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Research Article

Synthesis of (±) 3-(6-nitro-2-quinolinyl)-[9-methyl-^{11}C]-3,9-diazabicyclo-[4.2.1]-nonane ([^{11}C-methyl]NS 4194)

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Summary

(±) 3-(6-Nitro-2-quinolinyl)-[9-methyl-^{11}C]-3,9-diazabicyclo-[4.2.1]-nonane ([^{11}C-methyl]NS 4194), a selective serotonin reuptake inhibitor (SSRI), was synthesised within 35 min after end of bombardment with a radiochemical purity >98%. It had a decay-corrected radiochemical yield of 7% after preparative HPLC, and a specific radioactivity around 37 GBq/μmol (EOS). A typical production starting with 40 GBq [^{11}C]CO_2 yielded 800 MBq of radiolabelled [^{11}C-methyl]NS 4194 in a formulated solution. The synthesis of the precursor to [^{11}C-methyl]NS 4194, (±) 9-H-3-[6-nitro-(2-quinolinyl)]-3,9-diazabicyclo-[4.2.1]-nonane, as well as the unlabelled analogue (±) 9-methyl 3-[6-nitro-(2-quinolinyl)]-3,9-diazabicyclo-[4.2.1]-nonane (NS 4194), are also described. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: PET; ^{11}C; radiolabelling; selective serotonin reuptake inhibitor

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Introduction

Serotonergic neurotransmission plays an important role in many neuropsychiatric disorders.[1–3] Antidepressant actions of drugs are, for example, often associated with inhibitory action on the serotonin reuptake site.[4,5] Previously, we examined properties of some structurally related [^{11}C]-radiolabelled selective serotonin reuptake inhibitors (SSRIs) (Figure 1), but found them to have some downfalls for use in PET neuroimaging.[6,7] Due to our interest in evaluating serotonergic neurotransmission by positron emission tomography (PET),[8,9] we labelled (±) 9-H-3-[6-nitro-(2-quinolinyl)]-3, 9-diazabicyclo-[4.2.1]-nonane to obtain [^{11}C-methyl]-NS 4194, a new, high affinity, selective serotonin reuptake inhibitor (SSRI).[10]

Experimental

General

The NMR spectrum was recorded on a Bruker AM 500 MHz spectrometer. The mass spectrum was obtained on a JEOL JMS AX-505W double focusing mass spectrometer. The melting points were determined with a Griffin melting point apparatus. All chemicals were used without further purification unless otherwise stated.

