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Synthesis of Racemic [α-^{11}C]Amphetamine and [α-^{11}C]Phenethylamine from [^{11}C]Nitroalkanes

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SUMMARY

The synthesis of racemic [α-^{11}C]amphetamine and [α-^{11}C]-phenethylamine from on-line produced [^{11}C]nitroethane and [^{11}C]nitromethane, respectively, is described. The condensations of no-carrier-added [^{11}C]nitroalkanes with benzaldehyde to form [β-^{11}C]β-nitrostyrenes were investigated under basic and acidic conditions. The [β-^{11}C]β-nitrostyrenes were reduced with lithium aluminium hydride to produce the corresponding saturated amines. Purification was performed with semi-preparative reversed-phase HPLC using a Suplex pKb-100 column. The total radiochemical yields were 7-20% (from EOB and decay-corrected) and 24-35% (based on [^{11}C]nitroalkanes and decay-corrected) with a total synthesis time of 40-55 min. The specific radioactivity of [α-^{11}C]amphetamine and [α-^{11}C]phenethylamine at EOS was 200-1000 Ci/mmol (7.4-37 GBq/μmol) with a radiochemical purity >99%.

INTRODUCTION

Major neurotransmitters in the mammalian brain are the catecholamines (dopamine, noradrenaline and adrenaline) and the

Key Words: [α-^{11}C]amphetamine, [α-^{11}C]phenethylamine, [^{11}C]nitroethane, [^{11}C]nitromethane, [^{11}C]nitroalkane

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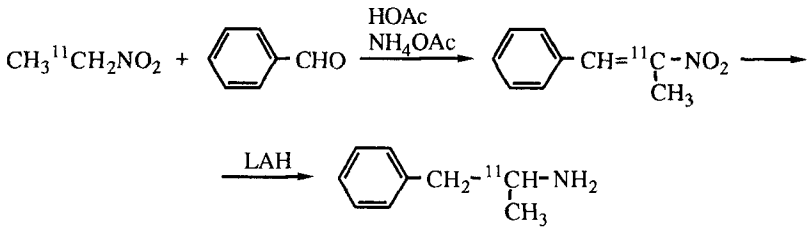
indoleamines (serotonin). Treatment of neuropsychiatric disorders with neuroactive compounds is often designed to increase or decrease the effects of these biogenic amines. In normal subjects a change in the levels of these amines may result in abnormal behaviour.

Amphetamine increases the synaptic levels of noradrenaline or dopamine via stimulation of their release. Administration of amphetamine results in a psychotic state, partially resembling paranoid schizophrenia (1). PET (positron emission tomography) examination of the human brain with ^{11}C-labelled amphetamine or related compounds may provide information on the mechanism of amphetamine psychosis in man and the pathiophysiology of schizophrenia.

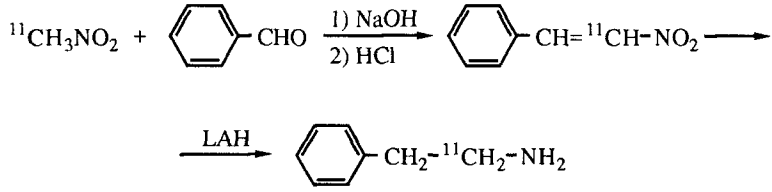
Amphetamine and several structurally related compounds have previously been prepared. Phenethylamine has been labelled from carrier-added (CA) [^{11}C]cyanide in the α-position (2). [^{11}C]Cyanide has also been found useful for a no-carrier-added (NCA) preparation of 3,4-dimethoxyphenethylamine labelled in the α-position (3). The NCA preparation of [^{11}C]dimethylphenethylamine labelled in two different positions using [^{11}C]methyl iodide and [^{11}C]phenethyl iodide, respectively, has also been reported (4). Amphetamine has been labelled both in the amino position with CA [^{13}N]ammonia (5) and in the methyl position by methylation of dimethyl 2-benzylmalonate with NCA [^{11}C]methyl iodide (6).

