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Synthesis and ^{11}C-Labelling of two Selective High Affinity Nicotinic Cholinergic Agonists for Evaluation as Radioligands for PET Studies

Frédéric DOLLE*, Lilian DOLCI, Héric VALETTE, Michel BOTTLAENDER,
Denis FOURNIER, Chantal FUSEAU, Françoise VAUFREY[§] and Christian CROUZEL

Service Hospitalier Frédéric Joliot, Département de Recherche Médicale, CEA
4 Place du Général Leclerc, F-91406 Orsay, France - [§]NMR section, SHFJ, same address.

Summary

ABT-418 ((S)-3-methyl-5-[1-methyl-2-pyrrolidinyl]isoxazole) and N-methylcytisine (N-methyl-1,2,3,4,5,6-hexahydro-1,5-methano-8H-pyrido-[1,2-a][1,5]diazocin-8-one) are two high affinity nicotinic cholinergic agonists. ABT-418 was synthesized in 7 steps from commercially available (S)-Boc-proline in 35% overall yield. Methylation of commercial cytisine cleanly gave N-methylcytisine. ABT-418 and N-methylcytisine were labelled using [^{11}C]methyl iodide by methylation of the corresponding nor-precursors for their *in vivo* evaluation as positron emission tomography (PET) probes of the nicotinic cholinergic receptors in baboon brain. As for [^{11}C]nicotine, specific binding *in vivo* could not be demonstrated for ABT-418. Therefore, further experiments are needed to determine the full PET pharmacological profile and the subsequent potential clinical applications of ABT-418 as a tracer for PET experiments. For labelled N-methyl-cytisine, radioactivity in the cerebral cortex and in the blood were similar. Thus, ^{11}C-labelled N-methylcytisine does not appear to be a suitable ligand for mapping brain nAChR.

Key Words : ABT-418, N-methylcytisine, carbon-11, positron emission tomography, brain nicotinic receptors

Introduction

Nicotinic acetylcholine receptors have been the focus of much research in the past years due to their implications in several neurodegenerative diseases. Biochemical and neuropathological studies have suggested that Alzheimer's disease or senile dementia of the Alzheimer type may be primarily related to brain dysfunction of the cholinergic system[1]. A consistent and severe loss of nicotinic acetylcholine receptors (nAChR) is found in these diseases[2,3].

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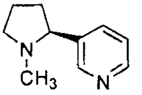
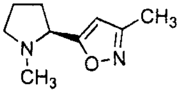
Several attempts have been made to visualize brain nAChRs with positron emission tomography (PET). The most important use of this radiotracer technique would be a practical one: the diagnosis of the disease in an early stage and the planning/monitoring of treatment with medications designed to modify brain chemistry.

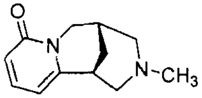
The most commonly used PET ligand has been [^{11}C]nicotine (1)[4-6]. In patients suffering from Alzheimer's disease or senile dementia of the Alzheimer type, a marked diminished brain uptake of [^{11}C]nicotine was observed when compared with age-matched controls[7]. However, several problems arise with [^{11}C]nicotine. The kinetics of [^{11}C]nicotine are rapid suggesting a very short interaction of this ligand with nAChR due to a high dissociation rate[6,8]. Nicotine is rapidly metabolized[6] but [^{11}C]cotinine, the main metabolite of nicotine, was not detected in the brain following injection in humans[6]. Also, nicotine is a rather toxic compound, and the amount that can be injected for characterization of the uptake *in vivo* (saturation studies) and for the determination of specific binding to receptors is limited[9]. *In vivo*, no saturation effect could be observed following co-injection of labelled and unlabelled nicotine in monkeys[4] and humans[8]. These characteristics limit the use of nicotine as a convenient ligand for PET imaging of brain nAChR.



Nicotine (**1**)



ABT-418 (**2**)



N-Methylcytisine (**3**)

As a part of our PET program to study neurodegenerative disease, an attempt was made to find other nAChR ligands.

The *in vitro* characteristics of the potent specific nicotinic agonist ABT-418 (**2**, (S)-3-methyl-5-[1-methyl-2-pyrrolidinyl]isoxazole[10,11]) showed high affinity for a single binding site (Kd = 2.85 nM) and a brain distribution on autoradiograhic slices in rats similar to that of the ligands nicotine and cytisine[12]. Furthermore, this compound shows a less toxic profile than nicotine (about 10 fold less toxic[13]). These properties make it a possibly suitable ligand for PET studies.

Another candidate, Amongst others for imaging nAChR with PET, is the agonist N-methylcytisine (**3**). In spite of a IC$_{50}$ of 0.05 µM for displacement of [^{3}H]nicotine (for comparaison, the IC$_{50}$ of cytisine is 0.14 µM[14]), this compound is pharmacologically active *in vivo* and appears to be less toxic than nicotine[15].

