Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **44**, 337-345 (2001)
DOI: 10.1002/jlcr.460

Highly Efficient Synthesis of [^{11}C]Me-QNB, a Selective Radioligand for the Quantification of the Cardiac Muscarinic Receptors using PET

Frédéric DOLLE*, Françoise HINNEN, Françoise VAUFREY, Stéphane DEMPHEL, Yann BRAMOULLE, Denis FOURNIER, Michel PONCHANT, Héric VALETTE and Christian CROUZEL

Service Hospitalier Frédéric Joliot - Département de Recherche Médicale - CEA
4 place du Général Leclerc - F-91401 Orsay - France

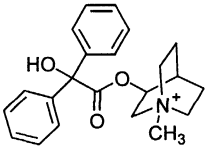
Summary

Me-QNB (*N*-methyl-quinuclidin-3-yl benzilate or *N*-methyl-quinuclidin-3-yl diphenylhydroxy acetate) is a hydrophilic, non-metabolized and highly specific muscarinic acetylcholinergic antagonist. Using this quaternary ammonium derivative of QNB, labelled with carbon-11, a positron-emitting isotope (half-life : 20.4 minutes), the potential for quantification of myocardial muscarinic receptors *in vivo* using the high-resolution, sensitive and quantitative imaging technique PET (positron emission tomography) was previously demonstrated in dogs and validated in humans. In this paper, the radiosynthesis of carbon-11-labelled Me-QNB is investigated and oriented towards the preparation of multi milliCuries of radiotracer. Typically, using no-carrier-added [^{11}C]methyl triflate as the alkylating agent and 0.64 mg (1.89 µmol) of QNB as precursor for labelling at 100°C for 1 minute lead to a 48.5% +/- 10% (15 runs) decay-corrected radiochemical yield (based on [^{11}C]methyl triflate). 183 mCi (+/- 39) of [^{11}C]Me-QNB ([^{11}C]-**1**) could be synthesized in only 27 to 28 minutes after EOB and occasionally, up to 340 mCi of [^{11}C]Me-QNB ([^{11}C]-**1**) were obtained, corresponding to a 85% decay-corrected yield. The associated decay-corrected specific radioactivities obtained were 2658 mCi/µmol (+/- 971) at EOB.

Key Words : Me-QNB, MQNB, carbon-11, positron emission tomography, PET, muscarinic receptor

Introduction

Me-QNB (**1**, *N*-methyl-quinuclidin-3-yl benzilate or *N*-methyl-quinuclidin-3-yl diphenylhydroxy acetate) is a hydrophilic, non-metabolized, and highly specific muscarinic acetylcholinergic antagonist (1). This quaternary ammonium derivative of QNB has been labelled with carbon-11 (2,3), a positron-emitting isotope (half-life : 20.4 minutes), and characterized as radiotracer for the *in vivo* imaging of the myocardial muscarinic receptors using the high-resolution, sensitive technique PET (positron emission tomography) in dogs, monkeys and humans (2,4,5). Furthermore, the potential for noninvasive quantification of ventricular muscarinic receptors using this imaging technique was first demonstrated and validated in dogs (6-8) but also in humans (9). This radiopharmaceutical has been used routinely for human studies in our institution assessing by PET the myocardial muscarinic receptor density and affinity constants in heart transplant patients (10), in



(1)
Me-QNB

Copyright © 2001 John Wiley & Sons, Ltd.

Received 18 December 2000
Accepted 3 January 2001

patients with chronic idiopathic dilated cardiomyopathy (11), or in familial amyloid neuropathy patients (12,13). It is also currently used in the assessment of parasympathetic innervation in several human diseases.

Mathematical compartmental ligand-receptor models using a kinetic approach based on a multi-injection protocol are used in order to analyze and fit the PET time-concentration curves obtained (14) with [^{11}C]Me-QNB ([^{11}C]-**1**). The typical simplified multi-injection protocol designed in our institution for routine [^{11}C]Me-QNB ([^{11}C]-**1**) PET aquisition requires the preparation, at a given time, of three syringes, S1, S2 and S3 respectively, consuming up to 50 mCi (at the beginning of the PET aquisition) of radiotracer, such as defined in the following table.

