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Research Article

Synthesis and preliminary evaluation of a carbon-11-labelled agonist of the α7 nicotinic acetylcholine receptor

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Summary

The lead compound of a new series of azabicyclic carbamates described by Astra Laboratories as ligands for the α7 nicotinic acetylcholine receptor subtype, namely *N*-(4-bromophenyl)carbamic acid quinuclidin-3-yl ester, has been labelled with carbon-11 using no-carrier-added [^{11}C]phosgene and the isocyanate pathway. Typically, 25–35 mCi (0.92–1.29 GBq) of the tracer was obtained within 30 min of radiosynthesis (HPLC purification included) with specific radioactivities ranging from 500 to 800 mCi/μmol (18.5–29.6 GBq/μmol). Biodistribution studies demonstrated a relatively good brain uptake of the compound (0.8–1.2% I.D./g tissue in various brain regions), but without preferential concentration in brain regions rich in α7-subtype nicotinic receptor (e.g. hippocampus, pons and colliculi). No specific binding could be demonstrated in pre-saturation studies performed with both the cold compound and nicotine. Therefore, this ligand is not suitable for further exploration in PET imaging.

Key Words: [^{11}C]isocyanate; α7 nAChR; carbon-11; positron emission tomography; PET

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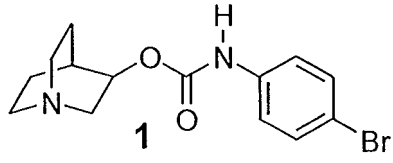
Introduction

The hypothesis that cholinergic dysfunction contributes to cognitive impairment in patients with senile dementia of the Alzheimer type or Parkinson disease[1] has prompted considerable exploration of positron emission tomography (PET) radioligands in order to visualize central nicotinic acetylcholine receptors (nAChRs) in human brain. Since a consistent and severe loss[2,3] of these receptors is found in these diseases, a radiotracer technique such as PET would find a practical use in the diagnosis of early-stage disease as well as in the planning and the monitoring of the treatment of patients. One of the predominant nAChR subtypes in the mammalian brain contains the $\alpha 7$ subunit.[4,5] These receptors are concentrated especially in telencephalic regions such as hippocampus and are involved in cognitive functions, schizophrenia and neuronal survival especially in the protection against the toxicity of β-amyloid peptides.[6]

Recently, a new series of azabicycle carbamates has been described by Astra Laboratories as ligands for the $\alpha 7$ nAChR subtype.[7,8] Due to its chemical structures, this whole series of carbamates presents the opportunity to be labelled with carbon-11, a positron emitter of 20.4 min half life, at the same site, the carbamate function, using the approach based on the radiosynthesis of isocyanates[9] from [^{11}C]phosgene.



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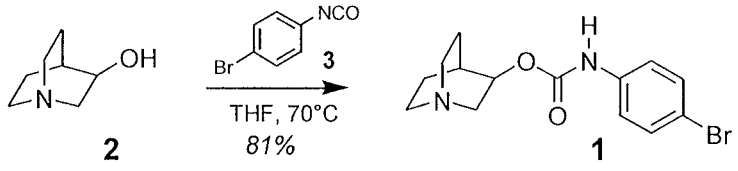
In this paper, we have investigated the carbon-11 radiosynthesis of the lead compound of this series, namely *N*-(4-bromophenyl)carbamic acid quinuclidin-3-yl ester (**1**).

