Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXVII, No. 11

SYNTHESIS OF [^{11}C]-3-QUINUCLIDINYLBENZILATE (QNB)

C. PRENANT, L. BARRE, C. CROUZEL

Service Hospitalier Frédéric Joliot, Département de Biologie,

Commissariat à l'Energie Atomique, 91406 Orsay, France.

SUMMARY

A method is described by which 1.11 to 1.48 GBq (30 to 40 mCi) of [^{11}C]QNB can be obtained from approximately 55.5 GBq (1.5 Ci) of $^{11}CO_2$ in 40 minutes.

The product, purified by HPLC chromatogaphy, is obtained with a specific activity of 18.5 to 33.3 GBq/μmol (500 to 900 mCi/μmol).

Carbon-11 labelling is performed in two stages :

- Formation of [^{11}C]benzilic acid by carbonation by $^{11}CO_2$ of the the pre-prepared benzophenone dianion.

- Esterification of [^{11}C]benzilic acid by 3-quinuclidinol in the presence of carbonyldiimidazole.

INTRODUCTION

QNB is a potent antagonist of acetylcholine ; the aim of its carbon-11 labelling is to study by Positron Emission Tomographie (P.E.T.) pathological conditions affecting muscarinic receptors such as Alzheimer's disease and schizophrenia.

0362–4803/89/111257–09$05.00

Received March 8, 1989
Revised May 30, 1989

Antagonist ligands of acetylcholine are not free of pharmacological activity ; nevertheless, the very high specific radioactivity obtained by carbon-11 labelling make it possible to use anticholinergic ligands in P.E.T.. The injected quantities of carbon-11 ligands do not induce physiological responses typical of these substances such as tachycardia or myoclonia of the atropinic type.

Dexetimide (1), Scopolamine (2, 3, 4) and MQNB (5) labellings have been described. Scopolamine and MQNB are both obtained by methylation, the first substance using [^{11}C]formol and the second one [^{11}C]methyl iodide. [^{11}C]MQNB is a cation which is too hydrophilic to cross the blood-brain barrier and is only suitable for studying peripheral cholinergic receptors (6).

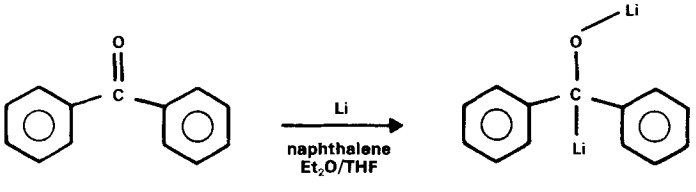
For a study of the central cholinergic receptors, we selected the ^{11}C-labelling of QNB, which is a very interesting ligand due to its high affinity for cholinergic receptors (Kd = 0.01 - 0.20 nM as per ref. 7).

This [^{11}C]-QNB synthesis has been reported at the symposium of Radiopharmaceutical chemistry at Groningen, The Netherlands, July, 1988 (Abstract ref. 8).

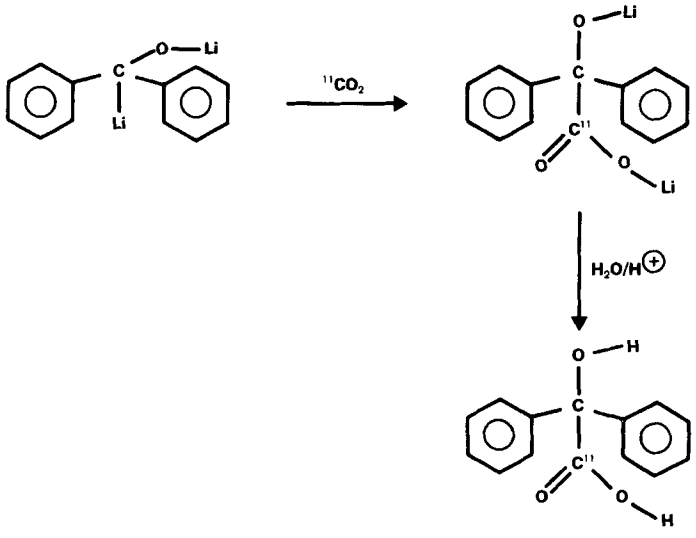
RESULTS AND DISCUSSION

Carbon-11 is obtained by the nuclear reaction : ^{14}N (p, α) ^{11}C and combines with oxygen traces present in the target to give $^{11}CO_2$.
Benzophenone in the form of a lithiate dianion reacts instantaneously with $^{11}CO_2$ by nucleophilic addition (6, 7, 8). Hydrolysis of lithium salt gives [^{11}C]benzilic acid.

