected fraction containing [^{11}C]Ro-647312 ([^{11}C]-**1**) was diluted with water (50 ml). The resulting solution was passed through a C18 Sep-pak® cartridge (Waters). The cartridge was washed twice with 5 ml of water and partially dried for 10 s by applying a nitrogen stream. The carbon-11 labelled tracer was eluted with 2 ml of EtOH (less than 10% of the total radioactivity was left on the cartridge) and filtered on a 0.22 μm GS-Millipore filter (vented). Finally, physiological saline was added to lower the EtOH concentration below 10%. This whole process was performed using a remote-controlled dedicated home-made device based on a literature procedure.[28]

*Quality control of [^{11}C]Ro-647312 ([^{11}C]-**1**)*

The radiopharmaceutical preparation was a clear and colourless solution with pH between 5 and 7. As demonstrated by HPLC analysis (HPLC B), the radiolabelled product was found to be >99% radiochemically pure and also co-eluted with a sample of authentic Ro-6473121 (**1**) (HPLC B; retention time: 2.5 min). The preparation was shown to be free of non-radioactive precursor (HPLC B; retention time: 1.5 min) and radiochemically stable for at least 30 min. Specific radioactivity was calculated from three consecutive HPLC analyses and determined as follows: the area of the UV absorbance peak corresponding to the radiolabelled product was measured (integrated) on the

HPLC chromatogram and compared to a standard curve relating mass to UV absorbance.

Biodistribution studies

Sprague-Dawley male rats weighing 200–250 g were used in all experiments. Animal procedures were in accordance with the recommendations of the EEC (86/609/CEE) and the French National Committee (decret 87/848) for the care and use of laboratory animals. Each animal received 15–30 μCi of the radioligand ([^{11}C]Ro-647312, [^{11}C]-**1**), dissolved in 0.1 ml saline, by injection in a tail vein. At designated times (5, 15, 30, 60 min) post-injection of the radioligand, animals ($n = 3$ per time point) were sacrificed by decapitation, the brains as well as peripheral organs were quickly removed, dissected, weighed and assayed for radioactivity in a γ-counter (Cobra Quantum, Packard). Samples of liver, kidney, heart, lung, muscle, plasma and for the brain, pons, cerebellum, thalamus, hippocampus, striatum, frontal cortex, posterior cortex were obtained for each animal. Results were expressed as % injected dose per gram of tissue (% ID/g tissue) after correction for the physical decay of the radioisotope.

Conclusion

The novel NR1/2B subtype-selective antagonist, [2-(3,4-dihydro-1H-isoquinolin-2-yl)-pyridin-4-yl]-dimethylamine, Ro-647312 (**1**), has been labelled with carbon-11 at its methyl tertiary-amine function using [^{11}C]methyl triflate. The evaluation of its *in vivo* pharmacological profile using biodistribution studies clearly indicates that [^{11}C]Ro-647312 ([^{11}C]-**1**) does not have the required properties for imaging NMDA receptors using positron emission tomography.

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