Results and discussion

Chemistry

N-(4-bromophenyl)carbamic acid quinuclidin-3-yl ester (**1**) was prepared for reference use in one chemical step from commercially

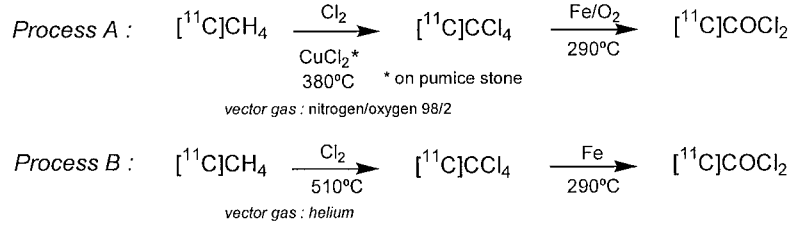
available 4-bromophenylisocyanate (**3**) and 3-quinuclidinol (**2**) in 81% yield. Analytical data (^{1}H NMR, ^{13}C NMR and MS) were in accordance with the described structure.



Derivative **1** was also prepared on a submicromolar scale, in order to verify the conditions which could be used in the carbon-11 radiosynthesis. As observed by HPLC, reaction of the isocyanate **3** with 3-quinuclidinol (**2**) in THF at 75–85°C was rapid (<3 min) and directly led to the desired carbamate **1** in high yield (70–90%).

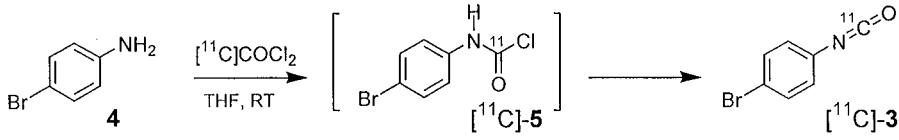
Radiochemistry

[^{11}C]Phosgene ([^{11}C]$COCl_2$) was synthesized form cyclotron-produced [^{11}C]methane ([^{11}C]CH_4), via [^{11}C]carbon tetrachloride ([^{11}C]CCl_4) using one of two different processes. In process A, [^{11}C]CH_4 was mixed with chlorine and the mixture was passed through a glass U-tube containing pumice stone impregnated with $CuCl_2$ at a temperature of 380°C.[10] In process B, the mixture of [^{11}C]CH_4 and chlorine was simply passed through an empty linear glass-tube at a temperature of 510°C.[11] Then, in both processes, the on-line synthesized [^{11}C]CCl_4 was passed through a glass U-tube containing iron filings at 290°C, giving [^{11}C]$COCl_2$.

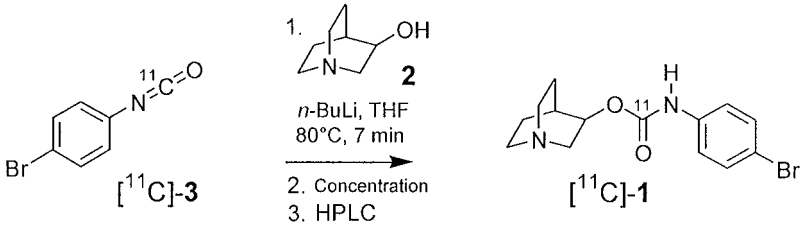


Regardless of the synthetic process employed, 275–480 mCi (10.2–17.7 GBq) of [^{11}C]$COCl_2$ is routinely obtained in our laboratory in 9 min after the end of bombardment (31–54% decay-corrected yield, based on starting [^{11}C]CH_4).

4-bromophenyl[^{11}C]isocyanate ([^{11}C]-**3**) was simply prepared by trapping [^{11}C]$COCl_2$ at room temperature in 100 µl of THF containing 0.4 µmol of 4-bromoaniline (**4**), probably via the corresponding carbamoyl chloride **5**.