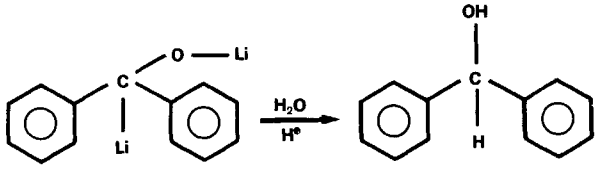
Reduction of the benzophenone with finely powdered lithium in the presence of naphthalene



Carbonation
followed by hydrolysis of the benzilate



The benzophenone's excess dianion gives benzhydrol :

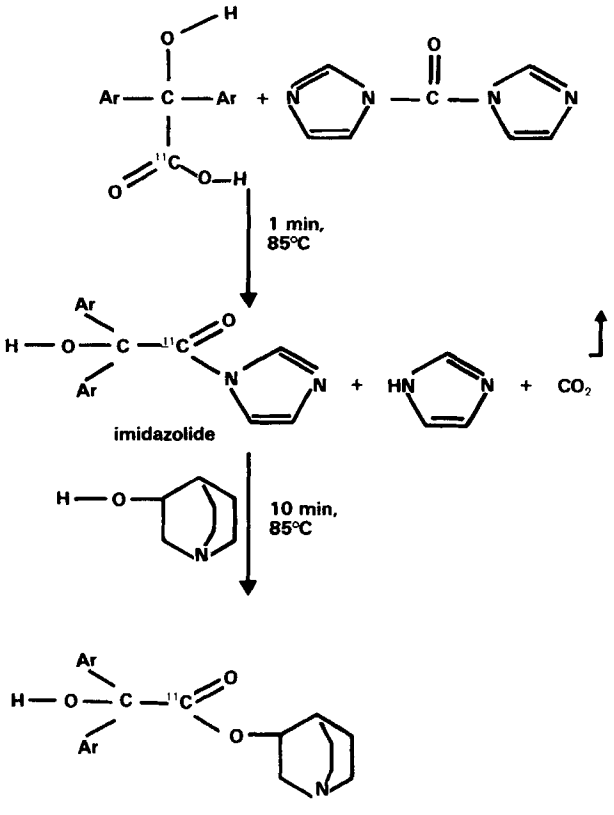


The benzilic acid then reacts with a dehydrating agent, carbonyldiimidazole, to give an unsymmetrical acid anhydride (9). This product evolves by releasing CO_2 to form an imidazolide which then undergoes nucleophilic attack by 3-quinuclidinol to give QNB.

1) Formation of [^{11}C]benzilic acid

The use of lithium to form benzophenone dianion seems preferable to the use of sodium when one compares the radioactivities produced in [^{11}C]benzilic acid for an identical benzophenone/metal weight ratio (ratio = 1.5).

Esterification after activation of the benzilic acid by carbonyldiimidazole



Percentage of radioactivity in benzilic acid by comparison with the total radioactivity in solution

Metal employed	Sodium	Lithium
	45 - 50 %	50 - 55 %

Moreover, for a given mass of benzophenone (3.5 mg, 20 µmol), a metal quantity larger than 5 mg causes a major fall in the benzilic acid yield through the formation of lithium carbonate.

However, if the quantity of metal is less than 2 mg, the dianion concentration of the benzophenone formed is too low.

2) Esterification of benzilic acid by 3-quinuclidinol

This condensation is rendered unsuitable by excessive dilution of the benzilic acid. Likewise, the presence of benzhydrol produced by excessive benzophenone becomes a problem during condensation if the molar ratio of 3-quinuclidinol/benzhydrol becomes less than 2.

3) Purification and identification

Chromatography of the reaction mixture shows two main radioactive peaks. The first one, probably a product of carbonation of naphthalene appears close to the column's dead volume and has very intense radioactivity. The third peak is that of [^{11}C]QNB. The other two radioactive peaks are unidentified.

