Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **XLI**, 567-576 (1998)

Synthesis of a ^{11}C-Labelled Derivative of the *N*-Methyl-D-Aspartate Receptor Antagonist MK-801.

Yvonne Andersson,[1,2] Niklas Tyrefors,[1] Sven Sihver,[1,2] Hirotaka Onoe,[2,3] Yasuyoshi Watanabe,[2,3] Hideo Tsukada [4] and Bengt Långström* [1,2]

[1] Uppsala University PET Centre, UAS, S-751 85 Uppsala, Sweden
Telephone +46 18 471 33 81 Telefax +46 18 471 33 90
[2] The Subfemtomole Biorecognition Project, JST, Uppsala
[3]Department of Neuroscience, Osaka Bioscience Institute, Osaka, Japan
[4]Hamamatsu Photonics, Central Research Lab, Hamakita, Japan

SUMMARY

A one-pot synthesis of (+)-3-[^{11}C]cyano-MK-801 was performed using a palladium-promoted reaction of hydrogen [^{11}C]cyanide with (+)-3-iodo-MK-801. The synthesis time was 32 min counted from end of bombardment, affording (+)-3-[^{11}C]cyano-MK-801 in 37 % decay-corrected radiochemical yield based on hydrogen [^{11}C]cyanide. The radiochemical purity was higher than 95 % and the specific radioactivity was in the range of 220-600 GBq/μmol. *In vitro* investigations in homogenate and frozen sections of rat brain indicated that (+)-3-[^{11}C]cyano-MK-801 may be used as tracer in studies of the NMDA receptor.

Keywords: NMDA-receptor, MK-801, palladium-promoted ^{11}C-cyanation, [^{11}C] cyano-MK-801, positron emission tomography, *in vivo* investigation

INTRODUCTION

The *N*-methyl-D-aspartate (NMDA) receptor is a ligand-gated ion-channel receptor that has been postulated to be involved in processes such as ischemic neuronal damage and chronic neuronal degeneration (1). The possibility of using NMDA receptor antagonists as therapeutic agents for treatment of neurodegenerative disorders has been subject to

* Author to whom correspondence should be addressed.

CCC 0362–4803/98/060567–10$17.50

Received 5 January 1998
Accepted 9 January 1998

extensive research during the last decade. 5-Methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine (MK-801, Fig.1) is a non-competitive NMDA receptor antagonist which has been widely used as a research tool for investigation of the receptor complex. Radiolabelled MK-801 and derivatives, *e.g.* [^{3}H]MK-801 or compounds labelled with ^{123}I, have been used in studies of the receptor employing autoradiographic techniques and single photon emission tomography (2,3). The prospect of *in vivo* imaging of the NMDA receptor in man may be realized by means of positron emission tomography (PET). Although attempts to develop an appropriate tracer labelled with short-lived radionuclides such as ^{11}C or ^{18}F (half-lives of 20.3 and 110 min respectively) have been described (4), the radioligands derived from MK-801 have so far been of limited usefulness for *in vivo* PET investigations due to decrease in affinity or poor *in vivo* specific binding (4a,5). As it has been shown that various modifications of the aromatic ring structure in MK-801 can be performed without substancial loss of affinity for the receptor (6), the introduction of a ^{11}C-labelled substituent in the aromatic part of MK-801 was considered a possible way to obtain a ^{11}C-tracer suitable for *in vivo* studies the of the NMDA receptor complex.