This paper fully describes :

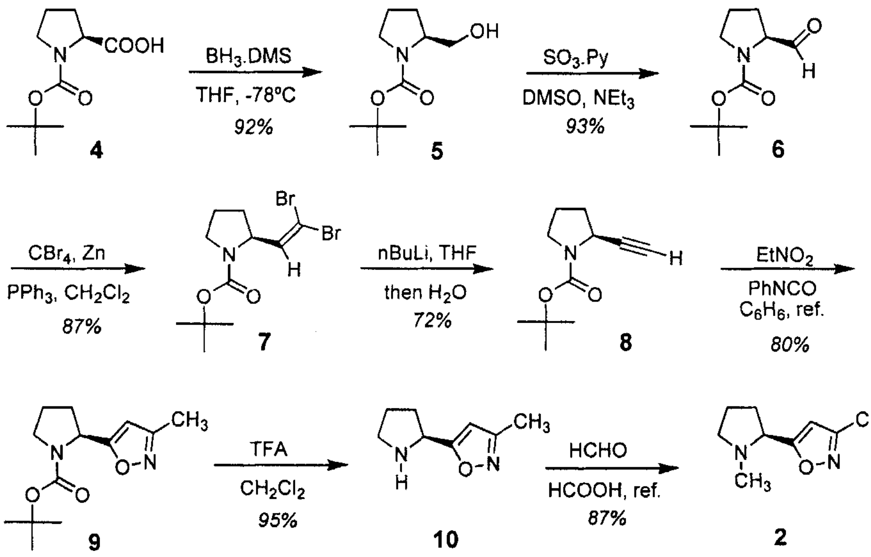
(1) : the straightforward synthesis of ABT-418 from commercially available (S)-Boc-Proline; (2) : the synthesis of N-methylcytisine from commercially available cytisine; (3) : the radiolabelling of both compounds using [^{11}C]methyl iodide; (4) : the preliminary PET experiment results.

Results and Discussion

Chemistry

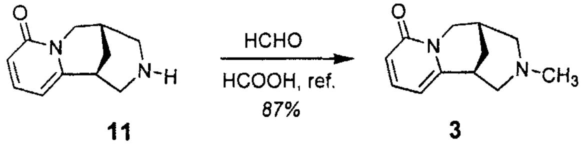
ABT-418 (**2**) was synthesized in 7 steps from commercially available (S)-Boc-Proline (**4**) in 35% overall yield. The synthesis followed the one briefly described in a short communication[11] and is outlined in the scheme below.

(S)-Boc-Proline (**4**) was cleanly reduced by an excess of diborane in THF at -78°C to give the prolinol derivative **5** with 92% yield. Parikh-Doering oxidation[16,17], using sulfur trioxide in a mixture of DMSO and triethylamine, gave (S)-Boc-prolinal (**6**) in high yield (93%). Using the Corey-Fuchs conditions[18,19] (carbon tetrabromide, triphenylphosphine and zinc dust), the dibromo vinyl intermediate **7** was synthesized from the aldehyde **6** in 87% yield. Compound **7** was then treated[18,19] with 2.1 equivalent of butyl lithium in THF at -78°C under an inert atmosphere to give the acetylide anion, which was quenched with water to give the terminal alkyne **8** in 72% yield.



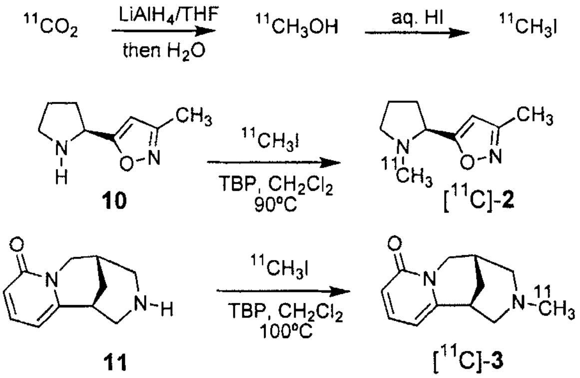
The isoxazole ring was synthesized by 1,3-dipolar cycloaddition of acetonitrile oxide[20], generated *in situ* from phenylisocyanate, nitroethane and triethylamine[21] to the acetylenic function of **8**. Derivative **9** was obtained in 80% yield. TFA deprotection of the tert-butoxycarbonyl function gave the free amine **10** in 95% yield. Methylation was performed using Eschweiler-Clarke[22,23] conditions (formalin and formic acid) to give the desired ABT-418 (**2**) in 87% yield.

N-methylcytisine (**3**) was synthesized in 87% yield from commercially available cytisine (**11**) using the same Eschweiler-Clarke[22,23] conditions (formalin and formic acid) as previously described.



Radiochemistry

ABT-418 was labelled with ^{11}C by reaction of [^{11}C]iodomethane[24] with the corresponding free secondary amine **10**. The reaction occurred cleanly at 90°C within 5 minutes in a mixture of dichloromethane and tributylphosphate. 100 to 190 mCi (3.70-7.03 GBq) of pure [^{11}C]-**2** were obtained at 25 to 30 minutes after End Of Bombardment (HPLC purification included) with specific radioactivities as high as 990mCi/µmol (36.6 GBq/µmol, typical values 600-900 mCi/µmol (22.2-33.3 GBq/µmol)).



N-methylcytisine was labelled with ^{11}C by reaction of [^{11}C]iodomethane[24] with the corresponding free secondary amine **11**. The reaction occured cleanly at 100°C within 5 minutes in a mixture of dichloromethane and tributylphosphate. 100 to 225mCi (3.70-8.30GBq) of pure [^{11}C]-**3** were obtained at 25 to 30 minutes after End Of Bombardment (HPLC purification included) with specific radioactivities as high as 1.2 Ci/µmol (44.4 GBq/µmol, typical values 500-1000 mCi/µmol (18.5-37.0 GBq/µmol)).

