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THE RADIOSYNTHESIS OF NCA [*O-METHYL*-^{11}C]VIQUALINE, THROUGH AN *N*-TRITYL-PROTECTED INTERMEDIATE, AS A POTENTIAL PET RADIOLIGAND FOR 5HT RE-UPTAKE SITES

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Summary

Viqualine, {4-[3-[3(*R*)-ethenyl-4(*R*)-piperidinyl]propyl]-6-methoxyquinoline}, has been labelled in the *O*-methyl position with no-carrier-added (nca) carbon-11 ($t_{1/2}$ = 20.4 min; β^+ = 99.8%) to provide a potential radioligand for PET studies of 5HT re-uptake sites. Direct treatment of *desmethyl*-viqualine with nca [^{11}C]iodomethane gave [*O-methyl*-^{11}C]viqualine as only a minor product. NMR data suggested that the dominant product is that from the [^{11}C]alkylation of the competing piperidino-nitrogen. Protection of this nitrogen with the trityl group, followed by treatment with nca [^{11}C]iodomethane in dimethyl sulphoxide with sodium hydroxide as base, and then rapid deprotection by mild acid hydrolysis gave nca [*O-methyl*-^{11}C]viqualine as the main crude product (27% radiochemical yield, decay-corrected from [^{11}C]iodomethane). Radiochemically and chemically pure nca [*O-methyl*-^{11}C]viqualine was obtained by work up on C18 Sep-pak followed by reverse phase HPLC. The radiosynthesis, including final formulation for *i.v.* administration, takes 56 min after producing the carbon-11 as [^{11}C]carbon dioxide by the ^{14}N(p,α)^{11}C reaction. This radiosynthesis illustrates the potential utility of the trityl group for the protection of nitrogen in rapid carbon-11 radiochemistry.

Key words: [^{11}C]viqualine, 5HT re-uptake site, radioliogand, PET, *N*-trityl

INTRODUCTION

Positron emission tomography (PET) (1) is now widely explored as a technique for investigating pathophysiology in living man, especially for measuring neurotransmitter systems whose dysfunction may play a role in the progress of poorly understood neuropsychiatric disease (for reviews see references 2-4). Much *in vitro* and *post mortem* evidence now implicates a dysfunction in serotonergic neurotransmission in clinical depression (for an overview see reference 5). One hypothesis is that depression results from a deficiency in serotonin (5HT) and hence that the well known tricyclic antidepressants counter this deficiency by specifically inhibiting the pre-synaptic re-uptake of 5HT. More recent hypotheses implicate abnormal levels of either pre-synaptic 5HT re-uptake sites or post-synaptic $5HT_2$ receptors.

Clearly, suitably radiolabelled markers of the serotonergic system might allow the aetiology of depression to be investigated by PET in living man. One approach is to develop a radioligand

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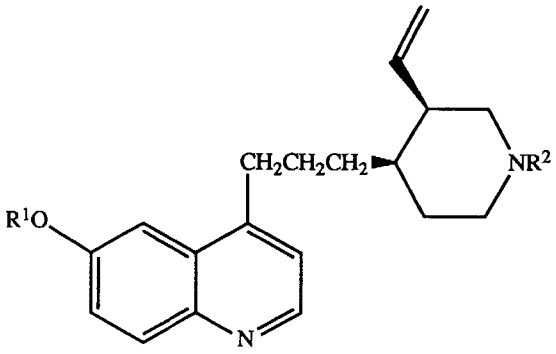
for PET that is specific for the pre-synaptic 5HT re-uptake site. Thus, tricyclic antidepressants [*e.g.* imipramine (6) and clomipramine (7)] and also some recent non-tricyclic antidepressants [*e.g.* fluoxetine (8), sertraline (9) and citalopram (10)] have been labelled with no-carrier-added (nca) carbon-11 ($t_{1/2}$ = 20.3 min, β^+ = 99.8%) to provide potential radioligands. Though the biologically active (+)-antipode of nca [*N-methyl*-^{11}C]citalopram behaves as a radioligand in rats *in vivo* (10), none of these radioligands is so far reported as efficacious for PET studies in man. Some, the labelled tricyclics and sertraline, are highly lipophilic, and might be expected to give high levels of non-specific binding compared to specific (re-uptake site) binding *in vivo*. Recent studies confirm this behaviour for sertraline in rats (11). Therefore, candidate radioligands should possess just sufficient lipophilicity to enable adequate penetration of the blood-brain barrier. Generally, a radioligand must also have high selectivity and high affinity (*e.g.* a K_D, K_I or $I.C._{50}$ value of less than 10 nM *in vitro*) for the target site in order to give a specific signal measureable with PET (3, 4). A further requirement is that it must be possible to synthesise the radioligand at a high enough specific activity to avoid the saturation of receptors *in vivo*.