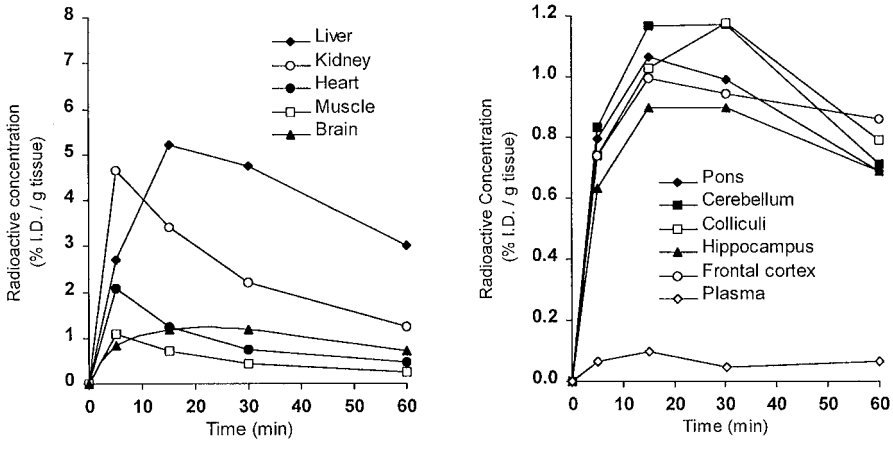
Finally, *N*-(4-bromophenyl)[^{11}C]carbamic acid quinuclidin-3-yl ester ([^{11}C]-**1**) was prepared by adding to the previous solution, 20 µmol of 3-quinuclidinol (**2**) and 16 µmol *n*-BuLi (10 µl of *n*-BuLi 1.6 M in hexanes) in 150 µl of THF. The resulting solution was heated at 80°C for 7 min and concentrated to dryness using a helium flow. The crude was diluted with 0.5 ml of the HPLC mobile phase and purified by HPLC.



Typically, 25–35 mCi (0.92–1.29 GBq) of *N*-(4-bromophenyl)[^{11}C]-carbamic acid quinuclidin-3-yl ester ([^{11}C]-**1**) was obtained within 30 min of radiosynthesis (HPLC purification included) with specific radioactivities ranging from 500 to 800 mCi/µmol (18.5–29.6 GBq/µmol)). As demonstrated by HPLC analysis, the radiotracer was found to be >95% chemically and radiochemically pure.

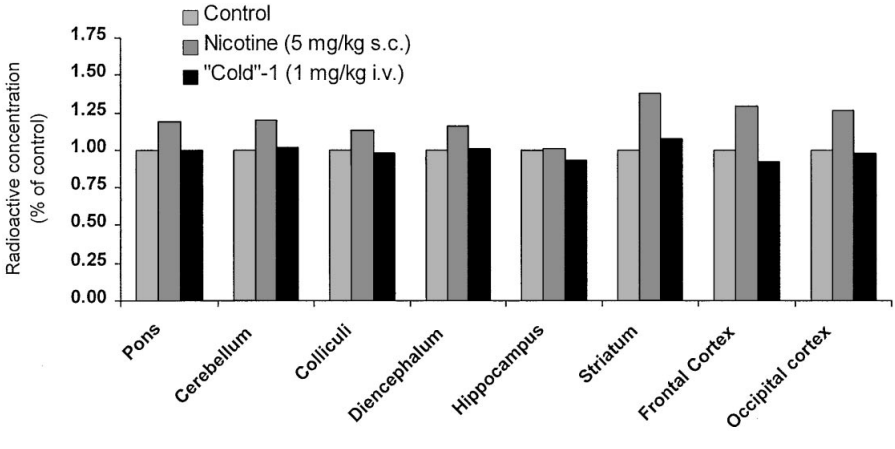
Pharmacology

As a preliminary characterization, a biodistribution study of the radiotracer was performed in rats. Adult male Sprague–Dawley rats weighing ~200 g were each injected with 24–28 µCi of the radiotracer [^{11}C]-**1** in the tail vein. The rats were sacrificed at selected time points after injection ($n = 3$ per time point). Tissues of different organs (brain, heart, lung, liver, kidney, skeletal muscle) were rapidly removed and weighed. Radioactivity of the samples was measured in a γ-counter and tissue concentrations were expressed as percent of injected does per gram of tissue (% I.D./g tissue).



