Synthesis and Brain Regional Distribution of [^{11}C]NPS 1506 in Mice and Rat: an *N*-Methyl-D-aspartate (NMDA) Receptor Antagonist

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NPS 1506 [3-fluoro-γ-(3-fluorophenyl)-*N*-methylbenzenepropamine] is representative of a non-psychotomimetic class of *N*-methyl-D-aspartate (NMDA) receptor antagonists. [^{11}C]NPS 1506 was prepared at high radiochemical purity (>98%) with a specific activity of around 50 GBq/μmol at the end of synthesis by methylation of the desmethyl precursor with [^{11}C]methyl iodide in the presence of NaH. Biodistribution of [^{11}C]NPS 1506 in mice and rat demonstrated that uptake into the brain was rapid and occurred at high levels. [^{11}C]NPS 1506 showed no appreciable specific binding in rodent brains under *in vivo* conditions, possibly because of both a large non-specific bound fraction and low *in vitro* binding affinity for NMDA receptors.

Key words NPS 1506; carbon-11; brain distribution; *N*-methyl-D-aspartate (NMDA) receptor

The glutamatergic *N*-methyl-D-aspartate (NMDA) receptors have been considered prime therapeutic targets for the development of useful neuroprotective strategies.[1] At present, many NMDA receptor antagonists, as exemplified by high-affinity channel blocker MK-801 and PCP, have serious unwanted effects, such as the induction of a psychotomimetic effect or cardiovascular dyregulation.[2]

Recently a novel non-psychotomimetic class of NMDA receptor open-channel blockers with a diphenylpropylamine skeleton has been described. NPS 1506 [3-fluoro-γ-(3-fluorophenyl)-*N*-methylbenzenepropamine] is representative of this class and currently used in human clinical trials for the treatment of acute ischemic stroke.[3,4] Pharmacological studies on NPS 1506 have shown[5]: (1) that this molecule is a moderate-affinity uncompetitive NMDA receptor antagonist (IC_{50}=664 nM at displacing radioligand from [^{3}H]MK-801 labeled binding sites); and (2) that NPS 1506 is neuroprotective in a variety of animal models of stroke and head injury; (3) that, importantly, at doses greater than those required for neuroprotection, NPS 1506 did not elicit in rodents the characteristic side effect profile and had no PCP-like psychotomimetic effects at doses between 1—5 mg/kg i.p. in rats.

Such a pharmacological profile, different from the previous NMDA antagonists, led us to label NPS 1506 with carbon-11 and study the distribution and pharmacokinetics of the tracer in animals. Positron emission tomography (PET) is an imaging modality for *in vivo* assessment of drug distribution and interaction with biochemical target systems by the use of tracers labeled with positron emitters such as carbon-11 ($T_{1/2}$=20.4 min).[6] In this paper, we describe the synthesis of ^{11}C-labeled NPS 1506 and preliminary results on regional brain distribution studies by the tissue dissection method in mice and by PET in a rat, with special attention to the ability of specific binding *in vivo*.

MATERIALS AND METHODS

Nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL GX-270 spectrometer 270 MHz with tetramethylsilane as an internal standard. All chemical shifts (δ) are reported in parts per million (ppm) downfield from the standard. Mass spectra were obtained on a JMS DX-610 spectrometer. Infrared (IR) spectra were recorded with a JASCO IR Report-100 spectrometer. Column chromatography was done on Merck Kieselgel 60 (70—230 mesh), and analytical thin layer chromatography (TLC) was carried out on Silica gel 60 F_{254} plates (Merck). In the synthetic procedures, organic extracts were routinely dried over anhydrous Na_2SO_4 and evaporated with a rotatory evaporator under reduced pressure. Radioactivity was quantified with a IGC-3R Curiemeter (Aloka). High-pressure liquid chromatography (HPLC) was done using a Waters HPLC system for nonradioactive runs or a JASCO HPLC system for radioactive runs. Effluent radioactivity from the HPLC was determined using a NaI (Tl) scintillation detector system. Carbon-11 was generated by the ^{14}N(p, α)^{11}C nuclear reaction using a CYPRIS HM-18 cyclotron (Sumitomo Heavy Industries, Ltd.). Preparation of [^{11}C]CH_3I and subsequent ^{11}C-methylation were carried out automatically by using a synthetic apparatus for ^{11}C-labeled compounds developed by Suzuki *et al.*[7] 3-Nitropropionic acid was purchased from Sigma Co. and all reagents were used as received unless otherwise stated. The animal experiments were carried out according to the recommendations of the committee for the care and use of laboratory animals, National Institute of Radiological Sciences.