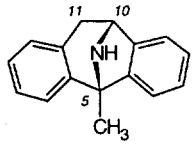
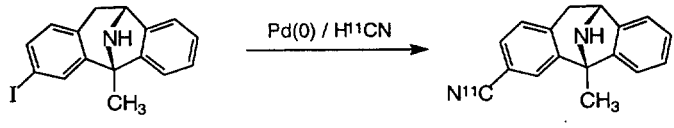


Figure 1. (+)-MK-801 (1)

In this paper the synthesis of (+)-3-[^{11}C]cyano-MK-801 (3) is reported.† The ^{11}C-labelled cyano-MK-801 was synthesized from the corresponding iodo compound (2) and hydrogen [^{11}C]cyanide as shown below, using a previously described method for palladium(0)-promoted ^{11}C-cyanation of aromatic halides (7). The use of liquid chromatography in combination with mass-spectrometry (LC-MS) as a versatile method for characterisation and quality control of the radiopharmaceutical is discussed. Furthermore, the preliminary results from *in vitro* studies using (+)-3-[^{11}C]cyano-MK-801 and from *in vivo* PET investigations of the tracer in Rhesus monkey are presented.



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† Preliminary results were presented at the Fifth International Symposium on Synthesis and Applications of Isotopes and Isotopically Labelled Compounds, Strasbourg 1994.

RESULTS AND DISCUSSION

Chemistry

Synthesis of (+)-3-[^{11}C]cyano-MK-801 was achieved by reaction of (+)-3-iodo-MK-801 with hydrogen [^{11}C]cyanide in the presence of tetrakis(triphenylphosphine)-palladium(0) [$Pd(PPh_3)_4$]. The synthesis was performed as a one-pot procedure, in which the labelled hydrogen cyanide was trapped at room temperature in a solution of palladium catalyst and substrate using tetrahydrofuran (THF) as solvent. After trapping, the mixture was heated at 90 °C for 4 min and then subjected to HPLC-purification. In palladium-promoted ^{11}C-cyanation of aromatic halides, high radiochemical yields can be obtained using a variety solvents such as acetonitrile, dimethyl sulfoxide (DMSO) or THF (7). The primary aim of this work was to introduce the ^{11}C-label into MK-801 as a cyano group, and for this purpose DMSO may be the most appropriate solvent with respect to reversed-phase purification procedures. The possibility of transforming the aromatic ^{11}C-nitrile into compounds with other labelled functional groups, *e.g.* amine, was however considered, *vide infra*, and THF was therefore chosen as solvent in the ^{11}C-cyanation.

The amount of substrate (received as the hydrochloride salt) employed was 0.2 mg, and for convenient manipulation of the substance, a solution of **2** in THF was prepared. Using this solution, the radiochemical yield of 3-[^{11}C]cyano-MK-801 was 40-50 % as determined by HPLC-analyses of samples withdrawn from the reaction mixture. By addition of one equivalent of aqueous potassium carbonate or potassium hydroxide to the substrate solution, a significant increase in the radiochemical yield was achieved. In these reactions, the radiochemical yield of **3** was 70-80 %, and similar results were obtained using either of the two bases. The dissolved reagent, with potassium hydroxide added, was stored at -20 °C for periods up to one month, without any observable decrease in radiochemical yield.

The 3-[^{11}C]cyano-MK-801 was characterised by radio-LC-MS analyses of the ^{11}C-labelled and ^{11}C/^{13}C-labelled product using the unlabelled 3-cyano-MK-801 as reference. Simultaneous monitoring of radioactivity, UV-absorbance and mass were performed, as shown in Figure 2. The concentration of (+)-3-cyano-MK-801 was calculated from a LC-MS calibration curve and found to be in the range of 100-300 ng/ml, which corresponded to a specific radioactivity of 220-600 GBq/μmol (11±5 Ci/μmol, n=6).