The isoquinoline derivative, viqualine (**I**), inhibits the uptake of 5HT into rat brain synaptosomes *in vitro* with an $I.C._{50}$ of 3 nM (12) and also the binding of another potent 5HT re-uptake site ligand,[^{3}H]indalpine, to rat brain sections with a K_i of 0.48 nM (13). This inhibition is highly selective for 5 HT (12). Viqualine (**I**) also inhibits 5HT uptake by human platelets (14), a system often used to model neuronal serotonin uptake. Our calculation, using the method of Hansch & Leo (15), suggests viqualine (**I**) to have modest lipophilicity (*i.e.* Log_{10}P = 2.7). Nca carbon-11 labelled viqualine was therefore considered as a prospective radioligand for PET studies of 5HT re-uptake sites. This paper describes the successful radiosynthesis of nca [*O-methyl*-^{11}C]viqualine (**II**) for evaluation as a radioligand *in vivo*.



I; R^1 = Me, R^2 = H

II; $R^1 = {}^{11}CH_3$, R^2 = H

III; R^1 = H, R^2 = H

IV; $R^1 = Ph_3C$, $R^2 = Ph_3C$

V; R^1 = H, $R^2 = Ph_3C$

VI; R^1 = H, $R^2 = {}^{13}CH_3$

EXPERIMENTAL AND RESULTS

Materials

Viqualine (PK 5078), {4-[3-[3(*R*)-ethenyl-4(*R*)-piperidinyl]propyl]-6-methoxyquinoline} (**I**), and *desmethyl*-viqualine (**III**) hydrochloride (PK 35004) were kindly provided by Dr C. Guérémy of Rhône-Poulenc Santé, Vitry Sur Seine (France). Other chemicals and solvents were purchased as follows: anhydrous dimethyl sulphoxide (DMSO, >99% purity) and triethylamine (99% purity) (Aldrich Chemical Co. Ltd); ^{13}C-enriched iodomethane (90 atom %) (Amersham International plc); hydroiodic acid (55%), hydrochloric acid, di-potassium hydrogen orthophosphate (>98% purity), acetic acid (99.5% purity), 4 Å molecular sieve and soda pellets (BDH Chemicals Ltd); ammonia solution (35%) (Hopkins and Williams Ltd); ethanol (>99.7% v/v) (F.A.D. Ltd); acetonitrile, methanol and dichloromethane, all HPLC grade (FSA Laboratory Supplies); triphenylmethyl chloride (Sigma Chemical Co. Ltd).

Preparation of 4-[3-[*N*-trityl-3(R)-ethenyl-4(R)-piperidinyl]propyl]-6-hydroxyquinoline (V)

A stirred suspension of *desmethyl*-viqualine (**III**) hydrochloride (120 mg, 0.36 mmol) in ice-cold dichloromethane (2 mL, dried over 4 Å molecular sieve) was treated with triethylamine (180 mL, 1.29 mmol), followed by triphenylmethyl chloride (138 mg, 0.49 mmol). The solution was allowed to warm slowly and left at room temperature under nitrogen for 140 min. The reaction was monitored by TLC (silica gel, CAMLAB Polygram SIL G/UV 254; eluted with AcOEt/pentane 3: 7 v/v), visualized by both UV irradiation and exposure to iodine. This showed the formation of two major products, with R_f values of 0.17 and 0.49 respectively.