PET experiments

PET studies of the brain uptake and distribution of [^{11}C]nAChR ligands were carried out in adult Papio papio baboons (n = 2 per ligand).

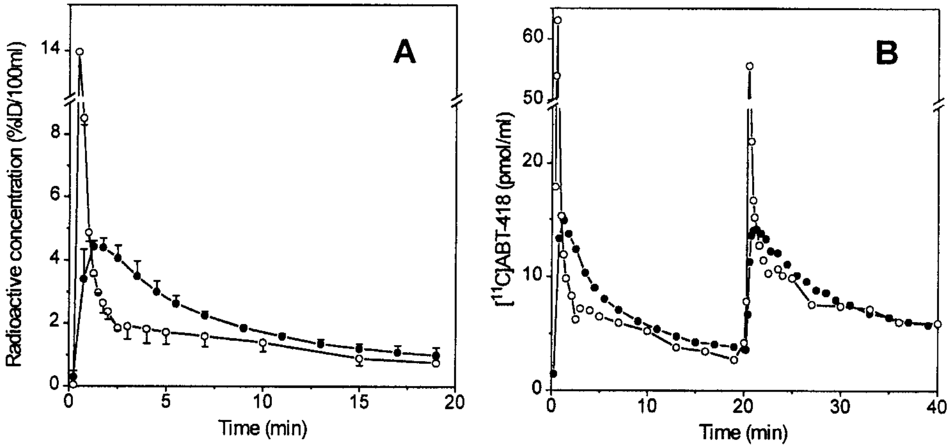


Figure : Plasma (open circles) and brain cortical (closed circles) kinetics of [^{11}C]ABT-418 in baboon during PET experiments. **A** : represents the mean curves (with standard deviation) obtained in two experiments. **B** : kinetics obtained after injection at 0 min of a tracer dose of [^{11}C]ABT-418 and co-injection at 20 min of [^{11}C]ABT-418 and a large amount (1 μmol/kg) of unlabelled ABT-418.

The *in vivo* behaviour of [^{11}C]ABT-418 appears to be close to that of [^{11}C]nicotine. The kinetics of ABT-418 are rapid suggesting a very short interaction of this ligand with nAChR due to a high dissociation rate (see figure). Following i.v. injection of ABT-418, uptake in the brain was rapid with a peak at 1-2 min (4.4 ± 0.2 % injected dose / 100 mL) followed by a rapid wash-out. Clearance of radioactivity from the blood was rapid. The regional distribution of radioactivity followed mainly the distribution of gray matter with high uptake in the cortex and a slightly lower uptake in the cerebellum. The regional distribution in the baboon brain, following injection of [^{11}C]ABT-418, is not consistent with the distribution of nicotinic receptors, as measured by brain autoradiography in rats using [^{3}H]ABT-418 or [^{3}H]cytisine[12] or by *in vivo* binding using [^{3}H]nicotine[9] in mice. The uptake and the shape of time-activity curves were unchanged by co-administration of labelled and unlabelled ABT-418 (1 μmol/kg : this dose was chosen as corresponding to one tenth of the amount producing seizures in rats[12]). Thus, essential criteria for visualizing receptor binding with PET could not be fulfilled.

Following i.v. injection of [^{11}C]N-methylcytisine, uptake in the brain was low and not significantly different from blood radioactivity (0.86 ± 0.01 % injected dose / 100 mL and 1.0 ± 0.1 % injected dose / 100 mL respectively). The *in vivo* binding of [^{11}C]N-methylcytisine appears to be rather different from that of [^{3}H]cytisine[25].

Experimental

General

(S)-Boc-Proline was purchased from Bachem, France and cytisine was purchased from Sigma, France. Other chemicals were purchased from Aldrich, Fluka or Sigma and were used without further purification.

TLC was run on pre-coated plates of silicagel 60F254 (Merck). The compounds were localized (1) when possible at 254 nm using an UV-lamp and/or (2) by iodine staining and/or (3) by dipping the tlc-plates in 1% ninhydrin in ethanol (or 1% aqueous $KMnO_4$) and heating on a hot plate. Flash chromatography was conducted on silicagel 63-200μm (Merck) at 0.3 bar. HPLC was run on a Waters system equipped with a 510 pump and a 490E UV-multiwavelength detector. The eluent was also monitored for radioactivity with a Geiger-Müller counter.

NMR spectra were recorded on a Bruker AMX (300 MHz) apparatus using the hydrogenated residue of the deuteriated solvents (DMSO-d_6, $\delta = 2.50$ ppm and CD_2Cl_2, $\delta = 5.32$ ppm) and/or TMS as internal standards for ^1H NMR as well as the deuteriated solvents (DMSO-d_6, $\delta = 39.5$ ppm and CD_2Cl_2, $\delta = 53.8$ ppm) and/or TMS as internal standards for ^{13}C NMR. The chemical shifts are reported in ppm, downfield from TMS. Mass spectra (MS, DCI/NH_4^+) were measured on a Nermag R10-10 apparatus; an ionization potential of 70eV was used. Optical rotations ($[\alpha]_D$) were measured on a Atago Polax-D.