3-Fluoro-γ-(3-fluorophenyl)-*N*-methylbenzenepropamine (NPS 1506) To a stirred solution of the hydrochloride salt of 3-fluoro-γ-(3-fluorophenyl)benzenepropamine (**1**)[8] (100 mg, 0.35 mmol) in a mixture of water and methanol (4 : 1, 2.5 ml) was added cyclopentadiene (130 μl, 1.58 mmol) and an aqueous solution of 37% formaldehyde (64 μl, 0.78

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mmol). After 66 h, the reaction mixture was neutralized with a 5% sodium bicarbonate solution and extracted with methylene chloride (20 ml×3). The combined extracts were washed with a saturated NaCl aqueous solution (30 ml), dried and evaporated to dryness. The crude product was chromatographed on a silica gel with $CHCl_3$: MeOH : NH_4OH= 49 : 1 : 0.1 to provide 2-azanorbornene adduct (**2**) (4.4 mg, 91%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (2H, t, J=6.4 Hz), 2.37—2.06 (7H, m), 3.19 (1H, q, J=3.9 Hz), 3.96 (1H, t, J=7.5 Hz), 5.93 (1H, dd, J=5.6, 2.0 Hz), 6.31 (1H, dd, J=5.6, 3.0 Hz), 7.02—6.84 (6H, m), 7.24 (2H, dt, J=14.3, 7.5 Hz). IR ($CHCl_3$) cm^{-1}: 2864, 1610, 1589. FAB-MS (m/z): 326 $(M+H)^+$.

To a solution of the 2-azanorbornene (**2**) (35 mg, 0.108 mmol) in $CHCl_3$ (1 ml) was added trifluoroacetic acid (1 ml) and triethylsilane (165 μl, 1.03 mmol). The resulting mixture was stirred at room temperature for 72 h. The solvent was removed under reduced pressure. The crude product was dissolved in chloroform (0.5 ml), treated with 10% hydrochloric acid (5.0 ml), and washed with a mixture of hexane and ether (1 : 1). The aqueous layer was neutralized with a 5% sodium bicarbonate solution and then the product was extracted with methylene chloride. The combined extracts were washed with a saturated NaCl aqueous solution (20 ml), dried and evaporated to dryness. The residue was purified by HPLC (column; nacalai tesque COSMOSIL 5C-18-MS, 10×250 mm, mobile phase; CH_3CN: 0.2% TFA in H_2O=35 : 65, flow rate; 4.0 ml/min) to give NPS 1506 (23 mg, 81%) as a colorless oil. ^{1}H-NMR ($CDCl_3$) δ: 2.23 (2H, q, J=7.5 Hz), 2.41 (3H, s), 2.56 (2H, t, J=7.3 Hz), 4.04 (1H, t, J=7.8 Hz), 7.03—6.85 (6H, m), 7.24 (2H, dt, J=14.2, 6.2 Hz). IR (KBr) cm^{-1}: 3022, 1676. FAB-MS (m/z): 262 $(M+H)^+$.