In this paper the syntheses of NCA [α-^{11}C]amphetamine and [α-^{11}C]phenethylamine are described (Scheme 1 and 2). [^{11}C]Nitroethane and [^{11}C]nitromethane, on-line produced from [^{11}C]ethyl iodide and [^{11}C]methyl iodide (7), respectively, were condensed with benzaldehyde using basic or acidic reaction conditions. A Sep-Pak (C-18) procedure was used to purify the intermediate products, [β-^{11}C]β-nitrostyrenes, which were subsequently reduced with lithium aluminium hydride (LAH) to produce [α-^{11}C]amphetamine and [α-^{11}C]phenethylamine. The

purification of both compounds was performed by semi-preparative reversed-phase HPLC using a Suplex pKb-100 column (8), which has been especially designed for separation of basic compounds.



Scheme 1.



Scheme 2.

EXPERIMENTAL

General

The [^{11}C]carbon dioxide was produced at the Karolinska Hospital with a Scanditronix RNP 16 cyclotron using the ^{14}N(p, α)^{11}C reaction. [^{11}C]Carbon dioxide produced was trapped in a stainless steel coil cooled with liquid nitrogen before being transferred to the one-pot ^{11}C-alkyl iodide system. [^{11}C]Methyl iodide was synthesized from [^{11}C]carbon dioxide utilizing a one-pot reaction set-up (9). Tetrahydrofuran (THF) was refluxed over sodium/benzophenone for 30 minutes and distilled under nitrogen. A solution of LAH was prepared from THF and stored

under nitrogen. Hydroiodic acid (57%) was distilled over red phosphorous in an atmosphere of nitrogen and stored in septum-sealed glass vessels (10 mL) protected from light at -26°C. Nitromethane, nitroethane and benzaldehyde were obtained from Merck. β-Nitrostyrene, β-methyl-β-nitrostyrene, phenethylamine and racemic amphetamine were prepared according to the literature (10-12). Other chemicals were obtained from commercial sources and were of analytical grade.

Analysis of the intermediate products (Figs. 1 and 2) and determination of radiochemical purity of [α-^{11}C]amphetamine and [α-^{11}C]phenethylamine were performed by a gradient analytical HPLC system using two Kontron 420 pumps, a Kontron 491 solvent mixer, a Rheodyne injector (7125 with a 1 mL loop), a Kontron UV 432 detector (254 nm) and a Beckman 170 radioactivity monitor connected to a Kontron 450 Multitasking computer system. The following three different analytical systems were used: (1) a Waters μ-Bondapak C-18 column (300 x 7.8 mm, 10 μm) eluted with acetonitrile/0.01 M phosphoric acid at a flow rate of 6.0 mL/min, linear gradient during 6 min from (35/65) to (60/40), linear gradient during 0.5 min to (80/20) and then isocratic elution at 3 min (Figs. 1 and 2), (2) same column, mobile phase and flow rate as above, isocratic elution during 6 min at (10/90), linear gradient during 0.5 min to (80/20) and then isocratic elution at 3 min and (3) a Suplex pKb-100 column (250 x 10 mm, 12 μm) eluted isocratically at a flow rate of 4.0 mL/min with acetonitrile/0.05 M potassium phosphate, pH 7.0 (2/98).

Semi-preparative reversed-phase HPLC was performed using a Kontron 420 pump, an automatic sample injector (Type Vici with a 1 mL loop) and a Kontron 432 UV-detector (254 nm) in series with a GM tube for radiation detection. Two different HPLC columns were tested in order to separate [α-^{11}C]amphetamine and [α-^{11}C]phenethylamine: (1) a Waters μ-Bondapak C-18 column (300 x 7.8 mm, 10 μm) eluted isocratically at a

flow rate of 4.0 mL/min with acetonitrile/0.01 M phosphoric acid (10/90) and (2) a Suplex pKb-100 column (250 x 10 mm, 12 μm) eluted with acetonitrile/0.05 M potassium phosphate, pH 7.0 (2/98) at a flow rate of 4.0 mL/min (Figs. 3 and 4).