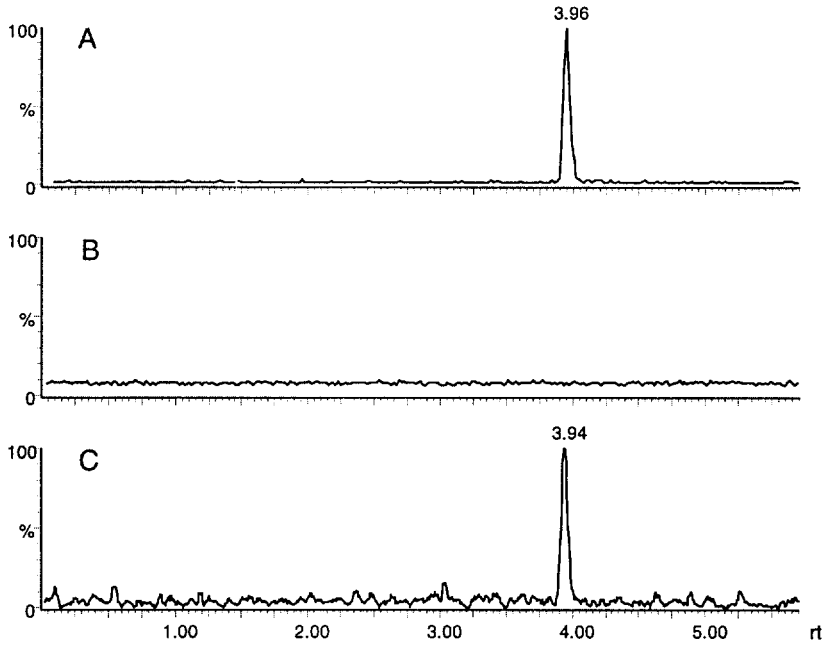

Figure 2. Analysis of (+)-3-[^{11}C]cyano-MK-801 (10 µl injection) by on-line detection of radioactivity (A), UV-absorbance (B) and positive electrospray mass-spectra with selected ion monitoring of *m/z* 247 $[M+H]^+$ (C).

In a typical experiment starting from 3.5 GBq (95 mCi) of hydrogen [^{11}C]cyanide, 1.4 GBq (38 mCi) of (+)-3-[^{11}C]cyano-MK-801 was obtained within 27 min counted from hydrogen [^{11}C]cyanide. The decay-corrected radiochemical yield of **3** was 37 ± 4 % (mean value ± range, n=8), based on hydrogen [^{11}C]cyanide, and the radiochemical purity >95 % as determined by analytical HPLC. The total synthesis time was 32 min counted from end of bombardment to product ready for administration.

The substrate 3-iodo-MK-801 has been reported to have high affinity for the NMDA receptor (3a), and the chemical purity of 3-[^{11}C]cyano-MK-801 was therefore checked to ensure that the starting material had been removed from the labelled product. In some experiments, small amounts of MK-801 (**1**) could be detected in the final product solution. Possible sources of this impurity may be the formation of **1** as a side product in the palladium-promoted coupling reaction, or from decomposition of the starting material during the reaction. No correlation between prolonged storage of the substrate solution and increased amounts of **1** in the product could be observed.

By LC-MS analysis the concentration of MK-801 in the product was determined to be in the range of 10-20 ng/ml. To investigate if this compound was formed during the reaction, experiments in which **2** was dissolved in freshly distilled THF and one equivalent of base in D_2O and thereafter immediately used in the ^{11}C-reaction were conducted. Although a small amount of MK-801 was detected in the isolated product, no trace of the deuterated MK-801 could be found. Analyses of the starting material **2** showed that this compound contained approximately 5% of MK-801. Since 0.2 mg of the substrate was used in the ^{11}C-labelling reaction, the amount of MK-801 that may origin from the precursor would be approximately 10 μg. However, this compound and the product **3** were base-line separated on the semi-preparative HPLC-system used, and the cause of the occasional MK-801-contamination of the labelled product is therefore not easily explained.

