JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
*J Label Compd Radiopharm* 2006; **49**: 163–170.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.1033

**Research Article**

# Synthesis and characterization of *N*-(2-chloro-5-methylthiophenyl)-*N*′-(3-methylthiophenyl)-*N*′-[$^{11}$C]methylguanidine [$^{11}$C]CNS 5161, a candidate PET tracer for functional imaging of NMDA receptors

Yongjun Zhao, Edward Robins, David Turton, Frank Brady, Sajinder K. Luthra and Erik Årstad*
*Hammersmith Imanet, Cyclotron Building, Du Cane Road, London W12 0NN, UK*

## Summary

*N*-methyl-D-aspartate (NMDA) receptors play a key role in excitatory neurotransmission and are linked to a variety of acute and chronic neurodegenerative diseases including epilepsy, schizophrenia, Parkinson's disease and drug abuse. *N*-(2-chloro-5-methylthiophenyl)-*N*′-(3-methylthiophenyl)-*N*′-methylguanidine (CNS 5161) is a high affinity ligand (Ki = 1.87 ± 0.25 nM) for the NMDA PCP site, which potentially can be used for functional imaging of this receptor. Herein we report the synthesis of the corresponding positron emission tomography (PET) tracer [$^{11}$C]CNS 5161 by means of [$^{11}$C]methylation of the desmethyl guanidine precursor. [$^{11}$C]CNS 5161 was synthesized with a decay corrected radiochemical yield of 10% within 45 min after end of bombardment (EOB). The final product was prepared in a sterile saline solution suitable for clinical studies with a radiochemical purity of >96% and a specific activity of 41 GBq/mmol at time of injection. Copyright © 2005 John Wiley & Sons, Ltd.

**Key Words:** NMDA; glutamate; CNS 5161; PET; radiotracer

## Introduction

NMDA receptors are gated ion-channels that mediate long-term potentiation by allowing prolonged influx of $Ca^{2+}$ ions, as well as $Na^{+}$ and $K^{+}$ ions, into the synapse.[1,2] They are tightly regulated, with a total of six binding sites for endogenous ligands controlling the frequency and duration of ion-channel opening.[3] Receptor activation requires depolarization of the neuron on which they are expressed and simultaneous binding of the co-agonists glutamate and

*Correspondence to: Erik Årstad, Hammersmith Imanet, Cyclotron Building, Du Cane Road, London W12 0NN, UK. E-mail: erik.arstad@csc.mrc.ac.uk



*Received 28 July 2005*
*Revised 2 November 2005*
*Accepted 5 November 2005*

glycine. The exact pharmacological and physiological properties of NMDA receptors are dictated by their subunit composition. Functional NMDA receptors are composed of heteromeric combinations of the ubiquitously expressed NR1 subunit, and the discretely distributed NR2 subunit, which has four subtypes (NR2A-D). Additional complexity arise from splicing of the NR1 subunit, which is known to exist in eight different isoforms, and from co-assembling of one or more NR3 subunits, of which two subtypes have been described to date (NR3A and NR3B).[4] Each subunit expresses distinct binding sites and different combinations produce receptors with unique permeation, kinetic and pharmacological properties.