[α-^{11}C]Amphetamine (Scheme 1)

[^{11}C]Nitroethane, prepared as described in detail elsewhere (7), was trapped at room temperature in a reaction vessel (1.0 mL mini-vial, Alltech), containing ammonium acetate (46 mg, 0.60 mmol), benzaldehyde (30 μL, 0.30 mmol), glacial acetic acid (200 μL) and methanol (100 μL). The vessel was sealed and heated at 140°C for 10 min. Methanol (0.3 mL) was added and the pale yellow solution was transferred to a syringe containing water (1.5 mL) connected to a Sep-Pak C_{18}-cartridge (prewashed with ethanol and water). The solution was eluted through the column followed by a wash with water (2 mL). [β-^{11}C]β-methyl-β-nitrostyrene was then eluted with n-hexane (3 mL, the first 4 drops discarded) and was subsequently passed through a Na_2SO_4-column (2.0 g, 5 mL-syringe) into a two-necked vessel (10 mL, connected with a Vigreux column). LAH/THF (250 μL, 0.2 M) was added dropwise at room temperature under a flow of nitrogen. After heating the vessel for 1 minute at reflux the solvent was evaporated at 120°C. The residue was cooled and dissolved in HCl (1.0 mL, 0.3 M) and injected on to the semi-preparative HPLC column. [α-^{11}C]Amphetamine eluted between 9-10.5 min using the Waters μ-Bondapak C-18 column and between 9-10 min with the Suplex pKb-100 column (Fig. 3), with the same retention time as a standard reference sample. After evaporation of the mobile phase, the residue was dissolved in sterile physiological phosphate buffer (pH 7.4, 8 mL) and filtered through a Millipore filter (0.22 μm), yielding a solution which was sterile and free from pyrogens.

[α-^{11}C]Phenethylamine (Scheme 2)

[^{11}C]Nitromethane, prepared as described in detail elsewhere (7), was

trapped at room temperature in a reaction vessel (1.0 mL mini-vial, Alltech), containing benzaldehyde (10 μL, 0.10 mmol), sodium hydroxide (4 μL, 10 M) and methanol (0.3 mL). After complete trapping, hydrochloric acid (50 μL, 6.0 M) in methanol (0.3 mL) was added and the pale yellow solution was transferred to a syringe containing water (1.5 mL) connected to a Sep-Pak C_{18}-cartridge (prewashed with ethanol and water). The subsequent experimental procedure was similar as described above for [α-^{11}C]amphetamine. [α-^{11}C]Phenethylamine eluted between 6.5-8.0 min using the Waters μ-Bondapak C-18 column and between 5.5-6.5 min with the Suplex pKb-100 column (Fig. 4), with the same retention time as a standard reference sample.

RESULTS AND DISCUSSION

The general approach to prepare secondary or tertiary α-^{11}C-labelled alkyl amines is by N-alkylation with a ^{11}C-alkyl iodide or by reductive N-alkylation with [^{11}C]formaldehyde. So far the access to primary α-^{11}C-labelled alkyl amines have exclusively been limited to the use of [^{11}C]cyanide. The development of a new class of ^{11}C-labelled precursors, ^{11}C-labelled nitroalkanes (7), has increased the range of suitable tools for labelling primary amines with ^{11}C. Nitroalkanes can readily be converted to anions, by the addition of base, and undergo condensation with aldehydes. The corresponding amines are formed by reduction of the condensation products. The potential of [^{11}C]nitromethane as a radiolabelling precursor in condensation reactions has already been demonstrated in the condensation with benzaldehyde (13) and with D-arabinose (14).

