[11C]CYANOGEN BROMIDE IN THE SYNTHESIS OF
1,3-DI(2-TOLYL)-[11C]GUANIDINE

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SUMMARY

The title compound was [11C]-labelled in a one-pot procedure using [11C]cyanogen bromide as the key intermediate. Synthesis time was 31 - 33 min counted from the end of bombardment, affording 1,3-di(2-tolyl)-[11C]guanidine (6) in 87 % decay-corrected radiochemical yield counted from [11C]cyanogen bromide. The specific radioactivity of the product at the end of synthesis was 118 GBq μmol^{-1} (3.2 Ci μmol^{-1}). Autoradiographic studies on slide-mounted rat brain sections showed specific binding of guanidine (6), but when injected intravenously into Rhesus monkey, brain uptake was very low, indicating that the 1,3-di(2-tolyl)-[11C]guanidine did not cross the blood-brain barrier.
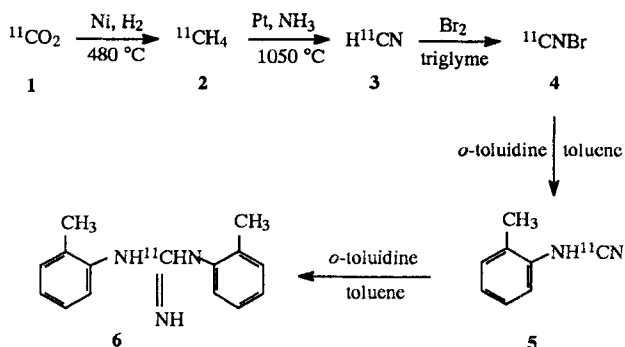
INTRODUCTION

The potent psychotomimetic and hallucinogenic properties of phencyclidine (PCP, "angel dust") and various benzomorphan opiates are thought to be mediated by a specific receptor system termed the haloperidol-sensitive σ-receptor.[1] Phencyclidine has been used as a drug model for schizophrenia, suggesting that σ-receptors may be involved in mental illness.[2] In the search for receptor ligands for mapping of σ-receptors, [3H]1,3-di(2-tolyl)-guanidine was shown in *in vitro* radioligand binding experiments to be highly selective for the σ-receptor, with a high degree of specific binding.[3] Positron emission tomography (PET) may be a useful method for the *in vivo*

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visualization and quantification of σ-receptors. In recent years, a number of potential σ-receptor ligands have been labelled with short-lived radionuclides.[4-6] In this paper we report a simple procedure for the synthesis of 1,3-di(2-tolyl)-[^{11}C]guanidine (**6**) using [^{11}C]cyanogen bromide as the key intermediate, Scheme 1.

$$^{11}CO_2 \xrightarrow[480\ °C]{Ni,\ H_2} {}^{11}CH_4 \xrightarrow[1050\ °C]{Pt,\ NH_3} H^{11}CN \xrightarrow[triglyme]{Br_2} {}^{11}CNBr$$

1   **2**   **3**   **4**

o-toluidine | toluene

CH$_3$—NH^{11}CHN—CH$_3$ (with ‖ NH below)   *o*-toluidine / toluene   CH$_3$—NH^{11}CN

6   **5**

Scheme 1

Materials and Methods

General

2-Methylaniline (*o*-toluidine) and 1,3-di(2-tolyl)guanidine were obtained from Aldrich Chem. Co. 2-Methylaniline was distilled from potassium hydroxide prior to use. Triethylene glycol dimethyl ether (triglyme, Merck, zur Synthese) was distilled from sodium hydride. All other chemicals were of analytical grade quality and used as received.