These biodistribution studies demonstrated a relatively good brain uptake of the compound (0.8–1.2% I.D./g tissue in various brain regions), but without preferential concentration in brain regions rich in α7-subtype nicotinic receptor (e.g. hippocampus, pons and colliculi).

In blocking studies, rats were pre-treated with the unlabelled compound (**1**, 1 mg/kg i.v. given 30 min before the radiotracer) and nicotine (5 mg/kg s.c. 5 min before the radiotracer). Each of the animals ($n = 3$ for each series) was then injected with 55–65 μCi of the tracer [^{11}C]-**1** in the tail vein and sacrificed 15 min after injection. Tissues were rapidly removed and processed as mentioned above. Radioactivity of the samples was measured in a γ-counter and tissue concentrations were expressed as percent of the control and corrected for plasma variation.



No specific binding could be demonstrated in pre-saturation studies performed with both the cold compound and nicotine. Therefore, this ligand is not suitable for further exploration in PET imaging.

Experimental

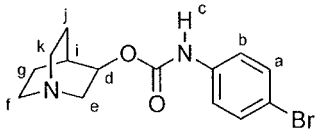
General

Chemicals were purchased from standard commercial sources (Aldrich, Fluka or Sigma France) and were used without further purification unless otherwise stated.

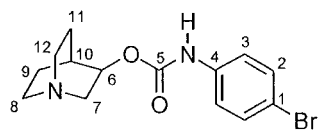
TLCs were run on pre-coated plates of silicagel $60F_{254}$ (Merck). The compounds were localized (1) when possible at 254 nm using a UV-lamp and/or (2) by iodine staining and/or (3) by dipping the TLC-plates in a 1% ethanolic ninhydrin solution (or in a 1% aqueous $KMnO_4$) and heating on a hot plate. Radioactive spots were detected using a Berthold TraceMaster 20 automatic TLC linear analyser. Flash chromatography was conducted on silicagel 63–200 μm (Merck) at 0.3 bars (compressed air).

HPLCs: *Equipment*: Waters or Shimadzu systems. For example, Waters systems equipped with a 510 pump, 440 UV detector or 481 and 486 UV-multiwavelength detectors; the effluent was also monitored for radioactivity with a Geiger-Müller counter; column: semipreparative C-18 Zorbax® SB, Hewlett-Packard (250×9.4 mm); porosity: 5 μm; conditions: isocratic elution with CH_3CN/H_2O/TFA, 30/70/0.1 (v : v : v); flow rate: 8.0 ml/min; UV detection at λ : 220 nm).

NMR spectra were recorded on a Bruker AMX (300 MHz) apparatus using the hydrogenated residue of the deuterated solvents (DMSO-d_6, δ: 2.50 ppm) and/or TMS as internal standards for ^{1}H NMR as well as the deuterated solvents (DMSO-d_6, δ: 39.5 ppm) and/or TMS as internal standards for ^{13}C NMR. For COSY, 128 experiments were recorded; before Fourier transform and symmetrization, the data were multiplied with an unshifted sine bell function in each dimension. For ^{1}H-^{13}C shift correlation, 256 experiments were recorded; before Fourier transform, the data were multiplied with an unshifted sine bell function in F2 (^{13}C) and with an exponential function in F1 (^{1}H). The chemicals shifts are reported in ppm, downfield either from TMS (s, d, t, dd, b for singlet, doublet, triplet, doublet of doublet and broad, respectively; $^{\#}$, *, $^{\circ}$: interchangeable assignments). The nomenclature used for the ^{1}H and ^{13}C NMR attribution is given below:



^{1}H-NMR



^{13}C-NMR

The mass spectra (MS) were measured on a Nermag R10-10 or a Quadripolair Finnigan 4600 instrument (DCI/NH_4^+).

Air- or moisture-sensitive reactions were conducted in heat-gun-dried glassware, under an inert atmosphere and with freshly distilled solvents.