The condensation reactions of [^{11}C]nitroalkanes investigated here are modifications of earlier published preparative synthetic work (10-11). Three different synthetic approaches were studied under NCA conditions. In the first approach, methanol containing hydroxide ion

gave a high incorporation (74-90%) of [^{11}C]nitromethane to [β-^{11}C]β-nitrostyrene (Fig. 2, **VII**). However, a significantly lower radiochemical incorporation was obtained (10-20%) when [^{11}C]nitroethane was condensed with benzaldehyde during similar reaction conditions in the preparation of [β-^{11}C]β-methyl-β-nitrostyrene (**III**). In a second approach, the yield of **III** (Fig. 1) was markedly increased (63-88%) under acidic conditions (acetic acid/ammonium acetate). The final approach tested was an intermediate Shiff base formation, using a primary amine in an alcoholic solvent (15-16), which turned out to be unsuccessful. Reaction parameters such as solvent composition, substrate concentration, temperature and reaction time were varied in the different approaches in order to optimize the incorporation of [^{11}C]nitromethane and [^{11}C]nitroethane.

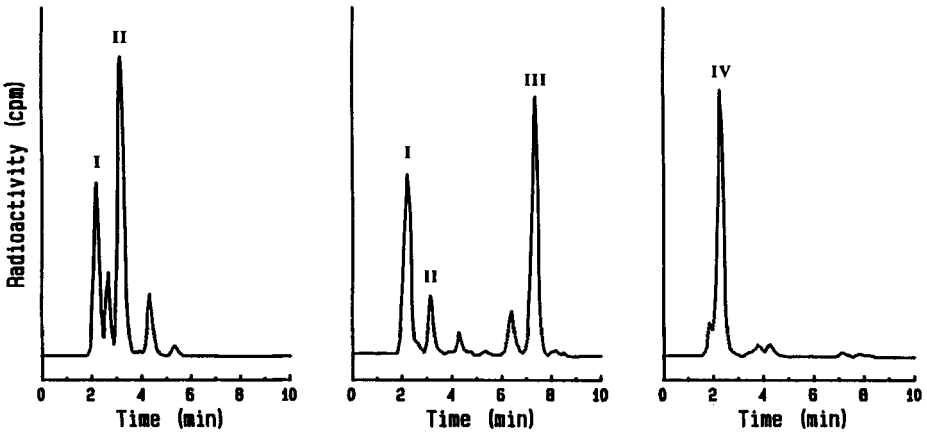


Figure 1. Analytical HPLC chromatograms (radioactivity vs time) using a Waters μ-Bondapak C-18 column in the preparation of [α-^{11}C]amphetamine (HPLC-system 1). Left: before condensation. Middle: after condensation and before Sep-Pak. Right: after reduction and before semi-preparative HPLC. **I**, [^{11}C]ethyl nitrite; **II**, [^{11}C]nitroethane; **III**, [β-^{11}C]β-methyl-β-nitrostyrene; **IV**, [α-^{11}C]amphetamine.

The intermediate products, [β-^{11}C]β-nitrostyrenes, were passed through a Sep-Pak C_{18}-cartridge to remove polar components and enable a solvent (n-hexane) more suitable for the subsequent reduction. The discarded fractions contained radioactivity mostly consisting of

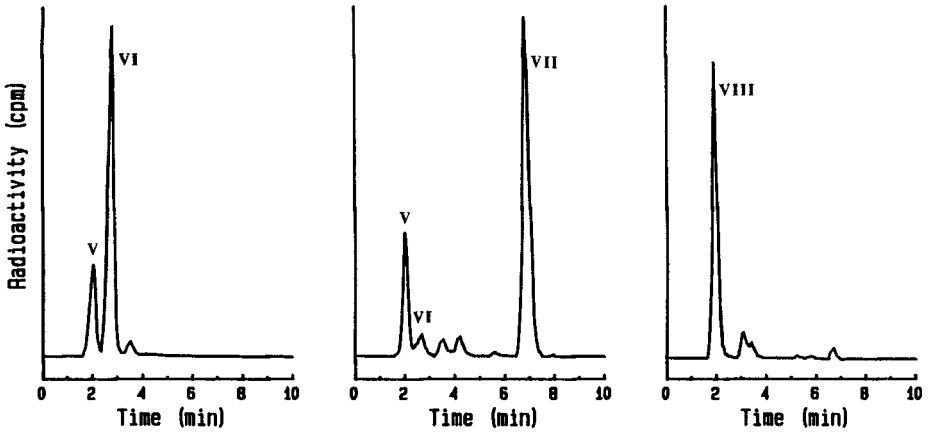


Figure 2. Analytical HPLC chromatograms (radioactivity vs time) using a Waters μ-Bondapak C-18 column in the preparation of [α-^{11}C]phenethylamine (HPLC-system 1). Left: before condensation. Middle: after condensation and before Sep-Pak. Right: after reduction and before semi-preparative HPLC. **V**, [^{11}C]methyl nitrite; **VI**, [^{11}C]nitromethane; **VII**, [β-^{11}C]β-nitrostyrene; **VIII**, [α-^{11}C]phenethylamine.