[^{11}C]Carbon dioxide was prepared by the ^{14}N(p,α)^{11}C nuclear reaction using a nitrogen gas (AGA, 6.0) target containing 0.1 % oxygen (AGA, 5.0) and 17 MeV protons produced by the Scanditronix MC-17 cyclotron at the Uppsala University PET Centre. The [^{11}C]carbon dioxide was converted to hydrogen [^{11}C]cyanide using the Scanditronix RNP-17 radionuclide production system according to published procedures.[7,8] HPLC was performed with a Beckman 126 pump and a Beckman 168 diode array detector in series with a β$^+$-flow detector. Data collection and decay correction were performed on a personal computer using the Beckman System Gold Chromatography Software Package. Thin-layer chromatography (TLC) was performed on Merck Silica gel F254 plates. Autoradiographic images of TLC plates and brain sections were obtained using a Molecular Dynamics PhosphorImager® (Sunnyvale, Cf., USA). All chromatographic analyses were performed at room temperature.

Synthesis of [^{11}C]cyanogen bromide

[^{11}C]Cyanogen bromide was produced according to a procedure described in detail elsewhere.[9] Hydrogen [^{11}C]cyanide was trapped in 800 μL triglyme; bromine (60 mg, 0.4 mmol) was dissolved in 300 μL triglyme immediately prior to use and added to the reaction vessel via a septum. The reaction mixture was heated to 180 °C under a nitrogen gas purge (80 mL/min), and the [^{11}C]cyanogen bromide transferred to a receiving vessel for further synthesis. Synthesis times were 9 - 10 min counted from the end of bombardment.

Synthesis of 1,3-di(2-tolyl)-[^{11}C]guanidine

[^{11}C]Cyanogen bromide was trapped at room temperature in a vial containing 250 μL toluene and 50 μL (470 μmol) *o*-toluidine. After trapping, the reaction vessel was heated for 7.5 min in a heating block held at 130 °C. The reaction mixture was then diluted with 300 μL 50 mM ammonium formate pH 3.5/acetonitrile, 70/30 (v/v), and injected on the semi-preparative HPLC column (Spherisorb ODS 1 C$_{18}$, 5 μm, 250×10 mm i.d., mobile phase 50 mM ammonium formate pH 3.5/acetonitrile 65/35 (v/v), linear gradient to 20/80 (v/v) from 5 to 10 min, flow 5 mL/min, UV-detection at 254 nm). The fraction containing 1,3-di(2-tolyl)-[^{11}C]guanidine (retention time 11.5 min) was collected and transferred to a rotary evaporator for removal of the solvent. The residue was redissolved in 4 mL sterile phosphate buffer (0.1 M, pH 7.4) and passed through a sterile filter (Dynagard ME, 0.22 μm) into a sterile ampoule to provide a solution ready for intravenous injection. Samples (20 μL) were analysed using a Beckman Ultrasphere C$_{18}$ 5 μm, 250× 4.6 mm i.d., mobile phase 50 mM ammonium formate pH 3.5/methanol 55/45 (v/v), flow 1.5 mL/min, UV-detection at 254 nm. The retention time for the 1,3-di(2-tolyl)-[^{11}C]guanidine was 6.2 min. TLC (methylene chloride/ethyl acetate/methanol/ammonium hydroxide (3M), 20/20/10/1) showed a single radioactive spot (Rf 0.24) co-eluting with authentic reference compound.

Autoradiographic radioligand binding studies

Slide-mounted cryosections (14 μm) from brains of Sprague-Dawley rats were incubated at room temperature for 15 min with 1,3-di(2-tolyl)-[^{11}C]guanidine using the procedure of Weber *et al.*[3] After incubation, PhosphorImager® plates were exposed for 30 min and then developed using the Molecular Dynamics PhosphorImager®.

PET studies

Female Rhesus monkeys (Macaca mulatta, 10 - 12 kgs) were placed in the PET scanner (GE 4096-15 WB-Plus). Anesthesia was induced by intravenous infusion of propofol (Diprivan®, ICI, 5 mg/kg/h) and muscular relaxation was provided by intravenous injection of atracurium (Tracrium®, Wellcome). Mechanical ventilation with oxygen in air was maintained throughout the studies. Radioactive doses (100 - 200 MBq) were administered intravenously in one hind leg of the monkey. Ten min prior to injection of the second dose of 1,3-di(2-tolyl)-[11C]guanidine, haloperidol (Haldol®, Janssen, 0.5 mg/kg) was injected intravenously.