After 4 h, dichloromethane (*ca* 20 mL) was added to the reaction mixture. The resultant solution was washed with water (3 x 10 mL), dried over anhydrous sodium sulphate and then evaporated to dryness. The residue was chromatographed on a column (2 cm i.d., 17 cm height) loaded with silica gel (70 - 230 mesh, Merk) using AcOEt/pentane (3: 7 v/v) as eluent. The first fraction collected was evaporated to give a foam, characterised as *N,O*-ditrityl-*desmethyl*-viqualine (**IV**): **TLC**: R_f = 0.49; **MS** (C.I.+ve, NH_3, 1 torr): *m/z* = 781 ([M+1]$^+$, 8.8%); 539 ([M+1-Ph_3C]$^+$, 22%); 297([M+1-2Ph_3C]$^+$, 6%); 262(100%); 243 (Ph_3C^+, 62%); **^{13}C-NMR** (^{1}H-decoupled with DEPT editing, 62.9 MHz, D_6-DMSO), δ_{TMS} (ppm): 153.2, 146.2, 143.5, 143.2, 128.8, 126.5, 76.7 (Ph_3CN=), 89.9 (Ph_3CO-) - all quaternary carbons; 147.9, 138.7, 130.5, 124.2, 121.0, 110.8, 39.5, 39.2, 128.2, 127.3, 43.8, 127.8, 127.3, 125.9 - all CH carbons; 115.7 (vinyl), 59.7, 53.8, 48.9, 31.9, 28.1, 26.5 - all CH_2 carbons; **yield**: 76 mg, 27%.

The second fraction collected was evaporated and gave *N*-trityl-*desmethyl*-viqualine (**V**): **TLC**: R_f = 0.17; **MS** (C.I. +ve, NH_3 1 torr) *m/z* = 539 ([M+1]$^+$, 100%); 297([M+1-Ph_3C]$^+$, 1.5%); 243 (Ph_3C^+, 7.3%) ; **^{1}H-NMR** (250 MHz, D_6-DMSO), δ_{TMS} (ppm): 1.14 - 2.97 (14 H, aliphatic); 5.04 (1 H, d, *J* =17.1 Hz, vinyl); 5.18 (1 H, d, *J* = 9.7 Hz, vinyl); 7.15 - 7.38 (18 H, aryl); 7.85 (1 H, d, *J* = 8.9 Hz, aryl); 8.50 (1 H, d, *J* = 4.4 Hz, aryl); 9.92 (1 H, phenol). For comparison **III** hydrochloride gave δ_{TMS} (ppm): 1.30 - 3.17 (14 H, aliphatic); 5.10 (1 H, d, *J* = 8.5 Hz, vinyl); 5.14 (1 H, d, *J* = 9 Hz, vinyl); 6.09 (1 H, m, vinyl); 7.23 - 7.35 (3 H, aryl); 7.86

(1 H, d, J = 9.71 Hz, aryl); 8.55 (1 H, d, J = 4.29 Hz, aryl); 8.8 - 9.5 (2 H, H_2+N-); 10.13 (1 H, phenol); **^{13}C-NMR** (^{1}H-decoupled with DEPT editing, 62.9 MHz, D_6-DMSO), δ_{TMS} (ppm): 155.3, 145.6, 142.9, 128.9, 128.2, 76.6 (Ph_3CN=) - all quaternary carbons; 146.6, 138.2, 131.0, 121.5, 120.6, 104.5, 39.5, 39.1, 127.3, 125.8, 46.7- all CH carbons; 115.7 (vinyl), 31.5, 28.3, 26.0 - all CH_2 carbons; 78.3, 54.8, 49.1 too weak for editing. For comparison **I** gave δ_{TMS} (ppm): 157.1, 146.4, 143.7, 127.9 - all quaternary carbons; 147.5, 135.4, 131.1, 121.1, 120.9, 102.3, 39.6, 35.0 - all CH carbons: 117.6 (vinyl), 46.0, 419, 31.0, 30.4, 26.1, 24.3 - all CH_2 carbons; 55.42 - methyl carbon, and **III** hydrochloride gave δ_{TMS} (ppm): 155.8, 145.8, 143.2, 128.5 - all quaternary carbons; 147.7, 135.6, 131.2, 121.5, 120.8, 104.8, 39.6, 38.6 - all CH carbons: 117.8 (vinyl), 46.3, 42.1, 31.5, 30.6, 26.4, 24.5 - all CH_2 carbons; **yield**: 38 mg, 19%.

Production of nca [^{11}C]carbon dioxide

Nca [^{11}C]carbon dioxide was produced with the MRC Scanditronix MC 40 (mark II) cyclotron by the ^{14}N(p,α)^{11}C nuclear reaction on nitrogen (99.95% purity; G and E Union Carbide; pre-dried by passage through magnesium perchlorate) at 1333 kN/m^2 (200 psi). Bombardment was carried out for 1 - 3 min with 19 MeV protons with a beam current set between 10 - 30 μA. At the end of bombardment (EOB) the target was vented over *ca* 1.5 min, either into a loop (2.4 cm diameter, 2 turns) of stainless steel tube (0.73 mm i.d.) that had been pre-flushed with dry nitrogen and kept immersed in liquid argon or, as previously described (16, 17), into a trap of molecular sieve (4Å) at room temperature.