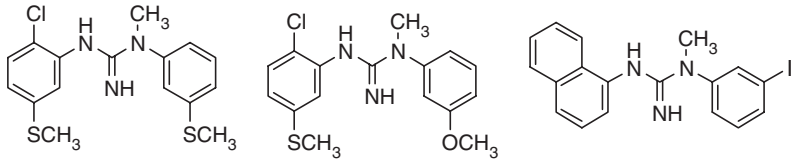
The role of NMDA receptors in pathological process has been extensively studied and NMDA dysfunction is linked to a variety of acute and chronic neurodegenerative diseases, including epilepsy, stroke, neuropathic pain, schizophrenia, Parkinson's disease, and drug abuse.[5–9] In this context, non-invasive molecular imaging and positron emission tomography (PET) in particular, represents a unique tool to study NMDA receptors in the living human brain. Imaging agents targeting the intrachannel PCP site are of particular interest as they selectively bind to NMDA receptors in the open state, and their distribution and density should therefore reflect dynamic changes in the channel itself.[10] This can potentially be used to quantify regional populations of activated receptors in the healthy and diseased brain, as well as to monitor the function of therapeutic drugs targeting this receptor system. A number of potential imaging agents for the PCP site have been synthesized (Figure 1),[11] of which the SPECT tracer [$^{123}$I]CNS 1261 (**1**) is the first to show promise in clinical trials.[12–14] Candidate PET tracers, including the related *N*,*N*′ diarylguanidine [$^{11}$C]GMOM (**2**), have suffered from low specific binding in addition to the usual pitfalls of rapid metabolism and low brain uptake.[11,15] We believe CNS 5161 (**3**), a lead therapeutic candidate from the *N*,*N*′diarylguanidine series, [16–18] is more attractive for PET tracer development due to its superior affinity for the PCP site ($1.87 \pm 0.25$ nM versus $5.2 \pm 0.3$ nM for GMOM) and favourable log *P* of 2.68. Herein, we report the synthesis and characterization of *N*-(2-chloro-5-methylthiophenyl)-*N*′-(3-methylthiophenyl)-*N*′[$^{11}$C]methylguanidine, an 11-carbon labelled derivative of CNS 5161.

## Materials and methods

### *General*

Reagents and solvents were obtained from Aldrich Chemical Co. and Fisher Scientific and used without further purification unless otherwise noted. NMR spectroscopy was carried out at the University of London Chemistry Department on a Varian Gemini-300 (300 MHz) instrument or at Imperial




**CNS 5161**
Ki= 1.87 nM

**GMOM**
Ki= 5.2 nM

**CNS 1261**
Ki= 4.65 nM

**Figure 1. Structures of selected *N,N′*-diarylguanidine ligands for the NMDA PCP site. Data from Dumont *et al.*[18]**

College, Hammersmith Campus on a JEOL GX500 (500 MHz) instruments and the chemical shifts are reported in parts per million (ppm) relative to residual solvent peaks. LRMS, HRMS and MS–MS analyses were performed by Mass Spectrometry Services at the School of Pharmacy, London. LRMS experiments were carried out using 50% acetonitrile/0.1% formic acid as solvent. HRMS and MS–MS experiments were performed using a Thermo-Quest Navigator mass spectrometer operated under ESI mode using a Micromass Q-TOF Global Tandem mass spectrometer under ESI mode using 50% acetonitrile/0.1% formic acid as solvent and calibrated using [Glu]-Fibrinopeptide B as internal standard. Flash column chromatography was performed on 230 mesh silica gel or an Iso Inc. CombiFlash Companion using pre-packed 4, 12 and 40 g RediSep silica columns. [$^{11}$C]CNS 5161 (**3**) was purified by reverse-phase HPLC using a Beckman 110B HPLC pump connected to a SA 6506 UV detector (Severn Analytical) set at 254 nm, a GM tube and a Servogor 124 twin pen chart recorder (Spectronic). Analytical HPLC for quality control was carried out on an Agilent 1100 series HPLC system equipped with a Bioscan GM tube detector.

*Synthesis*

3-Thiomethylphenylcyanamide (**4**), $^{18}$*N*-methyl-3-thiomethylphenylcyanamide (**5**), $^{18}$2-chloro-5-thiomethylaniline (**6**)[16] and CNS 5161 (**3**)[16] were synthesized according to published procedures.

*N-(2-chloro-5-methylthiophenyl)-N′-(3-methylthiophenyl)-guanidine HCl (**7**).* To 3-thiomethylphenylcyanamide (**4**) (0.46 g, 2.81 mmol) was added 2-chloro-5-thiomethylaniline hydrochloride (**6**) (0.54 g, 2.56 mmol) and chlorobenzene (2 ml), and the stirred reaction mixture was heated to 150°C under an inert atmosphere ($N_2$) for 4 h. The resulting solution was allowed to reach room temperature and concentrated under reduced pressure. Purification of the residue by means of flash chromatography (silica, $CH_2Cl_2$:MeOH gradient, 0–10%) provided the title

compound as a white solid (0.72 g, 76%). M.p. 168.5–170°C; $^1$H NMR ($CDCl_3$) δ (ppm) 2.49 (s, 6H, S–$CH_3$) and 6.8–7.3 (m, 7H, Aryl H).