	PET Time	[^{11}C]Me-QNB		Me-QNB
S1	T0	5-10 mCi	7-14 nmol	-
S2	T0 + 30 min	-	-	850 nmol $
S3	T0 + 60 min	20-40 mCi	29-58 nmol	850 nmol $

Syringue contents at beginning of the PET protocol : PET aquisition started with S1 (5-10 mCi of [^{11}C]Me-QNB) ; S2 is injected 30 minutes later ; S3 is injected 60 minutes after S1, leading to 2.6-5.2 mCi of [^{11}C]Me-QNB at the time of injection. $: 850 nmole of non-radioactive Me-QNB (**1**) correspond to about 300 µg of Me-QNB I (**1**.I).

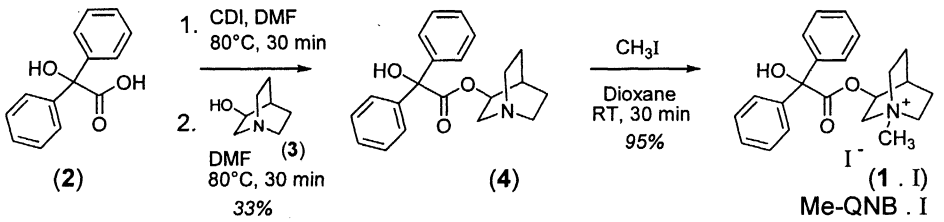
Recently we were confronted with an increasing demand of [^{11}C]Me-QNB ([^{11}C]-**1**) in the course of our PET program and therefore had the challenging opportunity to achieve two complete PET acquisition protocols in parallel on our two imaging systems with only one radiosynthesis and radiopharmaceutical batch preparation.

In order to increase our production capability, we have re-investigated the radiosynthesis of carbon-11-labelled [^{11}C]Me-QNB ([^{11}C]-**1**) and oriented our procedures towards the preparation of hundreds of milliCuries of radiotracer.

Results and discussion

Chemistry

Me-QNB (**1**), as reference compound and as carrier ligand in the PET protocol (see table above), was synthesized in two steps from commercially available benzilic acid (diphenylglycolic acid, **2**) and 3-quinuclidinol (**3**) in 31% yield. The synthesis started with the activation of the carboxylic acid function of benzilic acid (**2**) with CDI for 30 minutes at 80°C, followed by condensation with 3-quinuclidinol (**3**) for another 30 minutes at 80°C. Analytically pure QNB (**4**, free from residual CDI and imidazole) was obtained in 33% yield as a white powder after recrystallization.



Quaternization of the amine **4** using methyl iodide in dioxane for 30 minutes at room temperature afforded pure Me-QNB (**1**) as its iodide salt in 95% yield.

Copyright © 2001 John Wiley & Sons, Ltd.

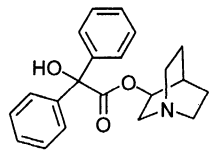
Radiochemistry

Me-QNB (**1**) was labelled with carbon-11 at its methyl ammonium function from the corresponding tertiary amine precursor **4** with [^{11}C]methyl iodide or [^{11}C]methyl triflate.

[^{11}C]Methyl iodide was prepared from [^{11}C]carbon dioxide using the well-known two step, one pot protocol, consisting of the trapping of [^{11}C]CO_2 and conversion into [^{11}C]methanol ($LiAlH_4$) followed by iodination using aqueous HI giving [^{11}C]methyl iodide (15). [^{11}C]Methyl triflate was prepared according to a literature procedure from [^{11}C]methyl iodide using silver triflate (16).

$$[^{11}C]CO_2 \xrightarrow[\text{then } H_2O]{LiAlH_4/THF} [^{11}C]CH_3OH \xrightarrow[165°C]{\text{aq. HI}} [^{11}C]CH_3I \xrightarrow[150\text{-}200°C]{AgOTf} [^{11}C]CH_3OTf$$

About 750 mCi (27.75 GBq) of [^{11}C]CH_3I or [^{11}C]CH_3OTf is routinely obtained in our laboratory in 7 to 8 minutes after the end of the bombardment (EOB) in 80% decay-corrected yield (960 mCi or 35.52 GBq, at EOB), based on starting [^{11}C]CO_2 (1.20 Ci or 44.40 GBq, at EOB).