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

$[^{11}C]CO_2$ was produced by irradiation of an ultrapure N60 Air Liquide N_2 target with a 20MeV proton beam via the $^{14}N[p,\alpha]^{11}C$ nuclear reaction on a CGR-MeV 520 cyclotron.

Complementary data for the preparation and characterisation of the synthesized compounds listed below are also available in the literature. See, for example, reference 26 for **5**, references 17 and 26 for **6**, reference 18 for **7** and **8**, reference 10 and recently published reference 27 for **2** and reference 15 for **3**. No characterisation for compouds **9** and **10** were found in the literature even though they are not new (see reference 11).

Synthesis of ABT-418 (**2**), *N-methylcytisine* (**3**) *and theirs derivatives*

N-(*tert*-butoxycarbonyl)-(S)-prolinol (**5**)

To a solution of N-(*tert*-butoxycarbonyl)-(S)-proline* (**4**) (40.0 g, MW : 215.25, 186 mmol) in THF (250 mL), stirred under argon and cooled to 0°C, were carefully added dropwise 88.1 mL of borane dimethylsulfide complex (5 eq). The mixture was stirred at 0°C for 2 h and at room temperature overnight. The mixture was cooled again to 0°C and the excess of borane was destroyed by careful addition of 80 mL of water. The solution was then transferred into a separatory funnel and diluted with 600 mL of EtOAc. After phase separation, the organic layer was washed with 10% aq K_2CO_3, water, brine and dried with $MgSO_4$. Concentration gave 34.4 g of crude N-(*tert*-butoxycarbonyl)-(S)-prolinol (**5**) as a dense oil (92%) which was used without further purification.

Rf (EtOAc/heptane : 50/50) : 0.2. ^{1}H NMR (DMSO-d_6, 370.0K) : δ : 4.22 (bs, $w_{1/2}$ = 15.0 Hz, 1H) ; 3.71 (m, 1H) ; 3.55 (dd, J = 10.5Hz & 3.9Hz, 1H) ; 3.40-3.15 (b, 3H) ; 1.90-1.65 (b, 4H) ; 1.41 (s, 9H). ^{13}C NMR (DMSO-d_6, 370.0K) : δ : 153.5 [C] ; 77.7 [C] ; 62.1 [CH_2] ; 58.1 [CH] ; 46.0 [CH_2] ; 27.7 [CH_3] ; 27.1 [CH_2] ; 22.4 [CH_2]. MS : 219 [M + NH_4^+] ; 202 [M + H^+].

*N-(*tert*-butoxycarbonyl)-(S)-proline (**4**), Rf ($CHCl_3$/MeOH/AcOH : 90/08/02) : 0.2. ^{1}H NMR (DMSO-d_6, 370.0K) : δ : 4.10 (dd, J = 10.5Hz & 3.9Hz, 1H) ; 3.42-3.25 (b, 2H) ; 2.30-2.10 (b, 1H) ; 1.95-1.75 (b, 3H) ; 1.35 (s, 9H). ^{13}C NMR (DMSO-d_6, 370.0K) : δ : 173.3 [C] ; 153.0 [C] ; 78.2 [C] ; 58.3 [CH] ; 45.7 [CH_2] ; 29.5 [CH_2] ; 27.7 [CH_3] ; 22.9 [CH_2].

N-(*tert*-butoxycarbonyl)-(S)-prolinal (**6**)

To a solution of N-(*tert*-butoxycarbonyl)-(S)-prolinol (**5**) (32.0 g, MW : 201.26, 159 mmol) in a mixture of DMSO (215 mL) at room temperature under argon, was added NEt_3 (80 mL) and stirring was continued for 15 min. The mixture was then cooled to 10°C and 89 g of sulfur trioxide-pyridine complex (3.5 eq) was added in portions over 20-25 min while maintaining the temperature around 15-17°C. The resulting yellow-brown mixture was stirred at room temperature for 2 h, diluted with 600mL of cold water and extracted several times with CH_2Cl_2. The organic layers were combined, washed twice with 1.0 M aq $NaHSO_4$, water, sat aq $NaHCO_3$, water, brine, dried with $MgSO_4$, concentrated to dryness and the residue was chromatographed on silica gel. Elution with heptane/EtOAc (90/10 to 50/50) gave 29.4 g of pure N-(*tert*-butoxycarbonyl)-(S)-prolinal (**6**) as a yellow oil which solidifies on standing at -20°C (93%).

Rf (EtOAc/heptane : 50/50) : 0.4. ^{1}H NMR (DMSO-d_6, 360.0K) : δ : 9.45 (d, J = 1.80Hz, 1H) ; 4.07 (b, 1H) ; 3.39 (b, 2H) ; 2.15-2.00 (b, 1H) ; 2.00-1.70 (b, 3H) ; 1.40 (s, 9H). ^{13}C NMR (DMSO-d_6, 360.0K) : δ : 199.6 [CH] ; 153.1 [C] ; 78.6 [C] ; 64.2 [CH] ; 46.0 [CH_2] ; 27.4 [CH_3] ; 26.3 [CH_2] ; 23.1 [CH_2]. MS : 217 [M + NH_4^+] ; 200 [M + H^+].