Several reports on the structure-activity relationship of MK-801 derivatives have shown that modifications of the aromatic ring structure in MK-801 can be performed without substantial loss of receptor affinity, for instance, derivatives with halides or hydroxy substituents in different positions have been described as potent compounds (6). Preliminary results from *in vivo* and *in vitro* studies suggest that (+)-3-[^{11}C]cyano-MK-801 may be a useful tracer for studying the NMDA receptor complex. Although some substituents in MK-801 can be present without major influence on the receptor affinity, different ^{11}C-substituents may alter the *in vivo* behaviour of the tracer dramatically by modification of lipophilicity or hydrogen bonding potential. In order to provide a suitable tool for evaluation of such prospects, synthesis of other ^{11}C-labelled tracers, such as 3-[^{11}C]methylamino-MK-801, is now under investigation.

Positron emission tomography studies

Prior to the PET study, the specificity, saturability, and affinity of **3** were investigated in crude membrane fraction of rat brain by binding assay with the aid of a cell harvester and a phosphor imager system (methods and detailed results will be submitted elsewhere, 8). The *in vitro* studies revealed that the (+)-3-[^{11}C]cyano-MK801 binding was saturated with time and with increasing concentration of (+)-MK-801, and that the IC_{50} value in the presence of glutamate and glycine calculated from the displacement study with MK-801 was less than 40 nM. By the use of *in vitro* autoradiography technique with frozen rat brain sections (8), the specific and high-affinity binding of **3**

was observed mainly in the hippocampus, cerebral cortices, thalamus, and striatum. In the PET studies in Rhesus monkey, a high uptake of **3** was observed in the striatum, cerebral cortices and thalamic regions (8). As shown in time-activity curve, Figure 3, the initial high uptake was followed by a significant wash-out. Compared to the data from PET-studies in baboons using [^{18}F] methyl-MK-801 (5), the results obtained with (+)-3-[^{11}C]cyano-MK-801 are promising, although these preliminary data are not sufficient to validate whether or not (+)-3-[^{11}C]cyano-MK-801 may be a useful tracer for dynamic studies of the NMDA receptor.

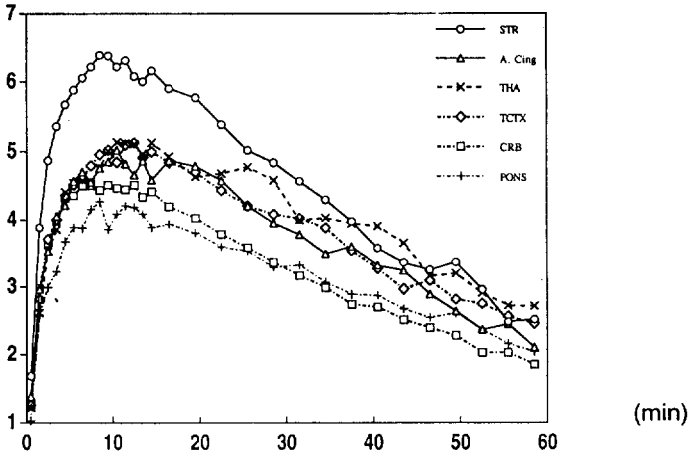

Figure 3. A typical time-activity curve (uptake of radioactivity as a function of time after injection) of (+)-3-[^{11}C]cyano-MK-801 in several brain regions of Rhesus monkey. The tracer (123 MBq) was injected intravenously, and PET scans were obtained according to the protocol in the experimental section. STR=striatum, A Cing=anterior cingulate cortex, THA=thalamus, TCTX=temporal cortex and CRB=cerebellum.

CONCLUSIONS

An efficient synthesis of (+)-3-[^{11}C]cyano-MK-801 of high specific radioactivity has been developed using hydrogen [^{11}C]cyanide in a palladium-promoted one-pot reaction with 3-iodo-MK-801. The synthetic strategy presented could be useful in production of other ^{11}C-labelled MK-801 compounds, substituted in different positions with either a ^{11}C-cyano group or with functional groups derived from the ^{11}C-nitrile. The preliminary data from *in vitro* experiments indicate that (+)-3-[^{11}C]cyano-MK-801 may be used as tracer for investigating functional aspects of the NMDA receptor complex.