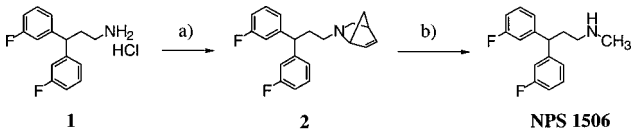
3-Fluoro-γ-(3-fluorophenyl)-*N*-[^{11}C]methylbenzenepropamine ([^{11}C]NPS 1506) The hydrochloride salt of (**1**) was further purified by HPLC (column; nacalai tesque COSMOSIL 5C-18-MS, 10×250 mm, mobile phase; CH_3CN: 0.2% TFA in H_2O=35 : 65, flow rate; 4.0 ml/min) and the trifluoroacetic acid salt obtained (10 mg, 0.027 mmol) was neutralized with a 5% sodium bicarbonate solution (5 ml) and extracted with ether (5 ml×4). The combined extracts were washed with a saturated NaCl aqueous solution (20 ml), dried and evaporated to dryness to give the free base of (**1**) (7 mg, 100%). Preparation of [^{11}C]CH_3I and subsequent ^{11}C-methylation of (**1**) to [^{11}C]NPS1506 were achieved automatically using specially designed equipment.[7] The [^{11}C]CH_3I prepared was trapped in anhydrous DMF (250 μl) containing the free base of (**1**) (1.0 mg) and NaH (4.5 μl, 1 M in DMF) at −15—−20 °C. The reaction vessel was heated at 30 °C and kept there for 3 min. After addition of an HPLC mobile phase (500 μl), the radioactive mixture was transferred onto an HPLC (column; nacalai tesque COSMOSIL 5C-18-MS, 10×250 mm; mobile phase; CH_3CN : 0.2% TFA in H_2O= 35 : 65, flow rate; 4.0 ml/min). A radioactive fraction having a retention time of 10 min was collected in a flask. After evaporation of the solvents from the flask under reduced pressure, the pure [^{11}C]NPS 1506 was reconstructed in sterile 0.9% saline. By this procedure, radiochemical pure (>98%) [^{11}C]NPS 1506 (300—370 MBq) was obtained in a synthesis time of 20 min from end of bombardment (EOB) after a 15—20 min proton bombardment at a beam current of 15 μA. Radiochemical purity was assayed by analytical HPLC (column; nacalai tesque COSMOSIL 5C-18-AR, 4.6×250 mm, UV at 254 nm, mobile phase; CH_3CN : 0.2% TFA in H_2O= 35 : 65, flow rate; 1.5 ml/min). Confirmation of the identity of [^{11}C]NPS 1506 was achieved with authentic NPS 1506. The specific activity at end of synthesis (EOS) was calculated by UV spectroscopy (254 nm) to be an average of 50 GBq/μmol.

Brain Distribution in Mice [^{11}C]NPS 1506 (in 0.2 ml saline, *ca.* 7.3 MBq) was injected intravenously *via* a tail vein into ddY mice (40—42 g). These mice (n=3 per time point) were killed at 1, 20, and 60 min by decapitation, the whole brains were rapidly removed and dissected into the cerebral cortex, striatum, hippocampus, and cerebellum. The samples were weighed and their radioactivities were measured with a Packard γ-counter and corrected for decay. A sample of blood was also taken. The results were expressed as the percent of administered dose per gram of tissue (% dose/g). In separate experiments, the effect of the carrier dose on the brain uptake was investigated. Unlabeled NPS 1506 (3 mg/kg) was co-injected with [^{11}C]NPS 1506 into the mice, which were sacrificed at 20 min after injection. The brain was removed, dissected and regional radioactivity was determined as described above. The control mice were treated with a saline solution under identical conditions.

PET of [^{11}C]NPS 1506 in a Rat One conscious rat (male Sprague-Dawley, 10 weeks old) was used for control scan and subsequent drug-pretreatment scan. The rat was fixed with a specifically designed head-hold device,[9] and PET scans were performed with a high-resolution SHR-7700 PET camera (Hamamatsu Photonics Shizuoka, Japan) which provides with 31 transaxial slices 3.6 mm (center-to-center) apart and a 14.8 cm field of view. After transmission scan for attenuation correction, dynamic emission scans were performed for 60 min (30 s×6 scans, 1 min×7 scans, 2 min×10 scans, 5 min×6 scans) following the [^{11}C]NPS 1506 injection (30 MBq and 54 MBq for control and pretreatment experiment, respectively) *via* the tail vein. The scan images were reconstructed with a Hanning filter of 4 mm, and circular regions of interest (ROIs) with a 5 mm diameter were placed over the striatum and hippocampus using image analysis software (in-house software PET Analyzer). The ROI values were expressed in kBq/ml, normalized to the injected radioactivity of 37 MBq, and plotted against time (Fig. 2). In the pretreatment experiment, 3-nitropropionic acid (20 mg/kg, 11.6 mg/0.8 ml saline) was injected intraperitoneally 3 h prior to the [^{11}C]NPS 1506 injection.