2(S)-(2,2-dibromoethenyl)-N-(*tert*-butoxycarbonyl)pyrrolidine (7)

To a mixture of triphenylphosphine (110.5 g, 421 mmol, 3 eq) and carbon tetrabromide (93.4 g, 281 mmol, 2 eq) in 650 mL of CH_2Cl_2 was added, under argon and with a cooling bath, slowly 18.4 g zinc dust (<10 μm, 280 mmol, 2 eq). After the mixture had been stirred for 15 min, 27.9 g of N-(*tert*-butoxycarbonyl)-(S)-prolinal (**6**, MW : 199.25, 140 mmol) in 50 mL of CH_2Cl_2

was slowly added and the mixture was stirred at room temperature for 2 h. The reaction mixture was then diluted with 700 mL of heptane/EtOAc (50/50) and filtered through basic alumina (3 cm thick) / silica gel (5 cm thick). The filter cake was then washed with 1 L of CH_2Cl_2/EtOAc/heptane (40/30/30). The filtrate was concentrated, the residue was taken up in 200 mL of heptane/EtOAc (50/50) and the resulting precipitate was filtered off. After concentration of the filtrate, the residue was chromatographed on silica gel. Elution with heptane/EtOAc (95/05 to 80/20) gave 43.5 g of pure 2(S)-(2,2-dibromoethenyl)-N-(*tert*-butoxycarbonyl)-pyrrolidine (**7**) as a white solid (87%).

Rf (heptane/EtOAc : 80/20) : 0.25. ^{1}H NMR (DMSO-d_6, 360.0K) : δ : 6.54 (d, J = 6.0Hz, 1H) ; 4.28 (b, 1H) ; 3.31 (b, 2H) ; 2.20-2.05 (b, 1H) ; 1.95-1.60 (b, 3H) ; 1.40 (s, 9H). ^{13}C NMR (DMSO-d_6, 360.0K) : δ : 152.8 [C] ; 140.4 [CH] ; 86.7 [C] ; 78.1 [C] ; 58.7 [CH] ; 45.7 [CH_2] ; 30.6 [CH_2] ; 27.7 [CH_3] ; 22.7 [CH_2]. MS : 375 [M + NH_4^+] ; 373 [M + NH_4^+] ; 371 [M + NH_4^+] ; 358 [M + H^+] ; 356 [M + H^+] ; 354 [M + H^+].

1-[2(S)-N-(*tert*-butoxycarbonyl)-pyrrolidinyl]ethyne (**8**)

To a solution of 2(S)-(2,2-dibromoethenyl)-N-(*tert*-butoxycarbonyl)-pyrrolidine (**7**) (40.7 g, MW : 355.07, 115 mmol) in THF (350 mL) cooled to -78°C under argon, was added dropwise 150 mL of butyllithium (1.6 M in hexane) while maintaining the temperature under -70°C (addition over 30 min). The mixture was then stirred at -78°C for 30 min and for another 30 min at 0°C. To this cold solution was carefully added 100 mL of cold water followed by 100 mL of brine and the mixture was then extracted several times with EtOAc. The organic layers were combined, washed once with water, twice with brine, dried with $MgSO_4$, concentrated to dryness and the residue was chromatographed on silica gel. Elution with heptane/EtOAc (98/02 to 85/15) gave 16.2 g 1-[2(S)-N-(*tert*-butoxycarbonyl)-pyrrolidinyl]ethyne (**8**) as a clear pale-yellow oil (72%)

Rf (heptane/EtOAc : 80/20) : 0.20. ^{1}H NMR (DMSO-d_6, 360.0K) : δ : 4.37 (bd, 1H) ; 3.26 (b, 2H) ; 2.90 (d, J = 2.1Hz, 1H) ; 2.15-2.00 (b, 1H) ; 2.00-1.75 (b, 3H) ; 1.41 (s, 9H). ^{13}C NMR (DMSO-d_6, 360.0K) : δ : 152.6 [C] ; 84.1 [CH] ; 78.2 [C] ; 70.9 [C] ; 47.1 [CH] ; 44.9 [CH_2] ; 32.5 [CH_2] ; 27.6 [CH_3] ; 22.9 [CH_2]. MS : 213 [M + NH_4^+] ; 196 [M + H^+].

(S)-3-methyl-5-[1-(*tert*-butoxycarbonyl)-pyrrolidinyl]isoxazole (**9**)

To a solution of 1-[2(S)-N-(*tert*-butoxycarbonyl)-pyrrolidinyl]ethyne (**8**) (10.5 g, MW : 195.26, 54 mmol) in 50 mL of benzene under argon at room temperature, was added 4.7 mL of nitroethane (1.2 eq), 14.1 mL of phenylisocyanate (2.4 eq) and 4 mL of NEt_3. The mixture was stirred for 15 min at room temperature and then refluxed for 1 h. After cooling to room temperature, again 3.0 mL of nitroethane (0.8 eq), 7.8 mL of phenylisocyanate (1.3 eq) and 4 mL of NEt_3 were added. The mixture was stirred for 15 min at room temperature and then refluxed for another 1 h. After cooling, the mixture was filtered, concentrated to dryness and the residue was chromatographed on silica gel. Elution with heptane/EtOAc (90/10 to 50/50) gave 10.9 g of (S)-3-methyl-5-[1-(*tert*-butoxycarbonyl)-pyrrolidinyl]isoxazole (**9**) as a yellow oil (80%)

Rf (heptane/EtOAc : 50/50) : 0.35. ^{1}H NMR (DMSO-d_6, 360.0K) : δ : 6.03 (s, 1H) ; 4.90 (dd, J = 7.8Hz & 2.3Hz, 1H) ; 3.40 (b, 2H) ; 2.25-2.15 (b, 1H) ; 2.18 (s, 3H) ; 2.00-1.80 (b, 3H) ; 1.34 (s, 9H). ^{13}C NMR (DMSO-d_6, 360.0K) : δ : 173.1 [C] ; 158.5 [C] ; 152.8 [C] ; 100.6 [CH] ; 78.3 [C] ; 53.0 [CH] ; 45.6 [CH_2] ; 31.0 [CH_2] ; 27.5 [CH_3] ; 22.8 [CH_2] ; 10.1 [CH_3]. MS : 270 [M + NH_4^+] ; 253 [M + H^+].