The residual benzilic acid comes off the column of chromatography after elution with pure ethanol.

By HPLC chromatography, the QNB produced gives a fine shoulder-free peak when coinjected with the reference QNB.

A mass spectrum was taken on stable QNB synthesized by the method described and purified by the same HPLC chromatography system. The mass spectra for the synthetic product and the reference product are identical.

Irradiation of a nitrogen target with 20 MeV protons under 8 bar for 30 minutes at 30 µA gives about 55.5 GBq (1.5 Ci) $^{11}CO_2$. 1.11 to 1.48 GBq (30 to 40 mCi) of QNB are obtained 45 minutes after the end of bombardment with a specific radioactivity of 18.5 - 33.3 GBq/µmol (500 - 900 mCi/µmol).

MATERIALS AND METHODS

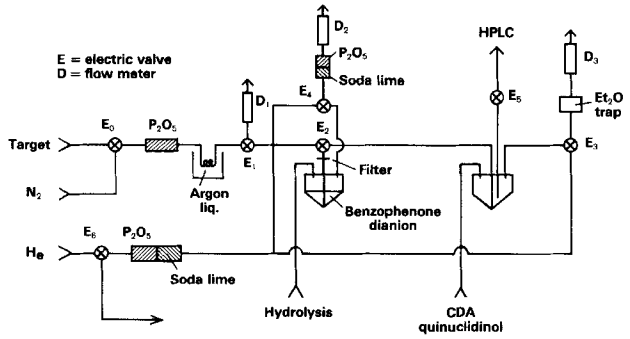
1) Apparatus

The reaction takes place in cylindroconical tubes (3 ml) closed by chromatographic septa (Touzart et Matignon) and interconnected by teflon tubes (int. ø : 0.08 cm) fixed onto medical needles (fig. 1).

The passage of gases was controlled by compressed air-operated electrovalves.

The reaction tubes were fixed on a mobile rod and may be transferred from a cold bath (-10°C) to a hot bath (85°C).

The radioactivity was measured by 3 ionisation chambers : one beside the $^{11}CO_2$ trap, another near the tube in which QNB was synthetized and the third at the chromatograph column outlet. All of the apparatus was contained in a hermetically closed shielded cell (5 cm lead).



SCHEME OF THE APPARATUS FOR ^{11}C-QNB

2) Preparation

A - Formation of benzophenone dianion

A solution of benzophenone (3.5 mg, 20 µmol) and naphthalene (1.3 mg, 10 µmol) in 150 µl of an ethyl ether/tetrahydrofuran mixture, freshly distilled (the former on sodium and the latter on lithium aluminium hydride) and containing 2.5 to 3 mg of lithium, degreased by ether, was left in suspension at room temperature in a dry helium atmosphere (Air Liquide N55) until complete evaporation. The dark blue solid deposited was then taken up by 300 µl of the ether-THF mixture. The solution under helium becomes blue and then red, which was the caracteristic colour of benzophenone dianion, within a few minutes.

B - Carbonation and formation of [^{11}C]benzilic acid

Nitrogen (Air Liquide, purity N60) was irradiated for 30 min. at a pressure of 8 bar, with 20 Mev protons at 30 µA intensity.

The CO_2 formed was carried by the nitrogen current through a P_2O_5 trap (ID = 0.4 mm, L = 5 cm) in which all traces of water were stopped and collected in a metal loop (ID = 0.1 cm, L = 40 cm) cooled by liquid argon (-186°C).

This operation, which took about 5 minutes, gives approximately 55.5 GBq (1.5 Ci) of $^{11}CO_2$.

The loop was then removed from the liquid argon and brought back to room temperature. The radioactivity was carried by a nitrogen flow (Air Liquide N48, 20 ml/min.) into the first tube containing the benzophenone solution in dianion form, and cooled to -10°C. Transfer was completed in less than 10 seconds.

A mixture of 10 µl of 85 % orthophosphoric acid, 50 µl of water and 100 µl of ethyl ether was then injected from outside the shielded cell to hydrolyze the lithium benzilate.

C - Esterification of [^{11}C]-benzilic acid by 3-quinuclidinol

After adding 700 µl of ethyl ether from outside the shielded cell, the solution was transferred to a second tube via a glass wool filter, while the first tube was held at the temperature of a dry-ice/acetone bath (-78°C) to retain the water by freezing. This operation was repeated after a further addition of 700 µl of ethyl ether.