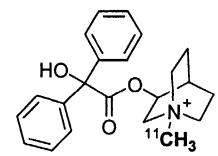


(4)

[^{11}C]CH_3I *or* [^{11}C]CH_3OTf

TBP (300 µL)

See below for the conditions



[^{11}C]Me-QNB ([^{11}C]-**1**)

[^{11}C]CH_3-X	Precursor (**4**)	Time $	Temp	mCi £	σ	Yield §	σ
CH_3-I	1.57 µmol	8 min	100°C	66.4	+/- 31	23.3% (10)	+/- 10%
CH_3-I	1.89 µmol	8 min	100°C	82.4	+/- 30	30.0% (5)	+/- 9%
CH_3-I	1.57 µmol	2 min	200°C	105.9	+/- 27	25.0% (3)	+/- 5%
CH_3-I	1.89 µmol	2 min	200°C	129.8	+/- 33	34.4% (3)	+/- 10%
CH_3-OTf #	1.89 µmol	1 min	100°C	183.4	+/- 39	48.5% (15)	+/- 10%

Conditions : (1) [^{11}C]methyl iodide or triflate trapping at room temperature for 2-3 min in 300 µL of TBP ; (2) heating the reaction mixture ; (c) Dilution of the crude with 0.5 mL of the HPLC mobile phase and (d) HPLC purification.

$: reaction time. £ : at the end of synthesis.
§ : decay-corrected, based on [^{11}C]methyl iodide or triflate ; the value between parentheses indicates the number of runs.
# : Occasionally up to 340 mCi were obtained (85% yield).

Reaction of the amine-compound **4** with [^{11}C]methyl iodide employing the standard conditions that had so far been used in our laboratory (1,2) yielded [^{11}C]Me-QNB in an unsatisfactory average yield of 23.3% (+/- 10%). The conditions used were the following : (1) trapping at room temperature of the [^{11}C]methyl iodide in 300 µL of tributylphosphate (TBP) containing 0.53 mg of precursor (**4**, QNB, 1.57 µmol) ; (2) heating the reaction mixture at 100°C for 8 minutes ; (3) dilution of the reaction mixture with 0.5 mL of the HPLC mobile phase and (4) HPLC purification. From an average production batch of 960 mCi (or 35.52 GBq, at EOB) of [^{11}C]CH_3I, only 66.4 mCi (+/- 31) of [^{11}C]Me-QNB ([^{11}C]-**1**) could be synthesized in 35 minutes after EOB (Yield, decay-corrected and based on [^{11}C]methyl iodide : 23.3%). Using the same procedure as described above but with 20% more precursor **4** - 0.64 mg, 1.89 µmol - lead to a

Copyright © 2001 John Wiley & Sons, Ltd.

significant increase of the yield : 30.0% +/- 9% (decay-corrected and based on [^{11}C]methyl iodide) corresponding to a final amount of [^{11}C]Me-QNB ([^{11}C]-**1**) of 82.4 mCi (+/- 30).

When the originally defined reaction mixture (0.53 mg of precursor **4**, 1.57 μmol) was heated at 200°C (instead of 100°C) for only 2 minutes, not only the total synthesis time was reduced, but also the yield was slightly increased : 25.0 +/- 5% (decay-corrected and based on [^{11}C]methyl iodide). As observed above, when 20% more precursor **4** (0.64 mg, 1.89 μmol) was used, the yield was significatively increased (34.4% +/- 10%, decay-corrected and based on [^{11}C]methyl iodide), leading to 129.8 mCi (+/- 33) of [^{11}C]Me-QNB ([^{11}C]-**1**) in less than 30 minutes of synthesis (HPLC included). However, when precursor **4** was further increased, the final yield was not affected and moreover, the HPLC separation of the radiotracer [^{11}C]Me-QNB ([^{11}C]-**1**) and the precursor **4** was not successful in all batches, leading to rejection of the radiopharmaceutical preparation by our *Quality Control* unit.

Using [^{11}C]methyl triflate as the alkylating agent and 0.64 mg (1.89 μmol) of precursor **4** at only 100°C for 1 minute immediately lead to a large increase of the radiochemical yield : 48.5% +/- 10% (decay-corrected and based on [^{11}C]methyl triflate). 183.4 mCi (+/- 39) of [^{11}C]Me-QNB ([^{11}C]-**1**) could be synthesized in only 27 to 28 minutes after EOB. Occasionally, up to 340 mCi of [^{11}C]Me-QNB ([^{11}C]-**1**) were obtained, corresponding to a 85% decay-corrected yield.

The associated decay-corrected specific radioactivities obtained were 2658 mCi/μmol (+/- 971) at EOB. Whatever the synthetic process employed, no significant differences could be observed.