(S)-3-methyl-5-pyrrolidinylisoxazole (**10**)

To a solution of (S)-3-methyl-5-[1-(*tert*-butoxycarbonyl)pyrrolidinyl]isoxazole (**9**) (5.0 g, MW : 252.31, 20 mmol) in 100 mL of CH_2Cl_2 at room temperature was added at once 30 mL of trifluoroacetic acid. The mixture was stirred for 1 h and diluted with 200 mL of cold water. The solution was washed twice with 50 mL of CH_2Cl_2. The aqueous phase was brought to pH 8 by careful dilution with sat aq $NaHCO_3$ and was extracted 5 times with CH_2Cl_2. These organic layers were combined, washed once with water, twice with brine, dried with $MgSO_4$, concentrated to dryness and the residue was chromatographed on silica gel. Elution with $CHCl_3$/MeOH/ aq 30% NH_3 : 95/05/0.3 gave 2.9 g of (S)-3-methyl-5-pyrrolidinylisoxazole (**10**) as a light oil (95%).

Rf ($CHCl_3$/MeOH/ aq 30% NH_3 : 92/08/01) : 0.25. ^{1}H NMR (DMSO-d_6, 360.0K) : δ : 6.06 (s, 1H) ; 4.22 (dd, J = 7.8Hz & 5.3Hz, 1H) ; 2.90 (b, 2H) ; 2.67 (b, w1/2 = 15.0Hz, 1H) ; 2.17 (s, 3H) ; 2.15-2.00 (b, 1H) ; 1.85-1.65 (b, 3H). ^{13}C NMR (DMSO-d_6, 360.0K) : δ : 175.7 [C] ; 158.4 [C] ; 100.2 [CH] ; 53.7 [CH] ; 45.7 [CH_2] ; 30.7 [CH_2] ; 24.6 [CH_2] ; 10.2 [CH_3]. MS : 170 [M + NH_4^+] ; 153 [M + H^+].

An aliquot was purified by HPLC as a standard for the labelling experiments.

Column : semipreparative SiO_2 Lichrosorb Merck (250 x 10 mm) ; Porosity : 7 μm ; Eluant : CH_2Cl_2/Solution A* (98/02) ; Flow rate : 8.0 mL/min ; Temperature : RT ; UV detection at λ : 254 nm. Retention time : 6.0 to 6.5 min.

*Solution A : EtOH/water/ethylamine (100/2/2, by vol.).

(S)-3-methyl-5-[1-methyl-2-pyrrolidinyl]isoxazole (**2**)

A solution of (S)-3-methyl-5-pyrrolidinylisoxazole (**10**) (1.6 g, MW : 152.19, 10 mmol) in 20 mL of 36.5% formalin and 20 mL of 98% formic acid was refluxed for 2 h. The mixture was then diluted and brought to pH 8 with 1.0 N aq NaOH, and extracted 5 times with CH_2Cl_2. The organic layers were combined, washed once with water, twice with brine, dried with $MgSO_4$, concentrated to dryness and the residue was chromatographed on silica gel. Elution with CH_2Cl_2/MeOH : 97/03 gave 1.5 g of (S)-3-methyl-5-[1-methyl-2-pyrrolidinyl]isoxazole (**2**) as a light oil (87%).

Rf ($CHCl_3$/MeOH : 95/05) : 0.5. ^{1}H NMR (DMSO-d_6, 360.0K) : δ : 6.11 (s, 1H) ; 3.44 (dd, J = 8.1Hz & 6.6Hz, 1H) ; 3.01 (b, 2H) ; 2.35 (b, 1H) ; 2.24 (s, 3H) ; 2.19 (s, 3H) ; 1.90-1.75 (b, 3H). ^{13}C NMR (DMSO-d_6, 360.0K) : δ : 173.5 [C] ; 158.5 [C] ; 101.1 [CH] ; 61.0 [CH] ; 55.3 [CH_2] ; 39.6 [CH_3] ; 30.6 [CH_2] ; 22.2 [CH_2] ; 10.2 [CH_3]. MS : 167 [M + H^+]. $[\alpha]_D^{28}$: -95 (MeOH) ; lit.[10] -101.

An aliquot was purified by HPLC as a standard for the labelling experiments. Column : semipreparative SiO_2 Lichrosorb Merck (250 x 10 mm) ; Porosity : 7 μm ; Eluant : CH_2Cl_2/Solution A* (98/02) ; Flow rate : 6.0 mL/min ; Temperature : RT ; UV detection at λ : 254 nm. Retention time : 5.5 to 6.0 min.

*Solution A : EtOH/water/ethylamine (100/2/2, by vol.).