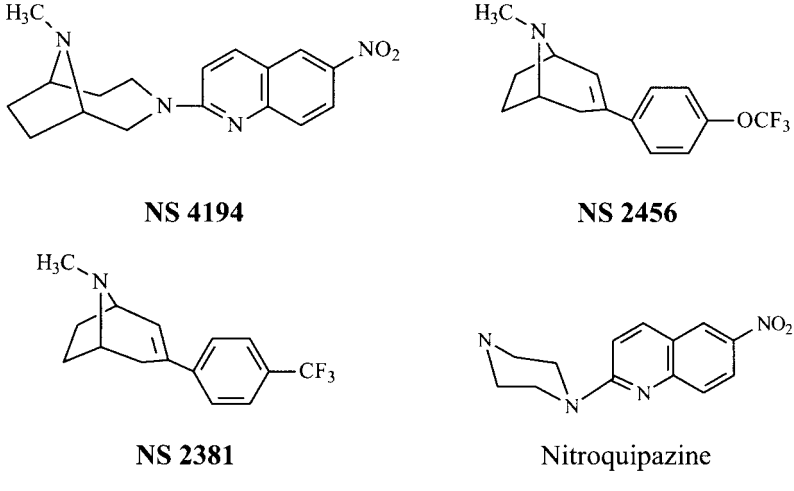


Figure 1. Structure of NS 4194, NS 2456, NS 2381 and nitroquipazine

(±) 9-Methyl-3,9-diazabicyclo-[4.2.1]-nonane[11] and 2-chloro-6-nitroquinoline[12] were prepared according to published procedures. Dimethylsulphoxide anhydrous (DMSO), 2,6-di-*tert*-butylpyridine (97%), *N*-iodosuccinimide, fumaric acid, acetonitrile (HPLC grade), and other solvents were purchased from Aldrich Chemical Co. Lithium aluminium hydride (LAH) was obtained from Fluka and was divided into 5 ml vials under argon in a glove box. The vials were sealed and were stored in a glove box. A fresh, saturated suspension of LAH was made before each experiment by adding 5 ml anhydrous Sure/Seal™ tetrahydrofuran (THF, Fluka) to the 5 ml vial containing the LAH powder. The suspension was diluted 4-fold under argon by THF in a second 5 ml vial before used. Acetic acid was obtained from Bie & Berntsen A/S, Århus, Denmark.

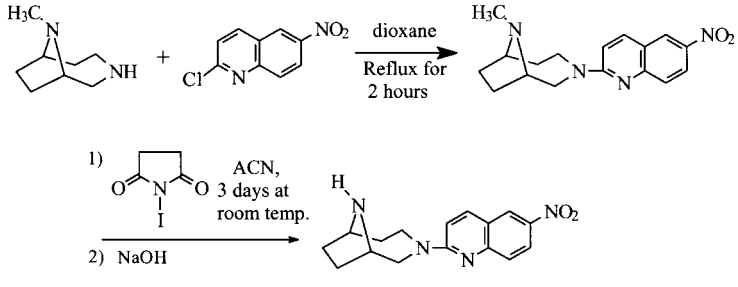
Biochemical procedure

Procedures involving animals were approved by the Danish National Committee for ethics in biomedical research. Studies of serotonin, dopamine and noradrenaline uptake were carried out *in vitro* using brain tissue obtained from male Wistar rats (150–200 g). Samples of cerebral corticies were used for serotonin uptake, corpus striata were used for dopamine uptake, and hippocampi were used for noradrenaline uptake. All samples were homogenized in ice-cold 0.32 M sucrose containing 1 mM pargyline. Preparation of synaptosomes and incubation of samples with [^{3}H]serotonin (1 nM), [^{3}H]dopamine (1 nM) or [^{3}H]noradrenaline (1 nM) were carried out according to conventional procedures.[13,14]

The concentrations of NS 4194 used for the incubations were 0.01–30 μM for noradrenaline and dopamine uptake, and 0.0001–1 μM for serotonin uptake. The samples were poured directly onto Whatman GF/C glass fibre filters under suction after incubation. Then, the filters were washed three times with 5 ml of ice-cold 0.9% (w/v) NaCl solution, and the amount of radioactivity on the filters was determined by conventional liquid scintillation counting. Specific uptake was calculated as the difference between total uptake and uptake measured in the presence of a selective uptake inhibitor (*rac*-citalopram, benztropine or desipramine at final concentration of 1 μM for inhibition of serotonin, dopamine or noradrenaline uptake, respectively).