Formulation of labelled [^{11}C]Me-QNB ([^{11}C]-**1**) for i.v. injection was effected as follows : Dilution of the HPLC-collected fraction (eluent : 0.9% aq. NaCl / EtOH : 65 / 35, see experimental section) with physiological saline to a 10% EtOH concentration followed by sterile filtration. As demonstrated by HPLC analysis, the radiopharmaceutical preparation was found to be > 95% chemically and > 99% radiochemically pure and was shown to be free of non-radioactive precursor and was radiochemically stable for at least 120 minutes.

Experimental

General

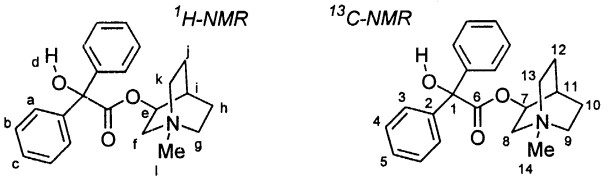
Chemicals were purchased from standard commercial sources (Aldrich, Fluka or Sigma France) and were used without further purification unless stated otherwise. QNB (quinuclidin-3-yl benzilate or quinuclidin-3-yl diphenylhydroxy acetate, **4**) and Me-QNB.I (*N*-methyl-quinuclidin-3-yl benzilate or *N*-methyl-quinuclidin-3-yl diphenylhydroxy acetate, iodide salt, **1**.I) were purchased as analytical standards (5 mg each) from RBI France.

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 analyzer. Flash chromatography was conducted on silicagel 63-200 μm (Merck) at 0.3 bars (compressed air).

Copyright © 2001 John Wiley & Sons, Ltd.

HPLCs : **A** : Equipment : Waters Alliance 2690 equipped with a UV spectrophotometer (Photodiode Array Detector, Waters 996) and a Berthold LB509 radioactivity detector ; column : analytical Spherisorb® SCX, Waters (150 x 4.6 mm) ; porosity : 5 µm ; conditions : isocratic elution with 0.9% aq. NaCl / EtOH : 50 / 50 (v:v) ; flow rate : 1.5 mL/min ; temperature : RT ; UV detection at λ : 207 nm ; **B** : Equipment : Waters or Shimadzu systems. For example, Waters systems equipped with a 510 pump, 440 UV detector or 481 & 486 UV-multiwavelength detectors ; the effluent was also monitored for radioactivity with a Geiger-Müller counter ; column : semipreparative Spherisorb® SCX, Waters (250 x 10 mm) ; porosity : 10 µm ; conditions : isocratic elution with : 0.9% aq. NaCl / EtOH : 65 / 35 (v:v) ; flow rate : 8.0 mL/min ; temperature : RT ; UV detection at λ : 214 nm ; **C** : Equipment : Waters Alliance 2690 equipped with a UV spectrophotometer (Photodiode Array Detector, Waters 996) and a Berthold LB509 radioactivity detector ; column : analytical Symmetry-M® C-18, Waters (4.6 x 50 mm, microcolumn) ; porosity : 5 µm ; conditions : isocratic elution with solvA / solvB : 45 / 55 (v:v) [solvA : H_2O containing Low-UV PIC® B7 reagent (Waters), 20 mL for 1000 mL ; solvB : H_2O / CH_3CN : 50 / 50 (v:v) containing Low-UV PIC® B7 reagent (Waters), 20 mL for 1000 mL] ; flow rate : 2.0 mL/min ; temperature : 30°C ; UV detection at λ : 214 nm.

NMR spectra were recorded on a Bruker AMX (300 MHz) apparatus using the hydrogenated residue of the deuteriated solvents (CD_2Cl_2, δ : 5.32 ppm ; DMSO-d_6, δ : 2.50 ppm) and/or TMS as internal standards for ^1H NMR as well as the deuteriated solvents (CD_2Cl_2, δ : 53.8 ppm ; 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 symetrisation, the data were multiplied with an unshifted sine bell function in each dimension. For ^1H-^{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 (^1H). The chemical shifts are reported in ppm, downfield either from TMS (s, d, t, dd, b for singlet, doublet, triplet, doublet of doublet and broad respectively ; #, *, ° : interchangeable assignments). The nomenclature used for the ^1H and ^{13}C NMR attribution is given below :



The mass spectra (MS), were measured on a Nermag R10-10 - or a Quadripolair Finnigan 4600 instrument (DCI/NH_4^+) or on a Esi/Tof Marinor Perspective (TOF). Elemental analysis, were performed by the Service Central de Microanalyses du CNRS, 91190 Gif-sur-Yvette, France, and were within 0.3% of the theoretical values calculated for C, H, N.