*N-methyl-(2-chloro-5-methylthio)aniline (***8***).* To a solution of (2-chloro-5-thiomethyl)aniline (0.599 g, 3.75 mmol) in THF (10 ml) cooled in an ice-bath was slowly added 1 equivalent of *n*-butyllithium (1 M hexanes, 3.75 mmol) under stirring. After 15 min methyl iodide (0.532 g, 3.75 mmol) was added dropwise and the resulting solution was allowed to reach room temperature over a period of 1 h. Solvents were evaporated and the residue separated between water (50 ml) and diethyl ether (50 ml). The organic phase was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The resulting crude product was purified by means of flash chromatography (silica, 1:1 dichloromethane/hexane) to give the title compound **8** (0.556 g, 79%) as a colourless oil. $^1$H NMR ($CDCl_3$) δ 2.53 (s, 3H, *S*-Me), 2.96 (s, 3H, *N*-Me), 4.40 (br s, 1H, NH), 6.59 (dd, 1H, Aryl H, $J = 2.2$ and 8.4 Hz), 6.65 (d, 1H, Aryl H, $J = 2.2$ Hz), 7.19 (d, 1H, Aryl H, $J = 8.4$ Hz). The corresponding hydrochloride salt was prepared by treating an ice-cold solution of **8** in diethyl ether (5 ml/mmol **8**) with a 2-fold molar excess of HCl in diethyl ether (anhydrous, 2 M). Filtration provided the salt as a bright white solid (0.621 g, 94%).

*N-(2-chloro-5-methylthiophenyl)-N′-(3-methylthiophenyl)-N-methylguanidine HCl (***9***).* To *N*-methyl-(2-chloro-5-methylthio)aniline (**8**) hydrochloride (0.100 g, 0.45 mmol) was added 3-methylthiophenylcyanamide (**4**) (0.074 g, 0.45 mmol), toluene (0.5 ml) and dichloromethane (0.5 ml) under an inert nitrogen atmosphere. The reaction mixture was heated to 120°C and concentrated by allowing the dichloromethane solvent to be removed from the reaction mixture by distillation. After 12 h the mixture was cooled to room temperature and the residue purified by column chromatography (silica, chloroform/methanol gradient, 0–10%). The resulting colourless oil was taken up in a minimum of chloroform and **9**.HCl was precipitated by addition of diethyl ether. The product was isolated by filtration as a white solid (0.054 g, 31%). $^1$H NMR ($d_6$-DMSO) δ 2.48 (s, 3H, *S*-Me), 2.52 (s, 3H, *S*-Me), 3.38 (s, 3H, *N*-Me), 7.14–6.97 (br m, 2H, Aryl H), 7.16 (m, 1H, Aryl H), 7.34 (m, 2H, Aryl H), 7.55 (m, 2H, Aryl H).

*Cold methylation of N-(2-chloro-5-methylthiophenyl)-N′-(3-methylthiophenyl)-guanidine (***7***).* To the desmethyl precursor **7** (1.4 mg, 4.14 μmol) added sodium hydride (4.0 mg, 0.17 mmol) and acetonitrile (0.6 ml). After stirring for several minutes, methyl iodide (1 μl, 16.06 μmol) was added and the mixture stirred for a further 10 min. The reaction mixture was diluted with $(NH_4)_2HPO_4$ (aq) (10 mM, 0.5 ml) and subjected to semi-preparative HPLC using a C18 μBondapak column (7.8 × 300 mm, Waters) with a $(NH_4)_2HPO_4$ (10 mM)/acetonitrile 50/50 (v/v) solvent system (3 ml/min, $\lambda = 254$ nm).

Samples of compounds **1**–**3** were concentrated under a flow of nitrogen and extracted into ethanol for analysis.