Synthesis of N-methylcytisine (**3**)

A solution of cytisine** (**11**) (70.0 mg, MW : 190.25, 0.37 mmol) in 10 mL of 36.5% formalin and 10 mL of 98% formic acid was refluxed for 5 h. The mixture was then diluted and brought to pH 8 with 1.0 N aq NaOH, and extracted 5 times with EtOAc. The organic layers were combined, washed once with water, twice with brine, dried with $MgSO_4$ and concentrated to dryness to give 65 mg of crude N-methylcytisine (**3**) (87%).

Rf (CH_2Cl_2/EtOH/Sol. A : 50/50/05) : 0.3. ^{1}H NMR (CD_2Cl_2, 295.0K) : δ : 7.26 (dd, J = 9.0Hz & 6.9Hz, 1H) ; 6.30 (dd, J = 9.0Hz & 1.2Hz, 1H) ; 5.97 (dd, J = 6.9Hz & 1.2Hz, 1H) ; 4.05-3.75 (AB system, 2H) ; 3.00-2.75 (b, 3H) ; 2.40 (b, $w_{1/2}$ = 15.0Hz, 1H) ; 2.22 (td, J = 10.5Hz & 2.1Hz, 2H) ; 2.12 (s, 3H) ; 1.90-1.65 (b, 2H). ^{13}C NMR (CD_2Cl_2, 295.0K) : δ : 163.6. [C] ; 152.3 [C] ; 139.0 [CH] ; 116.5 [CH] ; 104.6 [CH] ; 63.0 [CH_2] ; 62.6 [CH_2] ; 50.3 [CH_2] ; 46.3 [CH_3] ; 35.9 [CH] ; 28.5 [CH] ; 25.7 [CH_2]. MS : 222 [M + NH_4^+] ; 205 [M + H^+].

An aliquot was purified by HPLC as a standard for the labelling conditions. Column : semipreparative SiO_2 Lichrosorb Merck (250 x 10 mm) ; Porosity : 7 μm ; Eluant : CH_2Cl_2/Solution A* (96/04) ; Flow rate : 9.0 mL/min ; Temperature : RT ; UV detection at λ : 254 nm. Retention time : 4.5 to 5.0 min.

* Solution A : EtOH/water/ethylamine (100/2/2, by vol.).

** Cytisine (1,2,3,4,5,6-hexahydro-1,5-methano-8H-pyrido-[1,2-a][1,5]diazocin-8-one)

Rf (CH_2Cl_2/EtOH/Sol. A : 50/50/05) : 0.1. ^{1}H NMR (CD_2Cl_2, 295.0K) : δ : 7.26 (dd, J = 9.0Hz & 6.9Hz, 1H) ; 6.28 (dd, J = 9.0Hz & 1.2Hz, 1H) ; 5.95 (dd, J = 6.9Hz & 1.2Hz, 1H) ; 4.05-3.75 (AB system, 2H) ; 3.10-2.80 (b, 5H) ; 2.27 (b, $w_{1/2}$ = 15.0Hz, 1H) ; 1.90 (bt, $w_{1/2}$ = 9.0Hz, 2H) ; 1.47 (b, $w_{1/2}$ = 12.0Hz, 1H). ^{13}C NMR (CD_2Cl_2, 295.0K) : δ : 163.5. [C] ; 152.3 [C] ; 138.9 [CH] ; 116.3 [CH] ; 104.5 [CH] ; 54.4 [CH_2] ; 53.4 [CH_2] ; 50.0 [CH_2] ; 36.0 [CH] ; 28.3 [CH] ; 26.6 [CH_2].

Radiosynthesis of [^{11}C]ABT-418 (**2**) and *[^{11}C]N-methylcytisine* (**3**)

Preparation of [^{11}C]CH_3I

The [^{11}C]CO_2 was produced by irradiation of ultrapure nitrogen N60 Air Liquide with a 20 MeV proton beam from a 520-CGR-MeV cyclotron (30 µA, 30 min). After being separated from the target gas by trapping using liquid argon (-186°C), the [^{11}C]CO_2 was released from the trap by simply raising the latter to room temperature (with nitrogen as vector gas). The [^{11}C]CO_2 was converted into [^{11}C]CH_3OH by reduction at room temperature with a 1.0M THF solution of lithium aluminium hydride (5 µL) in THF (50 µL), followed by concentration and hydrolysis (50 µL). The [^{11}C]MeOH was distilled into 1 mL of a aqueous 57% HI solution, the [^{11}C]CH_3I thus synthesized was continously distilled away by a flow of nitrogen gas and trapped at 0°C into the reaction flask.

Labelling of ABT-418

5 µmol of ABT-418 precursor (**10**) were dissolved in a mixture of distilled CH_2Cl_2 (0.05 mL) and tributylphosphate (0.3 mL). The [^{11}C]iodomethane was trapped in this solution at 0°C for 5 min. The reaction vessel was then isolated, heated for 5 min at 90°C and cooled. The crude was then diluted with 0.5 mL of the HPLC mobile phase and was injected on the column. 100 to 190 mCi (3.70-7.03 GBq) of radiochromatographically pure [^{11}C]-**2** were obtained at 25 to 30 min after EOB (HPLC purification included) with specific radioactivities as high as 990 mCi/µmol (36.6 GBq/µmol, typical values 600-900 mCi/µmol (22.2-33.3 Gbq/µmol)).