Radiosyntheses were performed in a 5 cm-lead-shielded confinement. Specific radioactivity was determined as follows: the area of the UV absorbance peak corresponding to the radiolabelled product was measured on the HPLC chromatogram and compared to a standard curve relating mass to UV absorbance.

Chemistry

*N-(4-bromophenyl)carbamic acid quinuclidin-3-yl ester (**1**).* To 200 mg of 3-quinuclidinol (**2**, 1.57 mmol, MW: 127.19) in 20 ml of THF under a N_2 atmosphere at room temperature, was slowly added 310 mg of 4-bromophenyl isocyanate (**3**, 1.57 mmol, MW: 198.02, 1 equiv.). The mixture was stirred at room temperature for 5 min and then heated at 70°C for another 15 min. Finally, the reaction mixture was concentrated to dryness and the residue was then purified by chromatography on silica gel. Elution with heptane/AcOEt (90/10) gave 410 mg (81%) of pure *N*-(4-bromophenyl)carbamic acid quinuclidin-3-yl ester (**1**) as a white powder.

Rf (heptane/AcOEt 80/20): 0.3; Rt (HPLC): 7.0 min; ^{1}H NMR (DMSO-d6, 333.0 K: δ: 9.59 (s, 1H, *H-c*); 7.46 (s, 4H, *H-a,b*); 4.73 (bt, $w_{1/2}$: 10 Hz, 1H, *H-d*); 3.21 (bs, $w_{1/2}$: 20 Hz, 1H, *H-e*); 2.79 (multi sharp-peak system, $w_{1/2}$: 15 Hz, 1H, *H-k*); 2.66 (multi sharp-peak system, $w_{1/2}$: 15 Hz, 1H, *H-f*); 2.61 (multi sharp-peak system, $w_{1/2}$: 15 Hz, 1H, *H-e*); 2.10 (bs, $w_{1/2}$: 10 Hz, 1H, *H-i*); 1.84 (multi sharp-peak system, $w_{1/2}$: 25 Hz, 1H, *H-j*); 1.64 (multi sharp-peak system, $w_{1/2}$: 25 Hz, 1H, *H-g*); 1.56 (multi sharp-peak system, $w_{1/2}$: 25 Hz, 1H, *H-g*); 1.41 (multi sharp-peak system, $w_{1/2}$: 25 Hz, 1H, *H-j*); ^{13}C NMR (DMSO-d6, 333.0 K: δ: 153.2 (C-5); 138.6 (C-4); 131.3 (CH-3$^{\#}$); 120.2 (CH-2$^{\#}$); 113.8 (C-1); 71.5 (CH-6); 55.1 (CH_2-7); 46.9 (CH_2-12*); 46.0 (CH_2-8*); 25.3 (CH-10); 24.2 (CH_2-9°); 19.2 (CH_2-11°); MS (DCI/NH_4^+); $C_{14}H_{17}BrN_2O_2$; 327 [M + H^+]; 325 [M + H^+].

Radiochemistry

Preparation of [^{11}C]CH_4. [^{11}C]CH_4 was produced by irradiation of an ultrapure Air Liquide 95/5 mixture of N_2/H_2 target with a 20 MeV proton beam (30 μA) *via* the ^{14}N[p,α]^{11}C nuclear reaction on a CGR-MeV 520 cyclotron (54 000 μC in 30 min). At the end of the bombardment, the target contents were transferred to the 5 cm-lead-shielded hot cell dedicated to the radiosynthesis of the tracer and passed first through an empty tube (stainless-steel coil, 500 mm length, 4 mm internal diameter, cooled at −186°C using liquid argon) in order to remove traces of ammonia (produced during the irradiation) and then through a glass P_2O_5-guard (70 mm length, 3 mm internal diameter) in order to remove residual moisture. [^{11}C]CH_4 was then separated from the target gas by trapping in a copper-U-tube (150 mm length, 4 mm internal diameter) filled with Porapak-Q (80–100 mesh, Waters) and cooled at −186°C (liquid argon).