unreacted [^{11}C]nitroalkane and the corresponding [^{11}C]nitrite. Reduction of the nitro group and the double bond was performed by a dropwise reversed addition of LAH/THF to the stirred n-hexane solution under nitrogen. The reaction proceeded gently (70-90% conversion) and formed byproducts only to a low extent. After reduction the aluminium complex was decomposed by aqueous HCl giving a crude product ready for injection on to the HPLC column.

In the final semi-preparative HPLC-purification two different HPLC columns were tested. A Suplex pKb-100 column (8), especially designed for separation of basic compounds, generally gave a higher radiochemical purity (>99%) than a Waters μ-Bondapak-C18 column. Furthermore, the products eluted earlier and gave more reproducible retention times compared to the Waters μ-Bondapak-C18 column.

The total radiochemical yields were 7-20% (from EOB and decay-corrected) and 24-35% (based on [^{11}C]nitroethane and [^{11}C]-

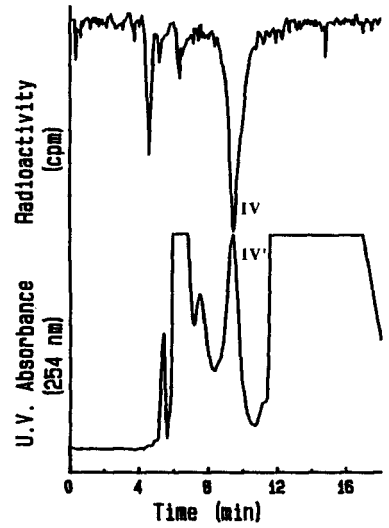


Figure 3. Semi-preparative HPLC chromatogram (radioactivity and U.V. vs time) using a Suplex pKb-100 column in the purification of [α-^{11}C]amphetamine. **IV**, [α-^{11}C]amphetamine; **IV'**, unlabelled amphetamine.

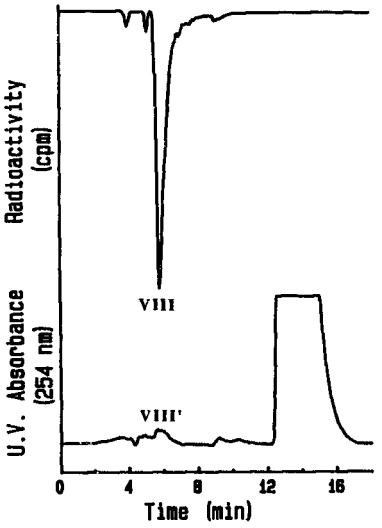


Figure 4. Semi-preparative HPLC chromatogram (radioactivity and U.V. vs time) using a Suplex pKb-100 column in the purification of [α-^{11}C]phenethylamine. **VIII**, [α-^{11}C]phenethylamine; **VIII'**, unlabelled phenethylamine.

nitromethane and decay-corrected) with a total synthesis time of 40-55 min (including HPLC purification and sterile filtration). The specific radioactivity of [α-^{11}C]amphetamine and [α-^{11}C]phenethylamine at EOS was about 200 and 400-1000 Ci/mmol (7.4 and 15-37 GBq/μmol),

respectively, with a radiochemical purity >99%. In typical production runs 4-7 mCi of racemic [α-^{11}C]amphetamine and 17-24 mCi of [α-^{11}C]phenethylamine were isolated in a pure and sterile form starting from 90-150 mCi [^{11}C]nitroethane and 250-300 mCi [^{11}C]nitromethane, respectively.

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