Column : semipreparative SiO_2 Lichrosorb Merck (250 x 10 mm) ; Porosity : 7 µm ; Eluant : CH_2Cl_2/Solution A* (98/02) ; Flow rate : 6.0 mL/min ; Temperature : RT ; UV detection at λ : 254 nm. Retention time : ABT-418 : 5.5 to 6.0 min, precursor : 12.0 to 15.0 min.

*Solution A : EtOH/water/ethylamine (100/2/2, by vol.).

Labelling of N-methyl cytisine

10 µmol of cytisine precursor (**11**) were dissolved in a mixture of distilled CH_2Cl_2 (0.05 mL) and tributylphosphate (0.3 mL). The [^{11}C]iodomethane was trapped in this solution at 0°C for 5 min. The reaction vessel was then isolated, heated for 5 min at 90°C and cooled. The crude was then diluted with 0.5 mL of the HPLC mobile phase and was injected on the column. 100 to 225 mCi (3.70-8.30 GBq) of radiochromatographically pure [^{11}C]-**3** were obtained at 25 to 30 min after EOB (HPLC purification included) with specific radioactivities as high as 1.2 Ci/µmol (44.4 GBq/µmol, typical values 500-1000 mCi/µmol (18.5-37.0 GBq/µmol)).

Column : semipreparative SiO_2 Lichrosorb Merck (250 x 10 mm) ; Porosity : 7 µm ; Eluant : CH_2Cl_2/Solution A* (95/05) ; Flow rate : 6.0 mL/min ; Temperature : RT ; UV detection at λ : 254 nm. Retention time : N-methylcytisine : 8.5 to 9.0 min, precursor : 20.0 to 23.0 min.

*Solution A : EtOH/water/ethylamine (100/2/2, by vol.).

Formulation

Formulation of both products for i.v. injection was effected as follows : (1) HPLC solvent removal by evaporation; (2) taking up the residue, while heating gently, in 5 mL of physiological saline to which had been added 100 µL of aqueous 1.0 M NaH_2PO_4/H_3PO_4; (3) filtration on a 0.22 µm Millipore filter. Injection in PET experiments were done within 15 min after End Of Synthesis (typically injected, 15 mCi in 1 mL of final solution, specific radioactivity of 700 mCi/µmol (25.9 GBq/µmol). The products were shown to be radiochemically stable for at least 45 min and free of non-radiactive precursor, as demonstrated by HPLC.

PET experiments

PET studies of the brain uptake and distribution of nAChR [^{11}C]ligands were carried out in adult Papio papio baboons (mean weight : 18 ±5 kg). Two hours before the PET acquisition, the animal received intramuscular ketamine (10 mg/kg). After being intubated, animals were artificially ventilated and maintained anaesthetized with N_2O (65%) and 1.5% isoflurane. PET experiments were performed with a CTI 953B/31 positron tomograph (CTI PET Systems, Knoxville, TN, USA). The baboon head was positioned in the tomograph using a custom-designed stereotaxic headholder. All the cerebral regions studied (cortex, caudate/putamen, thalamus, cerebellum) were contained in axial cross sections parallel to the orbito-meatal anatomical line of reference. For the standard kinetic study, baboons (n = 2 for each ligand) were i.v. injected with 10-15 mCi (370-555 MBq) of [^{11}C]ABT-418 (28.7 ± 6 nmol) or [^{11}C]N-methylcytisine (40.3 ± 28 nmol) and imaged for 20 min. The scanning protocol consisted of 15 images (4 x 30 s, 4 x 1 min, 7 x 2 min).

During PET acquisition, arterial blood samples were withdrawn from femoral artery at designated times. Blood and plasma radioactivity were measured in a gamma counting system and the blood-plasma time activity curves were corrected for ^{11}C decay from the time of the first injection.

We examined whether the [^{11}C]ABT-418 (but not N-methyl-cytisine, see Results and Discussion section) cerebral uptake could be blocked by competitive inhibition in an experiment by injecting, 20 min after the first radiotracer injection, a mixture of labelled (12.6 mCi (466 Mbq) or 26.3 nmol) and unlabelled (1 µmol/kg) ABT-418. For this dynamic sequence, the scanning protocol (total duration 40 min) consisted of 37 images (4 x 30 s, 4 x 1 min, 7 x 2 min, 6 x 10 s, 4 x 30 s, 7 x 1 min, 5 x 2 min).

For PET data analysis, regions of interests were delineated on early images on which anatomical structures could be clearly identified. The concentration of radioactivity in each region of interest was determined during each sequential scan, corrected for carbon-11 decay and expressed as percent of the injected dose / 100 mL of tissue (% ID / 100 mL).

Conclusion

ABT-418 and N-methylcytisine were both labelled with ^{11}C with high specific radioactivity for PET experiments. As for [^{11}C]nicotine, specific binding *in vivo* could not be demonstrated for ABT-418. Therefore, further experiments are needed to determine the full PET pharmacological profile and the subsequent potential clinical applications of ABT-418 as a tracer for PET experiments. For labelled N-methyl-cytisine, radioactivity in the cerebral cortex and in the blood were similar. Thus, ^{11}C-labelled N-methylcytisine does not appear to be a suitable ligand for mapping brain nAChR.

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