On average, about 1.20 Ci or 44.40 GBq (EOB) of [^{11}C]CH_4 is routinely obtained in our laboratory for a 30 μA, 30 min (54 000 μC) irradiation.

Preparation of [^{11}C]$COCl_2$. Process A: [^{11}C]CH_4 was released from the trap by simply warming the Porapak-Q-tube to room temperature (hot-air jet) and using helium as vector gas (40 ml/min). [^{11}C]CH_4 was then passed through a glass P_2O_5-guard (70 mm length, 10 mm internal diameter) and concentrated in a second copper U-tube (150 mm length, 2 mm internal diameter) filled with Porapak-Q (80–100 mesh, Waters) and cooled at −186°C (liquid argon) [^{11}C]CH_4 was released from the trap by warming the latter to room temperature (hot-air jet) and swept (15 ml/min) in a volume of 1–2 ml of helium into a gas-mixing chamber containing 10 ml of chlorine (99.99%, Air Liquide). Using nitrogen/oxygen (98/2) as vector gas (15 ml/mm), the [^{11}C]CH_4—chlorine mixture was passed through a glass U-tube (200 mm length, 6 mm internal diameter) containing 3.0 g of pumice stone impregnated with $CuCl_2$[†] at a temperature of 380°C. The on-line synthesized [^{11}C]CCl_4 was then passed through a glass U-tube (200 mm length, 4 mm internal diameter) containing 1.5 g of iron filings (Telar 57, Weber) at a temperature of 290°C (without changing the vector gas that is mentioned above)

[†] Preparation: pumice stone impregnated with $CuCl_2$: 65 g of $CuCl_2$ (Aldrich) was dissolved in 56 ml of milli-Q water. To this solution, pumice stone (40 g, Merck) was added and the mixture was stirred for 20 h. After filtration, the mass was dried for 2 h at 110°C. Usually, a 1/1 ratio (v/v) of this catalyst on pumice stone and non-impregnated pumice stone were used in the chlorination process.

converting it into [^{11}C]$COCl_2$. The [^{11}C]$COCl_2$ thus synthesized was continuously swept away and passed through a glass antimony-trap (70 mm length, 3 mm internal diameter, containing a 2/1 ratio (v/v) of antimony (400 mg) and glass beads (1 mm diameter)) in order to remove the excess of chlorine.

Process B: [^{11}C]CH_4 was released from the trap by simply warming the Porapak-Q-tube to room temperature (hot-air jet) and using helium as vector gas (40 ml/min). [^{11}C]CH_4 was then passed through a glass P_2O_5-guard (70 mm length, 10 mm internal diameter) and concentrated in a second copper U-tube (150 mm length, 2 mm internal diameter) filled with Porapak-Q (80–100 mesh, Waters) and cooled at −186°C (liquid argon). [^{11}C]CH_4 was released from the trap by warming the latter to room temperature (hot-air jet) and swept (15 ml/min) in a volume of 1–2 ml of helium into a gas-mixing chamber containing 10 ml of chlorine (99.99%, Air Liquide). Still using helium as vector gas (15 ml/mm), the [^{11}C]CH_4—chlorine mixture was passed through an empty glass-tube (215 mm length, 7 mm internal diameter) at a temperature of 510°C. The on-line synthesized [^{11}C]CCl_4 was then passed through a glass U-tube (200 mm length, 4 mm internal diameter) containing 1.5 g of iron filings (Telar 57, Weber) at a temperature of 290°C (using the same vector gas that is mentioned above) converting it into [^{11}C]$COCl_2$. The [^{11}C]$COCl_2$ thus synthesized was continuously swept away and passed through a glass antimony-trap (70 mm length, 3 mm internal diameter, containing a 2/1 ratio (v/v) of antimony (400 mg) and glass beads (1 mm diameter)) in order to remove the excess of chlorine.