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.

Copyright © 2001 John Wiley & Sons, Ltd.

Chemistry

Quinuclidin-3-yl benzilate (QNB, 4)

To 10.0 g of CDI (carbonyldiimidazole, 61.7 mmoles, MW : 162.15, dried over P_2O_5 under vacuum 24 h prior to use) in 50 mL of DMF (freshly distilled) under a N_2 atmosphere at room temperature, was slowly added 9.0 g of benzilic acid (diphenylglycolic acid, **2**) in 80 mL of DMF. The mixture was stirred at room temperature for 15 min and then heated at 80°C for another 15 min (strong evolution of CO_2). To this solution was then rapidly added 6.0 g of 3-quinuclidinol (**3**, 47.1 mmoles, MW : 127.19). The resulting mixture was stirred at 80°C for 2 h, diluted with water and extracted several times with dichloromethane. The organic layers were combined, washed once with brine, dried over Na_2SO_4 and concentrated to dryness. The residue was then semi-purified by chromatography on silica gel (eluent : CH_2Cl_2/MeOH 90/10), then redissolved in dichloromethane and the resulting solution was washed with 1N aq. NaOH (3 times), with water and brine, and dried over Na_2SO_4. Concentration to dryness gave a white solid (free from residual imidazole and CDI) which was recrystallized from EtOH / EtOAc. Vacuum drying at 100°C for 24 h gave 5.2 g (33%) of pure quinuclidin-3-yl benzilate (QNB, **4**) as a white powder.

Rf (CH_2Cl_2/MeOH 90/10) : 0.2 ; Rt (HPLC A) : 2.80 min ; ^{1}H NMR (CD_2Cl_2, 298.0 K) : δ : 7.40 (bs, $w_{1/2}$: 15 Hz, 4H, *H-a,b,c*$^{\#}$) ; 7.32 (bs, $w_{1/2}$: 25 Hz, 6H, *H-a,b,c*$^{\#}$) ; 4.92 (bt, $w_{1/2}$: 15 Hz, 1H, *H-e*) ; 3.09 (dd, J : 14.7 Hz & 8.4 Hz, 1H, *H-f*) ; 2.62 (bs, $w_{1/2}$: 20 Hz, 3H, *H-f*, H-g* & H-k**) ; 2.46 (bd, $w_{1/2}$: 20 Hz, 2H, *H-g* & H-k**) ; 1.96 (bs, $w_{1/2}$: 15 Hz, 1H, *H-i*) ; 1.61 (multi sharp-peak system, $w_{1/2}$: 25 Hz, 1H, *H-h°*) ; 1.51 (multi sharp-peak system, $w_{1/2}$: 25 Hz, 1H, *H-h°*) ; 1.35 (multi sharp-peak system, $w_{1/2}$: 25 Hz, 1H, *H-j°*) ; 1.27 (multi sharp-peak system, $w_{1/2}$: 25 Hz, 1H, *H-j°*) ; ^{13}C NMR (CD_2Cl_2, 298.0 K) : δ : 174.4 (C-6) ; 143.0 (C-2) ; 128.3 (CH-3$^{\#}$) ; 128.2 (CH-5) ; 127.8 (CH-4$^{\#}$) ; 81.3 (C-1) ; 74.6 (CH-7) ; 55.2 (CH_2-8) ; 47.3 (CH_2-9*) ; 46.5 (CH_2-13*) ; 25.6 (CH-11) ; 24.6 (CH_2-10°) ; 19.7 (CH_2-12°) ; MS (DCI/NH_4^+) : $C_{21}H_{23}N_1O_3$: 338 [M + H$^+$] ; Anal. (within 0.3% of the theoretical values) : $C_{21}H_{23}N_1O_3$. 0.2 EtOH : C, H, N.

N-Methyl-quinuclidin-3-yl benzilate, iodide salt (Me-QNB.I, 1.I)

To 4.5 g of 3-quinuclidinyl benzilate (**4**, QNB or 3-quinuclidinyl-α-hydroxydiphenylacetate, MW : 337.41) in 50 mL of dioxane, were added dropwise 23 mL of CH_3I (377 mmoles, d : 2.275, MW : 141.94). The reaction mixture immediately turned cloudy and a white precipitate slowly accumulated. After 1 h, the white precipitate was filtered off and washed with small portions of cold dioxane. Vacuum drying at 120°C for 48 h gave 6.1 g (95%) of pure methyl-3-quinuclidinyl benzilate (as its iodide salt, Me-QNB.I, **1**.I) as a white powder.