*Radiolabelling*

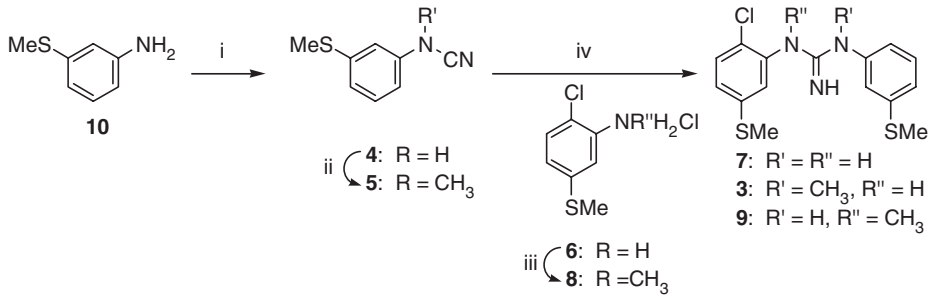
*N-(2-chloro-5-methylthiophenyl)-N′-(3-methylthiophenyl)-N′-[$^{11}$C]methylguanidine* (**3**), *[$^{11}$C]CNS 5161*. [$^{11}$C]Methyl iodide was prepared using the standard 'wet' method as described by Turton *et al.*[19] The radio-synthesis was carried out using a remotely controlled apparatus housed within a lead shielded enclosure. To the desmethyl precursor **7** (0.5 mg) was added sodium hydride (2 mg) and acetonitrile (anhydrous, 300 µl) under an inert atmosphere ($N_2$). After stirring for 1 min, [$^{11}$C]methyl iodide was distilled into the reaction vial and the resulting mixture was heated to 70°C for 3 min. The crude product was diluted with water (5 ml) and the resulting suspension was loaded on a reverse phase HPLC column (4.5 × 30 mm). The column was washed with water (8 ml) before the product mixture was eluted onto a semi-preparative C18 µBondapak column (7.8 × 300 mm, Waters) and purified using a solvent system of di-ammonium hydrogen orthophosphate (0.05 M)/acetonitrile 4/6 (v/v) (3 ml/min). The fraction containing [$^{11}$C]CNS 5161 (retention time ~17 min) was collected into a rotary evaporator flask containing a solution of ascorbic acid (1 mg) in water (1 ml). The product was evaporated to dryness under reduced pressure, the residue was dissolved in isotonic saline containing 5% ethanol (10 ml) and the solution sterilized by filtration (25 mm, 0.2 µm pore Acrodisc filter, Pall corp.) in a class A isolator. Analytical HPLC for product quality control was performed using a Prodigy C18 analytical column (4.6 × 150 mm) from Phenomenex and a solvent system of ammonium orthophosphate (0.05 M)/acetonitrile 45/55 (v/v) (1 ml/min). The identity and purity of the product was monitored with UV ($\lambda = 257$ nm) and a Bioscan GM tube detector. In this system [$^{11}$C]CNS 5161 (**3**) eluted after 4.5 min and the desmethyl precursor **7** eluted after 6 min.

## Results and discussion

CNS 5161 can potentially be labelled with [$^{11}$C]methyl iodide in three positions; either of the two *S*-methyl groups or the *N′*-methyl guanidine functionality. We rationalized that labelling of the desmethyl guanidine functionality provided the simpler route for precursor synthesis and therefore was preferred. The main concern with this strategy was formation of the *N*-[$^{11}$C]methyl guanidine regioisomer **9**, and whether this compound could be separated from [$^{11}$C]CNS 5161 (**3**) in a time-scale suitable for high activity routine production.

The desmethyl guanidine precursor **7**, CNS 5161 (**3**) and its *N*-methyl regioisomer **9** were synthesized using modified literature procedures as depicted in Scheme 1.