(±) 9-Methyl 3-[6-nitro-(2-quinolinyl)]-3,9-diazabicyclo-[4.2.1]-nonane (NS 4194) (Scheme 1). A mixture of 2-chloro-6-nitroquinoline[12] (2.0 g, 9.6 mmol), (±) 9-methyl-3,9-diazabicyclo-[4.2.1]-nonane[11] (1.4 g, 9.6 mmol) and dioxane (4 ml) was heated at reflux for 2 h. Aqueous sodium hydroxide (50 ml, 1 M) was added. The crystalline product was filtered and washed with diethyl ether (50 ml) to give NS 4194 (1.1 g, 3.5 mmol, 37%). M.p. 148–150°C. $R_f = 0.38$ (silica gel 60 F254 t.l.c using dichloromethane, methanol and conc. ammonia (89:10:1) as eluent). ^{1}H-NMR ($CDCl_3$): δ 8.81 (d, $J = 3$, 1 H), 8.36 (d, $J = 9$, 1 H), 8.34 (dd, $J = 9/3$, 1 H), 7.67 (d, $J = 9$, 1 H), 7.43 (d, broad, 1 H), 3.75–3.65 (m, 1 H), 3.48 (s, 3 H), 3.47–3.41(m, 2 H), 3.40–3.34 (m, 2 H), 2.65–2.62 (m, 1 H), 2.25–2.16 (m, 1 H), 2.09–1.99 (m, 2 H), 1.89–1.79 (m, 1 H), 1.67–1.57 (m, 1 H), 1.21–1.13 (m, 1 H). Ms (EI): $m/e = 312$.

(±) 9-H-3-[6-nitro-(2-quinolinyl)]-3,9-diazabicyclo-[4.2.1]-nonane fumaric acid salt. NS 4194 (1.85 g, 6.0 mmol), *N*-iodosuccinimide (4.0 g, 17.9 mmol), and acetonitrile (300 ml) were stirred at room temperature for 72 h. Aqueous sodium hydroxide (400 ml, 1 M) was added. The aqueous phase was extracted with dichloromethane (2×150 ml). Chromatography on silica gel with dichloromethane, methanol, and conc. ammonia (89:10:1) gave the title compound (0.20 g, 0.67 mmol, 11%). The corresponding salt was obtained by addition of a diethyl ether and methanol mixture (9:1) saturated with fumaric acid. M.p. 203–206°C. $R_f = 0.24$ (silica gel 60 F254 t.l.c. using dichloromethane, methanol and conc. ammonia (89:10:1) as eluent). ^{1}H-NMR ($(CD_3)_2SO$): δ 8.75 (d, $J = 3$, 1 H), 8.31 (d, $J = 9$, 1 H), 8.25 (dd, $J = 9/3$, 1 H), 7.59 (d, $J = 9$, 1 H), 7.42 (d, $J = 9$, 1 H), 6.55 (s, 2 H, fumaric acid),



Scheme 1. The synthesis of (±) 3-(6-Nitro-2-quinolinyl)-9-H-3, 9-diazabicyclo-[4.2.1]-nonane via unlabelled NS 4194

4.28–4.16 (m, 1 H), 4.07–3.95 (m, 2 H), 3.77–3.65 (m, 1 H), 3.48–3.37 (m, 2 H), 2.60–2.49 (m, 1 H), 2.08–1.94 (m, 3 H), 1.86–1.68 (m, 2 H), 1.28–1.18 (m, 1 H). Ms (EI): $m/e = 298$.

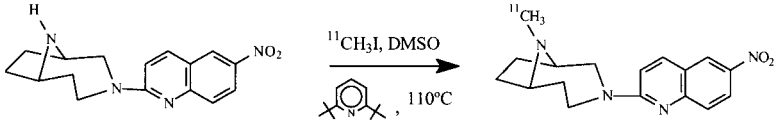
[^{11}C]Carbon dioxide. [^{11}C]Carbon dioxide was prepared by the ^{14}N(p,α)^{11}C nuclear reaction using a nitrogen gas target pressurised to 150 psi, and 16 MeV protons produced by the General Electric Medical Systems PETtrace 200 cyclotron at Aarhus University Hospital. Irradiation for 60 minutes with a beam current of 40 μA was typically used. The labelling procedure, including preparation of [^{11}C]methyl iodide, methylation, HPLC purification, rotary evaporation, and formulation, was performed using a fully automated set-up.[15]

Purification (HPLC system 1). Preparative HPLC was performed using an isocratic pump (Perkin Elmer model 200) equipped with a 1 ml injection loop and connected in series with a Phenomenex Nucleosil 5CN column, 250 × 10 mm, a variable wavelength UV detector (Applied Biosystems model 759A, $\lambda = 232$ nm), and a photodiode radiodetector of in-house design (HPLC system **1)**.