ACKNOWLEDGEMENTS

We wish to acknowledge the Swedish Natural Science Research Council for financial support, grant No. K-3436, and Research Biochemicals International for providing the 3-Iodo-MK-801 under the NIMH chemical synthesis program.

EXPERIMENTAL

General

[^{11}C]carbon dioxide was prepared by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction using a nitrogen (AGA Nitrogen 6.0) gas target (containing 0.1 % oxygen, AGA Oxygen 4.8) and 17 MeV protons produced by the Scanditronix MC-17 Cyclotron at the Uppsala University PET Centre. The [^{11}C]carbon dioxide was converted into hydrogen [^{11}C]cyanide according to published procedures (9), by use of the Scanditronix RNP-17 radionuclide production system.The LC-MS equipment consisted of a Beckman 126 solvent delivery module, a CMA 240 autosampler (CMA Microdialysis, Stockholm, Sweden) and a Fisons VG Quattro mass spectrometer equipped with pneumatically assisted electrospray and an RF ion bridge. The column was a 100 × 4.6 mm KromaSil C18. A post column 1:100 split was used, with 1% of the total flow delivered to the electrospray probe and 99% delivered to a Beckman 166 variable wavelength UV detector followed by a Bioscan Flow-Count β^+-detector. Mobile phases were (A) 5 mM trifluoroacetic acid in Nano-pure water (Barnsted) and (B) 5 mM TFA in acetonitrile. A linear solvent program from 5% to 100% B in 6 minutes was used.

HPLC was performed with a Beckman 126 pump and a Beckman 166 UV detector in series with a β^+-flow detector. A modified Gilson 231 Autosampler was used for injection and fraction collection, and data collection and decay-correction were performed using a personal computer and the Beckman System Gold Chromatography Software Package. The analytical column was a 250 x 4.6 mm Spherisorb ODS1 5µm. Semi-preparative columns were (C) Beckman Ultrasphere C-18 or (D) Spherisorb ODS1 C-18 5µm (250 x 10 mm). Mobile phases were (E) aqueous sodium chloride (9 mg/L), (F) 0.01 M potassium dihydrogen phosphate pH 4.7, (G) acetonitrile/water (500/70 v/v) or (H) methanol. (+)-MK-801, 3-cyano was synthesized at Central Research Laboratory, Hamamatsu Photonics K.K., Japan. (+)-MK-801 was purchased from Research Biochemicals International. (+)- and (-)-MK-801, 3-iodo, hydrochloride

were obtained from Research Biochemicals International under the NIMH chemical synthesis program. Tetrakis(triphenylphosphine)palladium(0) was purchased from Aldrich Chem. Co. THF was distilled from sodium and benzophenone. All other chemicals and solvents were of analytical or gradient grade and used as received.

Synthesis of (+)-3-[^{11}C]cyano-MK-801 (3)

0.6 mg (0.5 μmol) $Pd(PPh_3)_4$ was dissolved in 300 μl THF in a 3 mL septum-equipped glas vessel, whereafter 200 μl (0.5 μmol) of a solution containing 1 mg (+)-3-iodo-MK-801, hydrochloride (2) in 999 μl THF and 1 μl 2.5 M KOH was added. The reaction vessel was purged with nitrogen gas for 2 min, whereafter hydrogen [^{11}C]cyanide, passed through Sicapent® (100 x 10 mm) to reduce the amount of ammonia obtained in the production process, was trapped in the solution. After trapping, the reaction vessel was heated at 90 °C for 4 min. The labelled product was purified by semi-preparative HPLC using column C, isocratic elution 5 ml/min of solvent E/H 45/55, linear gradient to 10/90 from 10 to 15 min, column temperature 25 °C, wavelength 230 nm. The product fraction was collected at approximately 9 min and the organic solvent evaporated. To the residue was added 4 ml of 0.1 M sterile phosphate buffer, pH 7.4, and the resulting solution was passed through a sterile filter (Dynagard ME, 0.22 μm pore size) into a sterile vial. Samples of the formulated product were analysed by LC-MS and analytical HPLC as described below. Sterility and apyrogenicity were checked by an independent laboratory (The Microbiological Central Laboratory, Uppsala University Hospital).