Regardless of the synthetic process employed, 257–480 mCi (10.2–17.7 GBq) of [^{11}C]$COCl_2$ is routinely obtained in our laboratory in 9 min after end of bombardment in 31–54% decay-corrected yield and based on starting [^{11}C]CH_4.

*Preparation of N-(4-bromophenyl)[^{11}C]carbamic acid quinuclidin-3-yl ester ([^{11}C]-**1**).* [^{11}C]$COCl_2$, carried by a flow of vector gas (nitrogen/oxygen 98/2, Process A or helium, Process B), was trapped (bubbling through) at room temperature in a reaction vessel containing 0.4 μmol of 4-bromoaniline (**4**, MW: 172.02) in 80 μl of THF. Trapping of [^{11}C]$COCl_2$ was monitored using an ionization-chamber probe. When the reading had reached its maximum (3 min usually), a THF solution (150 μl) containing 2.5 mg of 3-quinuclidinol (**2**, 20 μmol, MW: 127.19) and 10 μl of *n*-BuLi (1.6 M in hexanes) was then rapidly added to the

previous reaction mixture. The reaction vessel was then sealed, heated at 80°C using a heating block for 7 min and the mixture concentrated to dryness using a helium flow. Finally, the reaction mixture was diluted with 1.0 ml of the HPLC mobile phase and was injected onto the column. (HPLC; Rt: [^{11}C]-(**1**): 7.0–7.5 min.)

Typically, 25–35 mCi (0.92–1.29 GBq, 7% decay-corrected yield based on starting [^{11}C]CH_4) of *N*-(4-bromophenyl)[^{11}C]carbamic acid quinuclidin-3-yl ester ([^{11}C]-**1**) with a radiochemical and chemical purity of more than 95%, was obtained within 30 min of radiosynthesis (HPLC purification included) with specific radioactivities ranging from 500 to 800 mCi/μmol (18.5–29.6 GBq/μmol).

*Formulation of N-(4-bromophenyl)[^{11}C]carbamic acid quinuclidin-3-yl ester ([^{11}C]-**1**).* Formulation of labelled product for i.v. injection was effected as follows: (1) Solvent removal by evaporation of the HPLC-collected fraction containing the carbon-11 labelled tracer; (2) taking up the residue, while heating gently (45°C), in 5 ml of physiological saline; (3) filtration on a 0.22 μm Millipore filter.

*Quality control of 4-N-(4-bromophenyl)[^{11}C]carbamic acid quinuclidin-3-yl ester ([^{11}C]-**1**).* As demonstrated by HPLC analysis, the radiolabelled product was found to be >95% chemically and radiochemically pure and also to co-elute with a sample of authentic *N*-(4-bromophenyl)carbamic acid quinuclidin-3-yl ester (**1**) (retention time: 7.2 min). The preparations were shown to be free of non-radioactive precursors and radiochemically stable for at least 120 min.

Conclusion

The lead compound of a new series of azabicyclic carbamates described by Astra Laboratories, namely *N*-(4-bromophenyl) carbamic acid quinuclidin-3-yl ester (**1**), has been labelled with carbon-11 using no-carrier-added [^{11}C]phosgene. Typically, 25–35 mCi (0.92–1.29 GBq) of the tracer was obtained within 30 min of radiosynthesis (HPLC purification included) with specific radioactivities ranging from 500 to 800 mCi/μmol (18.5–29.6 GBq/μmol). Biodistribution studies demonstrated a relatively good brain uptake of the compound (0.8–1.2% I.D./g tissue in various brain regions), but without preferential concentration in brain regions rich in α7 nAChR (e.g. hippocampus, pons and

colliculi). No specific binding could be demonstrated in pre-saturation studies performed with both the cold compound and nicotine. Therefore, this ligand is not suitable for further exploration in PET imaging.

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