Rt (HPLC A) : 3.89 min ; ^{1}H NMR (DMSO-d_6, 298.0 K) : δ : 7.38 (multi sharp-peak system, $w_{1/2}$: 35 Hz, 10H, *H-a,b,c*) ; 6.76 (s, 1H, *H-d*) ; 5.21 (bt, $w_{1/2}$: 15 Hz, 1H, *H-e*) ; 3.96 (bt, $w_{1/2}$: 20 Hz, 1H, *H-f*) ; 3.39 (multi sharp-peak system, $w_{1/2}$: 45 Hz, 4H, *H-g* & H-k**) ; 3.19 (bq, $w_{1/2}$: 25 Hz, 1H, *H-f*) ; 2.99 (s, 3H, *H-l*) ; 2.25 (bs, $w_{1/2}$: 15 Hz, 1H, *H-i*) ; 1.93 (b, $w_{1/2}$: 25 Hz, 2H, *H-h°*) ; 1.66 (multi sharp-peak system, $w_{1/2}$: 20 Hz, 1H, *H-j°*) ; 1.53 (multi sharp-peak system, $w_{1/2}$: 20 Hz,

Copyright © 2001 John Wiley & Sons, Ltd.

2H, *H-j°*) ; ^{1}H NMR (DMSO-d_6, 352.0 K) : δ : 7.35 (multi sharp-peak system, $w_{1/2}$: 35 Hz, 10H, *H-a,b,c*) ; 6.43 (s, 1H, *H-d*) ; 5.21 (bt, $w_{1/2}$: 15 Hz, 1H, *H-e*) ; 3.93 (dd, J : 11.4 Hz & 10.5 Hz, 1H, *H-f*) ; 3.37 (multi sharp-peak system, $w_{1/2}$: 50 Hz, 4H, *H-g* & H-k**) ; 3.19 (multi sharp-peak system, $w_{1/2}$: 25 Hz, 1H, *H-f*) ; 2.98 (s, 3H, *H-l*) ; 2.26 (bs, $w_{1/2}$: 15 Hz, 1H, *H-i*) ; 1.95 (bd, $w_{1/2}$: 25 Hz, 2H, *H-h°*) ; 1.70 (multi sharp-peak system, $w_{1/2}$: 40 Hz, 2H, *H-j°*) ; ^{13}C NMR (DMSO-d_6, 298.0 K) : δ : 172.3 (C-6) ; 142.9 (C-2) ; 127.9 (CH-3$^{\#}$) ; 127.8 (CH-3$^{\#}$) ; 127.6 (CH-5) ; 127.0 (CH-4$^{\#}$) ; 126.9 (CH-4$^{\#}$) ; 80.6 (C-1) ; 68.6 (CH-7) ; 61.2 (CH_2-8) ; 55.6 (CH_2-9*) ; 55.0 (CH_2-13*) ; 50.8 (CH_3-14) ; 23.1 (CH-11) ; 20.4 (CH_2-10°) ; 17.5 (CH_2-12°) ; ^{13}C NMR (DMSO-d_6, 352.0 K) : δ : 171.8 (C-6) ; 142.5 (C-2) ; 127.4 (CH-3$^{\#}$) ; 127.3 (CH-3$^{\#}$) ; 127.1 (CH-5) ; 126.6 (CH-4$^{\#}$) ; 126.5 (CH-4$^{\#}$) ; 80.4 (C-1) ; 68.3 (CH-7) ; 61.2 (CH_2-8) ; 55.7 (CH_2-9*) ; 55.0 (CH_2-13*) ; 50.8 (CH_3-14) ; 22.9 (CH-11) ; 20.2 (CH_2-10°) ; 17.3 (CH_2-12°) ; 2D NMR experiments (COSY and ^{1}H-^{13}C shift correlation) unequivocably confirm the proposed structure ; MS (TOF) : $C_{22}H_{26}INO_3$: 351 [M - I - H] ; Anal. (within 0.3% of the theoretical values) : $C_{22}H_{26}INO_3$: C, H, N.