Production of nca [^{11}C]iodomethane

Nca [^{11}C]iodomethane was prepared from the trapped [^{11}C]carbon dioxide, essentially as described previously (16) but using remotely controlled apparatus. Briefly, the procedure is performed in one pot and involves the reaction of [^{11}C]carbon dioxide with lithium aluminium hydride in THF (10 mg/mL, 200 μL), hydrolysis of the radioactive adduct to [^{11}C]methanol and conversion of this into [^{11}C]iodomethane by treatment with hydroiodic acid (55%, 150 μL). The preparation requires 10 min from EOB, giving [^{11}C]iodomethane in *ca* 60 - 80% radiochemical yield (decay-corrected) from [^{11}C]carbon dioxide.

Preparation of nca [*O-methyl*-^{11}C]viqualine (II)

1) By direct [^{11}C]methylation of desmethyl-*viqualine (III)*

A vial (volume 1.5 mL) was loaded with *desmethyl*-viqualine (**III**) hydrochloride (4.0 mg, 11.6 μmol), sodium hydroxide solution (5 M, 10 μL), DMSO (500 μL) and a magnetic follower and then sealed with a teflon-faced septum. The vial was temporarily vented with a needle to allow nca [^{11}C]iodomethane to be introduced in a slow stream of nitrogen over *ca* 1.5 min. The maximum radioactivity trapped in the reaction vessel was recorded at a known time. The vial was then re-sealed and heated to 80 ^{0}C with magnetic stirring. After 5 min, the vial was opened and nitrogen was bubbled through the solution to remove unreacted [^{11}C]iodomethane.

The solution was passed into a C18 Sep-Pak (Waters Associates) that had been pre-washed with ethanol (5 mL) and then water (10 mL). The Sep-Pak was eluted first with water (6 mL) and then with ethanol (6 mL). The aqueous eluate was found to contain only 7% of the radioactivity (decay-corrected) originally introduced into the reaction vessel. The ethanolic eluate was found to contain 78% of the original radioactivity (decay-corrected) in two major products, as shown by TLC (silica gel; CH_2Cl_2/EtOH/Et_3N, 10: 5: 0.2 by vol.) with autoradiography.

A preparation was carried out following the same procedure, but using ^{13}C-enriched (90 atom %) iodomethane (1.2 μL) in place of [^{11}C]iodomethane. The ethanolic eluate from the Sep-pak was evaporated to dryness and the residue examined by ^{1}H-decoupled Fourier transform ^{13}C-NMR spectroscopy (62.9 MHz) with multiplicity determined by DEPT editing. The spectrum revealed peaks at δ_{TMS} = 45.2, 46.3, and 55.4 ppm with relative peak heights of 14.6, 100 and 14.5%, respectively.

2) By [^{11}C]methylation of the N*-trityl compound (V) followed by deprotection*

A vial (volume, 1.0 mL) was loaded with the *N*-trityl compound (V) (*ca* 3.5 mg, 6.5 μmol), sodium hydroxide solution (5 M, 10 μL), DMSO (400 μL) and a magnetic follower and then sealed with a teflon-faced septum. The vial was temporarily vented with a needle to allow nca [^{11}C]iodomethane to be introduced in a slow stream of nitrogen. The radioactivity trapped in the reaction vessel reached a maximum within 1.5 min and was recorded at a known time. The vial was then re-sealed and heated to 110 - 130 ^{0}C with magnetic stirring. After 6 min the vessel was opened and allowed to cool to between 90 and 100 ^{0}C. Then hydrochloric acid (1 M; 100 μL) was added, followed, after 3 min, with ammonium hydroxide solution (18 M, 100 μL). The vial was left to cool for *ca* 2 min. The solution was diluted with water (0.5 mL) and passed into a C18 Sep-Pak that had been pre-washed by elution with ethanol (5 mL) and then water (10 mL). The Sep-Pak was first eluted with water (6 mL) and then with EtOH/CH_2Cl_2 (1: 3 v/v; 6 mL). The organic fraction contained 27% (decay-corrected, mean of 12 results) of the radioactivity initially introduced into the reaction vessel. This fraction was evaporated, the residue dissolved in HPLC eluent (EtOH/CH_2Cl_2; 1: 3 v/v; 0.7 mL) and injected through a Millex FH Luer filter (pore size 0.5 μm, Millipore) onto a silica gel column (30 x 0.7 cm i.d.; particle size 10 μm, "μ-Porasil", Waters Associates) eluted at 2.5 mL/min. The eluate was monitored continuously both for radioactivity and for absorbance at 254 nm. Figure 1 shows a typical chromatogram. The major radioactive peak had the same retention time (15 min) as authentic viqualine (**I**). This was collected between 14.8 and 15.8 min after injection and evaporated to dryness. The residue was formulated for intravenous injection by dissolution in a solution composed of normal saline for injection (9.8 mL; 0.9% v/v NaCl, BP, Boots Ltd) and absolute ethanol (0.2 mL). The final pH of this formulation was 6.5.