Identification (HPLC system 2). Analytical HPLC was performed using a Perkin Elmer model 250 pump with a 20 μl injection loop connected in series with a NucleosilSCN column (250 × 4.6 mm, 5 micron), a variable wavelength detector (Perkin Elmer model LC 295, $\lambda = 232$ nm), and a sodium iodide radiodetector of in-house design (HPLC system **2)**.

Production of [^{11}C]methyl iodide. [^{11}C]Carbon dioxide was purged from the target in a stream of nitrogen gas and trapped on 4 Å molecular sieves. The [^{11}C]CO_2 was released upon heating (ca. 250C°) and was passed through a solution of LAH (300 μl) in a stream of nitrogen gas (25 ml/min). On completion of [^{11}C]CO_2 transfer, the THF was evaporated and 1 ml hydriodic acid was added. The formed [^{11}C]methyl iodide was transferred in a stream of nitrogen gas (20 ml/min) to a solution of the precursor.

(±) 3-(6-nitro-2-quinolinyl)-[9-methyl-^{11}C]-3, 9-diazabicyclo-[4.2.1]-nonane ([^{11}C-methyl]-NS 4194) (Scheme 2). [^{11}C-methyl]NS 4194, (±) 3-(6-nitro-2-quinolinyl)-[9-methyl-^{11}C]-3, 9-diazabicyclo-[4.2.1]-nonane, was derived from (±) 3-(6-Nitro-2-quinolinyl)-9-H-3,



(±) 3-(6-Nitro-2-quinolinyl)-9-H-3, 9-diazabicyclo-[4.2.1]-nonane

[^{11}C-methyl]-NS 4194

Scheme 2. The synthesis of (±) 3-(6-nitro-2-quinolinyl)-[9-methyl-^{11}C]-3, 9-diazabicyclo-[4.2.1]-nonane, [^{11}C-methyl]NS 4194

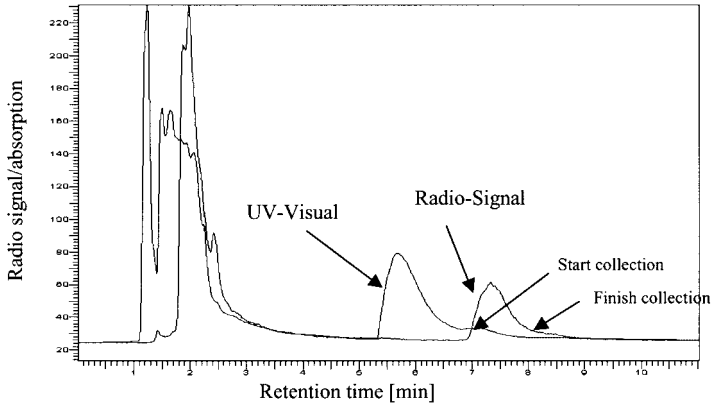


Figure 2. A typical semi-preparative HPLC chromatogram of the purification of [^{11}C-methyl]NS 4194 obtained using a UV-visual (λ = 232 nm) and a radio-detector

9-diazabicyclo-[4.2.1]-nonane as follows with a method described previously.[15] [^{11}C]Methyl iodide was reacted with (±) 3-(6-nitro-2-quinolinyl)-9-H-3,9-diazabicyclo-[4.2.1]-nonane (1.0 mg, 3.4 μmol) dissolved in DMSO (300 μl) and 2,6-di-*tert*-butylpyridin (10 μl, 50 μmol). After heating for 5 min at 110°C, the crude product was purified using HPLC system **1** with acetonitrile: 25 mM NaH_2PO_4 solution (pH = 3.5) (17:83) and with 0.1% acetic acid as eluent at a flow rate of 8 ml/min and with a UV detector wavelength of 232 nm. The fraction containing [^{11}C-methyl]NS 4194 was collected (Figure 2) and was transferred into a rotor vaporizer. Then, it was reduced to ca. 1 ml under reduced pressure at 110°C. The product fraction was formulated with a mixture of saline (9 ml) and 70% ethanol (1 ml). The radiochemical purity and product identity were determined by analytical HPLC using system **2** and the same eluent as described for HPLC system **1** at a flow of 2 ml/min.