RESULTS AND DISCUSSION

NPS 1506 presents in its chemical structure one *N*-methyl group, which was considered to the most accessible site for labeling with carbon-11 using [^{11}C]methyl iodide. The precursor for ^{11}C-labeling, 3-fluoro-γ-(3-fluorophenyl)benzenepropamine (**1**) was prepared according to the published method.[8] NPS 1506 as the reference compound was prepared by the two-step *N*-methylation *via* a retro aza Diels–Alder reaction (Chart 1).[10] The immonium ion generated *in situ* from the hydrochloride salt of (**1**) and aqueous formaldehyde in water underwent a facile cyclocondenzation with cyclopentadiene to give the 2-azanorbornene adduct (**2**) in 91% yield. Subsequent treatment of (**2**) in $CHCl_3$/CF_3CO_2H with triethylsilane gave the desired NPS 1506 in 81% yield. Com-



a) HCHO, cyclopentadiene, 66 h b) Et_3SiH, TFA, 72 h

Chart 1. Synthesis of Target Compound NPS 1506

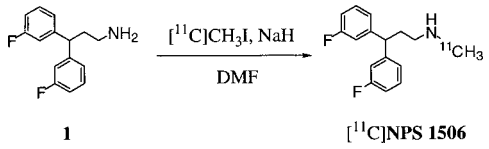


Chart 2. Radiosynthesis of [^{11}C]NPS 1506

Table 1. Biodistribution of [^{11}C]NPS 1506 in Mouse Brain and Blood

Tissue	% dose/g (mean)±S.D.[a)]		
	1 min	20 min	60 min
Blood	0.87±0.07	0.42±0.05	0.42±0.06
Cerebral cortex	1.98±0.32	3.84±0.35	4.16±0.53
Striatum	1.76±0.22	3.34±0.34	4.42±0.39
Hippocampus	1.69±0.25	3.31±0.44	4.00±0.67
Cerebellum	1.98±0.24	3.41±0.24	3.99±0.41

a) Average of three mice.

pound (**1**), (**2**) and NPS 1506 have analytical data such as ^{1}H-NMR and MS in agreement with the indicated structures.

[^{11}C]NPS 1506 was prepared by methylation of (**1**) with [^{11}C]methyl iodide in DMF in the presence of NaH at 30 °C for 3 min (Chart 2). The crude ^{11}C-reaction mixture was subjected to HPLC purification to give [^{11}C]NPS 1506 as an aqueous solution with >98% radiochemical purity. The precursor (**1**) was not detected by analytical HPLC system in the final solution of [^{11}C]NPS 1506. The total synthesis time was 20 min from EOB, and the specific activity was around 50 GBq/μmol at EOS. Although the reaction conditions have not been optimized, an average 330 MBq of [^{11}C]NPS 1506 after a 15—20 min proton bombardment at a beam current of 15 μA was obtained at EOS.

The brain biodistribution of [^{11}C]NPS 1506 after intravenous injection was investigated using mice (Table 1). Blood radioactivity was very low at 1 min (*ca.* 1% dose/g) and fell to <0.5% dose/g at 20 min after injection, indicating a rapid distribution of [^{11}C]NPS 1506 into the tissues. Radioactivity uptake in the brain was high and increased slightly with time, indicative of good blood–brain penetration. The regional brain distribution was fairly uniform and did not clearly reflect the expected distribution which is based on the known localization of NMDA receptors in the rodent brain.[11,12] We evaluated the effect of co-injection of unlabeled NPS 1506 (3 mg/kg per mouse) along with [^{11}C]NPS 1506 20 min after the tracer injection. As can be seen from Fig. 1, treatment with unlabeled NPS 1506 did not cause any change of the uptake in the mouse brain compared to the control group, suggesting that the brain uptake observed was unlikely to be mediated by a saturable, specific mechanism.