Synthesis of (-)-3-[^{11}C/^{13}C]cyano-MK-801

The reaction and purification was performed according to the procedure described above, with the following modifications: The hydrogen [^{11}C]cyanide was trapped in a mixture of 1.0 mg (1.2 μmol) $Pd(PPh_3)_4$, 400 ul (1.2 μmol) of (-)-3-iodo-MK-801-solution and 100 μl THF. After trapping, 1 mg (15 μmol) potassium [^{11}C]cyanide dissolved in 3 μl water was added to the reaction mixture. The reaction vessel was heated at 90 °C for 10 min, and the labelled product was purified by semi-preparative HPLC using column D, isocratic elution 5 ml/min of solvent F/G 15/85, linear gradient to 0/100 from 10 to 15 min. The retention time for (-)-3-[^{11}C/^{13}C]cyano-MK-801 was 11 min. The product was analysed by LC-MS as described below.

LC-MS and HPLC analyses

LC-MS analyses were performed at a flow rate of 1 ml/min, column temperature 40 °C and wavelength 230 nm. 10 µl injections of the formulated product **3**, of the unlabelled (+)-3-cyano-MK-801, of the ^{11}C/ ^{13}C-labelled (-)-3-cyano-MK-801and of (+)-MK-801 were analysed. (+)-3-[^{11}C]cyano-MK-801: Retention time 4.0 min, m/z 247.2 $[M+H]^+$, calculated molecular weight (m.w.) 246.3. (+)-3-cyano-MK-801: Retention time 4.0 min, m/z 247.2 $[M+H]^+$, calculated m.w. 246.3. (-)-3-[^{13}C]cyano-MK-801: Retention time 4.0 min, m/z 248.2 $[M+H]^+$, calculated m.w. 247.3. (+)-MK-801: Retention time 4.1 min, m/z 222.1·$[M+H]^+$, calculated m.w. 221.1. HPLC analyses of compounds **1**, **2** and **3** were performed using a linear gradient of solvent F/G from 26/74 to15/85 in 5 min, thereafter isocratic elution at 15/85, flow 2 ml/min, column temperature 25 °C, wavelenght 230 nm. Retention times for **1**, **2** and **3** were 8.5, 10 and 5.2 min respectively, and the radiochemical purity of **3** was >95 %.

Positron Emission Tomography

The studies were performed in Rhesus monkey (*Macaca Mulatta*, female, weighing 7.8 kg) under anesthestia by inhalation of nitrous oxide (60% v/v) and isoflurane (0.3% v/v), and throughout the study mechanically ventilated with 40% oxygen in air. The head was fixed in a holder with an acrylic ear bar for repeated PET studies in the same position. Horizontal and vertical slices were oriented by use of a laser alignment system. Catheters were inserted into the femoral vein bilaterally, one for injection of the radioligand and the other for venous blood sampling. The injected doses in three separate PET studies were 121, 123, and 109 MBq, and the positron emission tomograph (GE PC2048-15B) was started immediately following intravenous injection of the radioligand. The protocol for PET scan was as follows: 0-15 min, every 1 min (15 frames); 15-60 min, every 3 min (15 frames). Summation images were produced from 16th to 25th frames. After reconstruction of images, regions of interest (ROIs) were delineated on the reconstructed PET images with reference to a map of horizontal cryosections of the Rhesus monkey head (Uppsala University PET Centre). The time-activity curves were obtained from sequence data in ROIs. The animal experiments were carried out with permission from the Animal Ethical Comittee, Uppsala University (license No. C262/92).

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