Results and Discussion

[11C]Cyanogen bromide was produced from hydrogen [11C]cyanide according to a published procedure,[9] and reacted with *o*-toluidine in toluene according to Scheme 1. The [11C]cyanamide (5) was obtained in high radiochemical yield in a short synthesis time when using 0.1 - 0.2 M concentrations of *o*-toluidine. The further reaction to produce guanidine (6), however, required the use of a higher concentration (1.9 M) of *o*-toluidine to afford good yields. As seen in Fig. 1, the maximum radiochemical yield of 1,3-di(2-tolyl)-[11C]guanidine was obtained after 7.5 min reaction time. Thus, starting with 11.5 GBq (310 mCi) of [11C]cyanogen bromide, 4.74 GBq (128 mCi) of

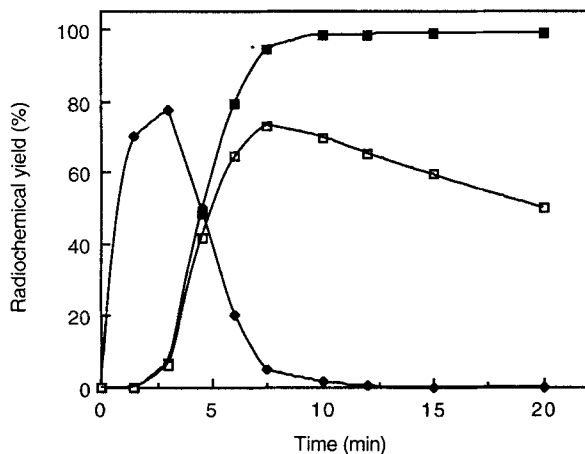Fig.1.   Decay-corrected radiochemical yield of (2-methyl)phenyl [11C]cyanamide (◆) and 1,3-di(2-tolyl)-[11C]guanidine (■). Also shown is the absolute radiochemical yield of 1,3-di(2-tolyl)-[11C]guanidine (▢).

1,3-di(2-tolyl)-[^{11}C]guanidine was obtained in 22 min synthesis time counted from [^{11}C]cyanogen bromide, corresponding to an overall decay-corrected radiochemical yield of 87 %. The specific radioactivity of the product at the end of synthesis was 118 GBq μmol^{-1} (3.2 Ci μmol^{-1}) as determined by HPLC.

In vitro autoradiographic studies

Incubating rat brain sections with nM concentrations of 1,3-di(2-tolyl)-[^{11}C]guanidine showed a heterogenous uptake of tracer with accumulation in cortical and thalamic regions, in accordance with the observations by Weber *et al.*[3] Binding was reversible and could be displaced by incubation with 10 μM unlabelled ligand.

Positron emission tomography studies

The radioactivity following intravenous injection of 1,3-di(2-tolyl)-[^{11}C]guanidine showed a low uptake in Rhesus monkey brain, whereas high uptake values were seen in temporal muscle. Upon intravenous administration of haloperidol, a slight increase of radioactivity uptake in brain was observed. These findings are consistent with a low tracer penetration of the blood-brain barrier. The increase of uptake following haloperidol administration can be rationalized by peripheral displacement of tracer, increasing the concentration of free ligand in blood.

CONCLUSIONS

A facile synthesis of 1,3-di(2-tolyl)-[^{11}C]guanidine has been developed using [^{11}C]cyanogen bromide in a procedure which should be useful also for the synthesis of other symmetrical *N,N'*-disubstituted guanidines by the proper choice of amine. Using a slightly different approach, [^{11}C]cyanogen bromide might also be useful for the synthesis of unsymmetrically substituted guanidines by treatment of the intermediate [^{11}C]cyanamide with an amine hydrohalide salt.[10]

Although the results obtained from *in vitro* experiments suggest that 1,3-di(2-tolyl)-[^{11}C]guanidine binds to the σ-receptor, the poor blood-brain barrier penetration of this ligand renders it less interesting as a tracer for *in vivo* PET investigations in the brain.

ACKNOWLEDGMENTS

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