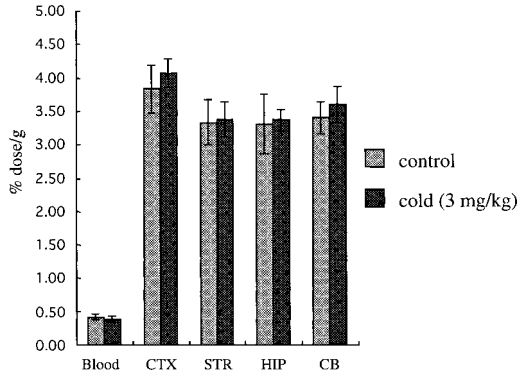


Fig. 1. Uptake of Radioactivity in Mouse Regional Brain and Blood at 20 min after Intravenous Injection of [^{11}C]NPS 1506 Together with NPS 1506 (3 mg/kg) or without NPS 1506 (Control)

The results are means (n=3)±S.D. expressed as % dose/g. No significant differences were detected. CTX: cerebral cortex, STR: striatum, HIP: hippocampus, CB: cerebellum.

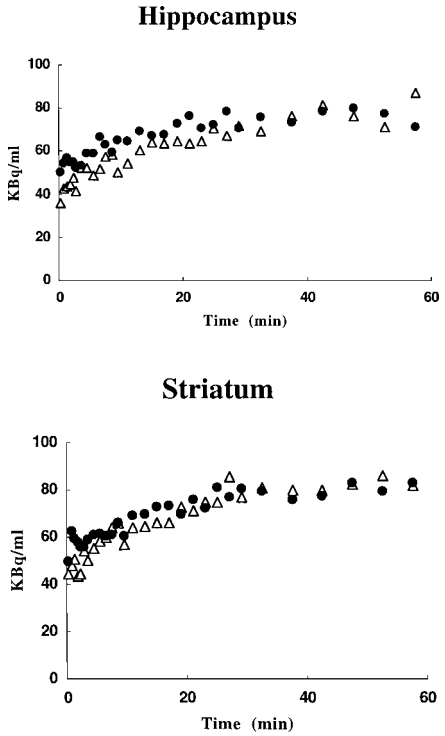


Fig. 2. Time Activity–Curves (TAC) in the Hippocampus and Striatum of Rat Obtained by PET after Intravenous Injection of [^{11}C]NPS 1506; Control (●) and Pretreatment with 20 mg/kg of 3-Nitropropionic Acid at 3 h before the Tracer Injection (△)

Each point was normalized to the injected dose of 37 MBq.

In vivo distribution was further assessed in the rat brain. Figure 2 shows the time–activity curves (TACs) in the hippocampus and striatum containing high densities of NMDA receptor for 60 min PET scanning after intravenous injection of [^{11}C]NPS 1506. Regional radioactivity in the hippocampus and striatum gradually increased with time, having a plateau from 30 min onwards, again reflecting good penetration of the tracer across the BBB. Recent studies have shown that the channel open probability of the NMDA receptors is not very high at the normal brain activity.[13] It is known that 3-nitropropionic acid (3-NP) causes NMDA receptor activation in the striatum and cortex when administrated acutely

to rats, as demonstrated by an increase in [^{3}H]MK-801 binding.[14] We examined the effect of prior treatment with 3-NP on the brain uptake of [^{11}C]NPS 1506. As can be seen from Fig. 2, pretreatment with 3-NP (20 mg/kg) given intraperitoneally 3 h prior to tracer injection did not produce any change in the regional uptake in the hippocampus nor the striatum.