**Scheme 1. Synthesis of precursor 7, the *N*-methylated standard 9 and its *N′*-methylated regioisomer 3. Reagents and conditions: (i) BrCN in ether, (ii) NaH and MeI, (iii) Butyl lithium and MeI and (iv) PhMe, reflux**

Commercially available 3-methylthioaniline (**10**) was reacted with cyanogen bromide (Caution: highly toxic) as described in the literature to give the corresponding cyanamide **4**.[18] For the preparation of CNS 5161 (**3**), this intermediate was methylated under basic conditions using methyl iodide. Subsequent condensation of either **4** or **5** with the chloroaniline **6**[16] provided the desmethyl precursor **7** and CNS 5161 (**3**), respectively. Initially, we attempted to synthesize the *N*-methylguanidine regioisomer **9** via the same route, i.e. transformation of the chloroaniline **6** to the corresponding cyanamide, followed by methylation and subsequent condensation with the aniline **10**. However, the chloroaniline **6** resisted all attempts to convert it into the corresponding cyanamide and instead the *N*-methylguanidine regioisomer **9** was prepared by deprotonation of the chloroaniline **6** with butyl lithium in THF, subsequent treatment with methyl iodide to provide the methylated chloroaniline **7**, and finally condensation with the cyanamide **4**.

When the desmethyl guanidine precursor **7** was reacted with [$^{11}$C]methyl iodide in acetonitrile in the presence of sodium hydride, HPLC analysis revealed formation of two major radioactive products which co-eluted with CNS 5161 (**3**) and the *N*-methyl regioisomer (**9**), respectively. When the reaction was repeated using non-radioactive methyl iodide an additional product was formed. To verify the nature of the products, samples were purified by preparative HPLC and subjected to electrospray mass-spectrometry (ES-MS) and ES-MS–MS fragmentation analysis. The main product was found to have an identical mass ($M + H^+ = 352$ amu) and fragmentation pattern to CNS 5161 (**3**), with C–*N′* cleavage giving rise to two characteristic ions of $m/z = 199$ and 154 amu (Figure 2). The molecular mass ($M + H^+ = 352$ amu) and fragmentation pattern of the second product were consistent with the *N*-methyl isomer **9**, with C–*N′* cleavage resulting in an ion of $m/z = 231$ amu, and C–*N* cleavage ions of $m/z = 188$ and 165 amu. The

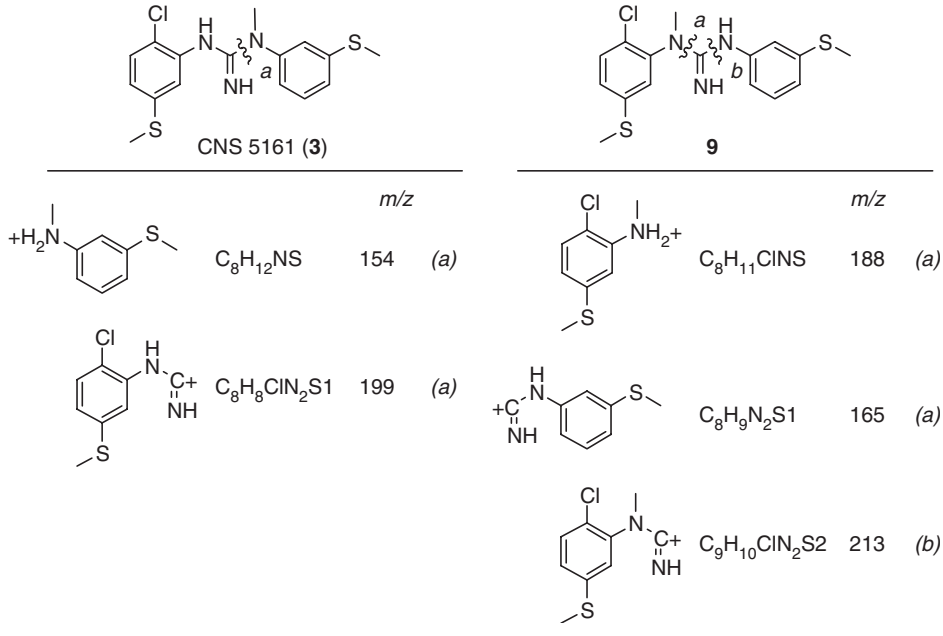



**Figure 2. Electrospray MS–MS fragmentation pattern of compounds 3 and 9**

product unique to the cold reaction was observed to have a molecular mass ($M + H^+ = 366$ amu) consistent with that of a di-methylated species, and we postulate that this is the *N*,*N*′-dimethylguanidine derivative. Overall, the data confirm that the major product from [$^{11}$C]methylation of the precursor **7** is [$^{11}$C]CNS 5161 (**3**) and that separation from the *N*-[$^{11}$C]methyl regioisomer **9** can be achieved by HPLC.