Radiochemistry

Preparation of [^{11}C]CO_2

[^{11}C]CO_2 was produced by irradiation of an ultrapure N60 Air Liquide N_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 (54000 μC in 30 minutes). At the end of the bombardment, the target content was transferred to the 5-cm lead-shielded hot cell dedicated to the radiosynthesis of the tracer and passed firstly through a glass P_2O_5-guard (70 mm length, 3 mm internal diameter) in order to remove moisture and secondly through an empty stainless-steel coil (1500 mm length, 0.51 mm internal diameter, cooled at -186°C using liquid argon) in order to trap the [^{11}C]CO_2.

Preparation of [^{11}C]CH_3I and [^{11}C]CH_3OTf

[^{11}C]CO_2 was then released from the trap by simply raising the latter to room temperature, swept away by a flow of nitrogen gas (40 mL/min) and trapped at -10°C (EtOH-ice bath) into 55 μL of THF containing 5 μL of 1.0M THF solution of lithium aluminium hydride. Concentration to dryness (evaporation of solvent at 165°C using a stream of nitrogen) followed by hydrolysis (100 μL of deionized water) of the formed aluminium complex afforded [^{11}C]CH_3OH, which was distilled using a flow of nitrogen gas into 1 mL of an aqueous 57% HI solution (heating block at 165°C). The [^{11}C]CH_3I thus synthesized was continuously swept away by a flow of nitrogen gas, passed through a combined 1/1 (v:v) soda lime/P_2O_5-guard (35 mm length each, 3 mm internal diameter) and converted into [^{11}C]CH_3OTf by passing through a glass column (33 cm length, 5 mm internal diameter), heated at 200°C and containing silver triflate impregnated graphitized carbon (200 mg). About 750 mCi (27.75 GBq) of [^{11}C]CH_3I (or [^{11}C]CH_3OTf) is routinely obtained in our laboratory in 7 to 8 minutes after EOB in 80% decay-corrected yield (960 mCi or 35.52 GBq, EOB), based on starting [^{11}C]CO_2 (1.20 Ci or 44.40 GBq, EOB).

Copyright © 2001 John Wiley & Sons, Ltd.

Preparation of [^{11}C]Me-QNB ([^{11}C]-1)

Typical procedures : [^{11}C]CH_3I or [^{11}C]CH_3OTf, carried a flow of nitrogen gas, was trapped (bubbling through) at room temperature in a reaction vessel containing 0.53-0.64 mg of the desmethyl-precursor **4** (1.57-1.89 μmol) dissolved in 300 μL of TBP. Trapping of [^{11}C]CH_3I or [^{11}C]CH_3OTf was monitored using an ionisation-chamber probe. When the reading had reached its maximum (2- to 3 minutes usually), the reaction mixture was heated at 100°C (or 200°C) using a heating block for 1 to 8 min and then cooled (EtOH-ice bath). Finally, the reaction mixture was diluted with 0.5 mL of the HPLC mobile phase and was injected onto the column (HPLC B ; Rt : (**1**) : 8.0 to 9.5 min ; (**4**) : 5.5 to 6.5 min).

Typically, using [^{11}C]CH_3OTf and 0.64 mg (1.89 μmol) of precursor **4** at 100°C for 1 minute, 180 to 200 mCi (6.7-7.4 GBq) of [^{11}C]Me-QNB ([^{11}C]-**1**) were routinely obtained within 28 minutes of radiosynthesis (including HPLC purification). Occasionally, up to 340 mCi (12.6 GBq) were obtained.

Formulation of [^{11}C]Me-QNB

Formulation of labelled product for i.v. injection was effected as follows : (1) Dilution of the HPLC-collected fraction (eluent : 0.9% aq. NaCl / EtOH : 65 / 35, see above) with physiological saline to a 10% EtOH concentration ; (2) filtration on a 0.22 μm GS-Millipore filter (vented). The first injection in PET experiments was done within 15 minutes after the end of synthesis.

Quality control of [^{11}C]Me-QNB

As demonstrated by HPLC analysis, the radiolabelled product was found to be > 95% chemically and > 99% radiochemically pure and also co-eluted with a sample of authentic Me-QNB (**1**) (HPLC C ; Rt : 1.75 to 1.80 min). The preparation was shown to be free of non-radioactive precursor (**4**, HPLC C ; Rt : 1.30 to 1.35 min) and to be radiochemically stable for at least 120 min.