In summary, the labeling of NPS 1506 with carbon-11 in the *N*-methyl position was accomplished with high specific activity and high radiochemical purity. [^{11}C]NPS 1506 administered in tracer doses readily crosses the BBB consistent with its partition coefficient ($\log P = 3.81$).[15] It has been reported that a number of NMDA receptor antagonists acting at the glycine site cross the BBB very poorly, despite their lack of dose-limiting side effects.[16] The good access of NPS 1506 to brain tissue gives it an advantage as an effective therapeutic agent over other antagonists. We could not prove that the accumulation of [^{11}C]NPS 1506 in rat and mouse brains was due to the specific binding to NMDA receptor binding sites. This could possibly result from both low *in vitro* affinity for NMDA receptors and large non-specific bound fractions, which might have masked the interaction with specific binding sites. In the present work, no metabolite studies were undertaken, and therefore whether the unspecific pattern of brain distribution of [^{11}C]NPS 1506 was due to radiolabeled metabolites that might have been formed in brain or penetrate the BBB remains uncertain. The observed brain retention of radioactivity may be related mainly to the affinity of [^{11}C]NPS 1506 itself for high-capacity, nonspecific binding sites in brain tissues, similar to the accumulation mechanism proposed for other lipophilic amines such as *N*-isopropyl-4-[^{123}I]-iodoamphetamine.[17] Further experiments are necessary to characterize the *in vivo* behavior of NPS 1506.

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REFERENCES AND NOTES

1) Brauner-Osborne H., Egebjerg J., Nielsen E. O., Madsen U., Krogsgaard-Larsen P., *J. Med. Chem.*, **43**, 2609—2645 (2000).
2) Kemp J. A., Mckernan R. M., *Nature Neurosci. Suppl.*, **5**, 1039—1042 (2002).
3) Moe S. T., Shimizu S. M., Smith D. L., Van Wagenen B. C., DelMar E. G., Balandrin M. F., Chien Y. (E)., Raszkiewicz J. L., Artman L. D., Mueller A. L., Lobkovsky E., Clardy J., *Bioorg. Med. Chem. Lett.*, **9**, 1915—1920 (1999).
4) Moe S. T., Smith D. L., DelMar E. G., Shimizu S. M., Van Wagenen B. C., Balandrin M. F., Chien Y. (E)., Raszkiewicz J. L., Artman L. D., White H. S., Mueller A. L., *Bioorg. Med. Chem. Lett.*, **10**, 2411—2415 (2000).
5) Mueller A. L., Artman L. D., Balandrin M. F., Brady E., Chien Y. (E)., DelMar E. G., George K., Kierstead A., Marriott T. B., Moe S. T., Newman M. K., Raszkiewicz, J. L., Sanguinetti E. L., Van Wagenen B. C., Wells D., *Ann. N.Y. Acad. Sci.*, **890**, 450—461 (1999).
6) Gibson R. E., Burns H. D., Hamill T. G., Eng W., Francis B. E., Ryan C., *Curr. Pharm. Design*, **6**, 973—989 (2000).
7) Suzuki K., Inoue O., Hashimoto K., Yamasaki T., Kuchiki M., Tamate K., *Appl. Radiat. Isot.*, **36**, 971—976 (1985).
8) Moe S. T., Smith D. L., By K., Egan J. A., Filer C. N., *J. Labelled Compds. Radiopharm.*, **41**, 535—543 (1998).
9) Okauchi T., Suhara T., Maeda J., Kawabe K., Obayashi S., Suzuki K., *Synapse*, **41**, 87—95 (2001).
10) Griecok P. A., Bahsas A., *J. Org. Chem.*, **52**, 5746—5749 (1987).
11) Wong E. H. F., Knight A. R., Woodruff G. N., *J. Neurochem.*, **50**, 274—281 (1988).
12) Ferrarese C., Guidotti A., Costa E., Miletich R. S., Rice K. C., de Costa B. R., Fulham M. J., Di Chiro G., *Neuropharmacol.*, **30**, 899—905 (1991).
13) Chen N., Luo T., Raymond L. A., *J. Neurosci.*, **19**, 6844—6854 (1999) and references cited therein.
14) Wüllner U., Young A. B., Penney J. B., Beal M. F., *J. Neurochem.*, **63**, 1772—1781 (1994).
15) Calculated value using Advanced Chemistry Development (ACD) Software Solaris V4.67, which is shown in STN databases.
16) Danysz W., Parsons C. G., *Pharmacol. Rev.*, **50**, 597—664 (1998).
17) Winchell H. S., Horst W. D., Braun L., Oldendorf W. H., Hattner R., Parker H., *J. Nucl. Med.*, **21**, 947—952 (1980).