Encouraged by the results, we developed an automated procedure for high activity routine production of [$^{11}$C]CNS 5161, which is given in the experimental part. [$^{11}$C]CNS 5161 is now routinely obtained in 10% decay corrected yield within 45 min after EOB, with a radiochemical purity >96% and a specific activity of 41 GBq/mmol at time of injection. The biological evaluation of [$^{11}$C]CNS 5161 will be published elsewhere.

## Conclusions

[$^{11}$C]CNS 5161 is a candidate PET tracer for functional imaging of the NMDA receptor. Herein we report that *N*-[$^{11}$C]methylation of the *N*-desmethyl guanidine precursor is a viable route to [$^{11}$C]CNS 5161, and describe an automated synthesis based on this chemistry. The radiosynthesis of [$^{11}$C]CNS 5161 is highly reproducible and allows for the tracer to be produced in high specific activity, good radiochemical purity and sufficient quantities for *in vivo* evaluations.

## References


1. Dingledine R, Borges K, Bowie D, Traynelis SF. *Pharmacol Rev* 1999; **51**: 7–61.
2. Riedel G, Platt B, Micheau J. *Behav Brain Res* 2003; **140**: 1–47.
3. Siegel GJ, Agranoff BW, Albers RW, Fisher SK, Uhler MD. *Basic Neurochemistry*: *Molecular*, *Cellular and Medical Aspects* (6th edn). Lippincott Williams & Wilkins: Philadelphia, 1999.
4. Kew JNC, Kemp JA. *Psychopharmacology* 2005; **179**: 4–29.
5. Waxman EA, Lynch DR. *Neuroscientist* 2005; **11**: 37–49.
6. Loftis JM, Janowsky A. *Pharmacology & Therapeutics* 2003; **97**: 55–85.
7. Konradi C, Heckers S. *Pharmacology & Therapeutics* 2003; **97**: 153–179.
8. Parson CG, Danysz W, Quack G. *Drug News Perspect* 1998; **11**: 523–567.
9. Heresco-Levy U. *Int J Neuropsychopharmacol* 2000; **3**: 243–258.
10. Bressan RA, Pilowsky LS. *Eur J Nucl Med* 2000; **27**: 1723–1731.
11. Waterhouse RN. *Nucl Med Biol* 2003; **30**: 869–878.
12. Owens J, Tebbutt AA, McGregor AL, Kodama K, Magar SS, Perlman ME, Robins DJ, Durant GJ, McCulloch J. *Nucl Med Biol* 2000; **27**: 557–564.
13. Bressan RA, Erlandsson K, Mulligan RS, Gunn RN, Cunningham VJ, Owens J, Ell PJ, Pilowksy LS. *Ann N Y Acad Sci* 2003; **1003**: 364–367.
14. Bressan RA, Erlandsson K, Mulligan RS, Gunn RN, Cunningham VJ, Owens J, Cullum ID, Ell PJ, Pilowsky LS. *Nucl Med Biol* 2004; **31**: 155–164.
15. Waterhouse RN, Dumont F, Sultana A, Simpson N, Laruelle M. *J Label Compd Radiopharm* 2002; **45**: 955–964.
16. Hu LY, Guo J, Magar SS, Fischer JB, Burke-Howie KJ, Durant GJ. *J Med Chem* 1997; **40**: 4281–4289.
17. Walters MR, Bradford AP, Fischer J, Lees KR. *Br J Clin Pharmacol* 2002; **53**: 305–311.
18. Dumont F, Sultana A, Waterhouse R. *Biorg Med Chem Lett* 2002; **12**: 1583–1586.
19. Turton DR, Brady F, Pike VW, Selwyn AP, Shea MJ, Wilson RA, Landsheere CM. *Int J Appl Radiat Isot* 1984; **35**: 337–344.