Further evidence for the identity of the radiolabeled products was achieved by co-injection with authentic non-radioactive material.

Results and Discussion

Table 1 shows the effects of NS 4194 on neuronal uptake of monoamines measured *in vitro* in rat brain synaptosomes. NS 4194 was significantly more potent as inhibitor of serotonin uptake than of either noradrenaline or dopamine uptake (p's < 0.01); IC_{50} values were 3000-fold lower for [^{3}H]serotonin than for either [^{3}H]dopamine or [^{3}H]noradrenaline.

NS 4194 was synthesised by a condensation reaction between 2-chloro-6-nitroquinoline and 9-methyl-3,9-diazabicyclo-[4.2.1]-nonane (Scheme 1). Demethylation of NS 4194 with *N*-iodosuccinimide followed by aqueous sodium hydroxide gave (±) 3-(6-nitro-2-quinolinyl)-9-H-3,9-diazabicyclo-[4.2.1]-nonane, the precursor for the ^{11}C-methyl labelling reaction.

[^{11}C]Methylation of (±) 3-(6-nitro-2-quinolinyl)-9-H-3,9-diazabicyclo-[4.2.1]-nonane in DMSO at 110°C for 5 min using 2,6-di-*tert*-butylpyridine as base produced [^{11}C-methyl]NS 4194 as a major radioactive product (ca. 35%) with a total decay-corrected yield of 7% (EOB).

Reverse-phase semi-preparative HPLC was used to purify [^{11}C-methyl]NS 4194. The retention time of [^{11}C-methyl]NS 4194 was around 15 min in the absence of 0.1% acetic acid, and the peaks were broad, which made it difficult to separate [^{11}C-methyl]-NS 4194 from (±) 3-(6-nitro-2-quinolinyl)-9-H-3,9-diazabicyclo-[4.2.1]-nonane. However, when 0.1% acetic acid was added to the eluent the peaks appeared much faster (i.e. after 7–8 min) and were sharper, which made it easier to separate the product, [^{11}C-methyl]NS 4194, from the precursor, (±) 3-(6-Nitro-2-quinolinyl)-9-H-3,9-diazabicyclo-[4.2.1]-nonane. For a typical production starting with 40 GBq [^{11}C]CO_2, 800 MBq [^{11}C-

Table 1. IC_{50} values (μM) of NS 4194 on monoamine uptake in rat brain synaptosomes (Data are means for N = 3)

Compound	Serotonin	Noradrenaline	Dopamine
NS 4194	0.0037	17	21

methyl]NS 4194 was obtained in a synthesis plus preparative HPLC time of 35 min (counting from EOB). Analytical HPLC showed the product to be >98% radiochemically pure and to co-elute with the authentic sample of unlabeled (±) 3-(6-nitro-2-quinolinyl)-9-methyl-3, 9-diazabicyclo-[4.2.1]-nonane (NS 4194**)**. Approximately equivalent amounts of product and precursor were found in the final product.

Conclusions

^{11}C-labelled (±) 3-(6-nitro-2-quinolinyl)-[9-methyl-^{11}C]-3,9-diazabicyclo-[4.2.1]-nonane, [^{11}C-methyl]NS 4194, was produced with high radiochemical purity and high specific activity in sufficient quantities for in vivo studies. PET studies on the biodistribution of [^{11}C-methyl]NS 4194 in the living brain are currently in progress.

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