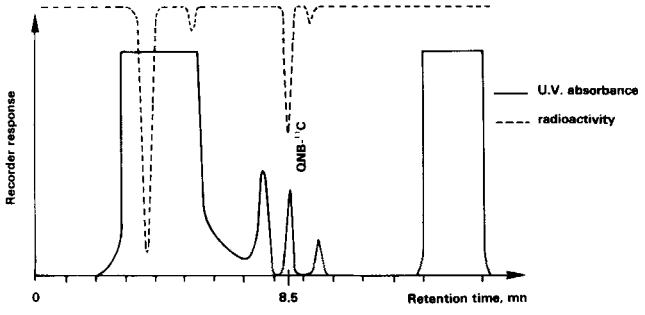
The second tube was then placed in an oil bath at 85°C while maintaining a nitrogen flow of approximately 20 ml/min. to evaporate the ether. This takes place in 6 or 7 minutes. The carbonyldiimidazole (8.1 mg, 50 µmol) in solution in 50 µl of anhydrous DMF was then injected from outside the shielded cell and the tube was heated for 2 minutes to 85°C before adding the 3-quinuclidinol (9 mg, 70 µmol) in solution in 100 µl of anydrous DMF. The tube was again heated to 85°C for 10 minutes.

After cooling and increasing its volume by adding 500 µl of dichloromethane, the solution was sent by nitrogen pressure via a teflon tubing to the injector of a high-performance chromatographic device (Waters pump 6000A).

The eluant used for separation on a 50 cm Whatman M9 partisil column

consisted of 94 % dichloromethane and 6 % ethanol (the latter containing 1.5 % ethylamine and 2.5 % water). The flow rate was 8 ml/min.

Recording was performed in radioactivity and in UV absorption at 254 nM (Waters detector M441).



CHROMATOGRAM OF [^{11}C]-QNB SYNTHETIC MIXTURE

The eluant fraction corresponding to the [^{11}C]QNB (t_R = 8 min.) was collected and the solvent evaporated in an oil bath at 120°C by nitrogen bubbling. The product was redissolved in 500 µl of ethanol and 4.5 ml of physiological serum buffered to a pH ≃ 3 by a 2.10^{-3} M phosphate buffer and sterilized on 0.22 µm filter (Millipore).

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Pichat, Research Director at the CEA, for the assistance he kindly provided.

REFERENCES

1. Dannals R.F., Langstrom B., Ravert H.T., Wilson A.A. and Wagner H.N. - Appl. Radiat. Isot., 39 (4) : 291 (1986).

2. Mulholland G.K., Jervett D.M. and Toorongian D.M. - Appl. Radiat. Isot., 39 (5) : 373 (1988).

3. Frey K.A., Keeppe R.A., Mulholland G.K., Jervett D.M., Hichwa R.D., Agranoff B.W. and Kuhl D.E. - Proceeding of the 35th Annual Meeting SNM (1988) (Abstract).

4. Vora M.M., Finn R.D. and Boothe T.E. - J. Label. Compds Radiopharm. XX (11) : 1229 (1983).

5. Mazière M., Berger G., Godot J.M., Prenant C., Sastre J. and Comar D. - J. Radioanal. Chem., 76 (2) : 305 (1983).

6. Syrota A., Paillotin G., Davy J.M. and Aumont M.C. - Life Sciences, 35 : 937 (1984).

7. Watson M., Roeske W. and Yamamura H. - (Meltzer H.Y. ed.) Raven Press, New York (1987).

8. Prenant C., Barré L. and Crouzel C. - J. Label. Comp. Radiopharm., 26 : 199 (1989).

9. Angelo B. - Bull. Soc. chim., 5 : 1710 (1969).

10. Selman S. and Eastham J.F. - J. Org. Chem., 30 : 3804 (1965).

11. Bulat A.D., Usaevich Yu.Ia., Askinazi B.Z. and Vekshina L.I. - Khim. Farm. Z.H., 11 : 86 (1977).

12. Staab H.A. - Angew. Chem. Internat. Edit. Vol.1, 7 : 351 (1962).