Conclusion

In this paper, the radiosynthesis of carbon-11 Me-QNB (*N*-methyl-quinuclidin-3-yl benzilate or *N*-methyl-quinuclidin-3-yl diphenylhydroxy acetate), a hydrophilic, non-metabolized and highly specific muscarinic acetylcholinergic antagonist for PET, was investigated and oriented towards the preparation of multi milliCuries of radiotracer. Typically, using no-carrier-added [^{11}C]methyl triflate as the alkylating agent and 0.64 mg (1.89 μmol) of QNB as precursor for labelling at 100°C for 1 minute lead to a 48.5% +/- 10% (15 runs) decay-corrected radiochemical yield (based on [^{11}C]methyl triflate). 183 mCi (+/- 39) of [^{11}C]Me-QNB ([^{11}C]-**1**) could be synthesized in only 27 to 28 minutes after EOB and occasionally, up to 340 mCi of [^{11}C]Me-QNB ([^{11}C]-**1**) were obtained, corresponding to a 85% decay-corrected yield. The associated decay-corrected specific radioactivities obtained were 2658 mCi/μmol (+/- 971) at EOB.

Copyright © 2001 John Wiley & Sons, Ltd.

Acknowledgement

The authors wish to thank cyclotron operators Mr Daniel Gouel, Mr Christophe Peronne and Mr Christophe Lechêne for performing the irradiations, as well as the "Section Analyses Instrumentation Spectroscopiques - CEA" (Henri Virelizier) and the "Laboratoire d'Analyses - Service des Molécules Marquées - CEA" (Alain Valleix) for MS recording.

References

1. Syrota A., Merlet P. and Delforge J. - *Cardiac Neurotransmission* in *Principles of Nuclear Medicine*, 2nd Ed., Chapter 37 (The Heart), Section 2, Wagner H.N. Ed., W.B. Saunders Company, Philadelphia - London - Toronto - Montreal - Sydney - Tokyo, pp 759-773, 1995
2. Mazière M., Comar D., Godot J.-M., Collard P.H., Cepedat C. and Naquet R. - *Life Sci.* 29: 2391-2397 (1981).
3. Mazière M., Berger G., Godot J.-M., Prenant C., Sastre J. and Comar D. - *J. Radioanal. Chem.* 76: 305-309 (1983).
4. Syrota A., Paillotin G., Davy J.M. and Aumont M.C. - *Life Sci.* 27: 937-945 (1984).
5. Syrota A., Comar D., Paillotin G., Davy J.M., Aumont M.C., Stulzaft O. and Mazière B. - *Proc. Natl. Acad. Sci. USA* 82: 584-588 (1985).
6. Delforge J., Janier M., Syrota A., Crouzel C., Vallois J.M., Cayla J., Lançon J.-P. and Mazoyer B. - *Circulation* 82: 1494-1504 (1990).
7. Valette H., Deleuze P., Syrota A., Delforge J., Crouzel C., Fuseau C. and Loisance D. - *J. Nucl. Med.* 36: 140-146 (1995).
8. Valette H., Syrota A. and Fuseau C. - *J. Nucl. Med.* 38: 1430-1433 (1997).
9. Delforge J., Le Guludec D., Syrota A., Bendriem B., Crouzel C., Slama M. and Merlet P. - *J. Nucl. Med.* 34:981-991 (1993).
10. Le Guludec D., Delforge J., Syrota A., Desruennes M., Valette H. Gandjbakhnch I. and Merlet P. - *Circulation* 90:172-178 (1994).
11. Le Guludec D., Cohen-Solal A., Delforge J., Delahaye N., Syrota A. and Merlet P. - *Circulation* 96: 3416-3422 (1997).
12. Delahaye N., Dinanian S., Delforge J., Slama M., Samuel D., Colin P., Syrota A., Merlet P. and Le Guludec D. - *Circulation* (suppl.), abstract (N°1943) from the 71st Scientific Sessions of the American Heart Association (November 8-11, 1998, Dallas, Tx, USA).
13. Delahaye N., Dinanian S., Slama M., Mzabi H., Samuel D., Adams D., Merlet P. and Le Guludec D. - *Eur. J. Nucl. Med.* 26:416-424 (1999).
14. Delforge J., Pappata S., Millet P., Samson B., Bendriem B., Jobert A., Crouzel C. and Syrota A. - *J. Cereb. Blood Flow Metab.* 15: 284-300 (1995).
15. Crouzel C., Langstrom B., Pike V.W. and Coenen H.H. - *Appl. Radiat. Isot.* 38: 601 (1987).
16. Jewett D.M. - *Appl. Radiat. Isot.* 43: 1383-1385 (1992).

Copyright © 2001 John Wiley & Sons, Ltd.