The radiochemical yield (decay-corrected) of the formulated radioactive product (**III**) from [^{11}C]iodomethane averaged 9.4% over 4 preparations. The overall synthesis time was 56 min from the end of radionuclide production (EOB).

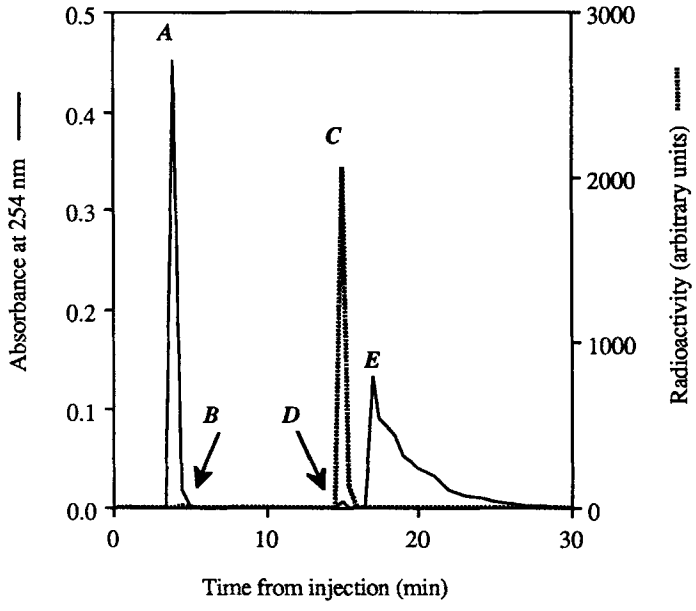


Figure 1. The preparative HPLC separation of [*O-methyl*-^{11}C]viqualine (**II**). ***A*** and ***B*** are unknowns. ***C*** is [*O-methyl*-^{11}C]viqualine (**II**), ***D*** is viqualine (**I**) and ***E*** is *desmethyl*-viqualine (**III**). Chromatographic conditions are described in the text.

Analysis of nca [*O-methyl*-^{11}C]viqualine (II)

Deprotected product from the [^{11}C]methylation of the *N*-trityl compound (**V**) was analysed as follows.

1) High performance liquid chromatography

A sample of the radioactive fraction collected from semi-preparative HPLC was analysed by re-injection on the same HPLC system. A single radioactive peak with the same retention time (15 min) as authentic viqualine (**I**) was observed.

A sample of the formulated product was also analysed on a reverse-phase column (30 x 0.7 cm i.d.; particle size 10 μm, "μ-Bondapak C_{18}", Waters Associates) eluted at 3.0 mL/min with K_2HPO_4 soln. (0.01 M) / MeOH /MeCN / AcOH (70: 22: 7: 1 by vol.). A single radioactive peak with the same retention time (12 min) as authentic viqualine (**I**) was observed.

The specific activity of [*O-methyl*-^{11}C]viqualine (**II**), decay-corrected to EOB, (S_v) was calculated from the radioactivity (A_v GBq) in the collected peak, measured in a calibrated high pressure ionisation chamber at t min from EOB, and from the mass of compound (M_v μmol) represented by the UV absorbance peakusing the expression:

$$S_v = (A_v/M_v)e^{0.034\,t} \qquad \text{GBq/μmol}$$

The chromatography system was pre-calibrated for this purpose by injecting known quantities of authentic viqualine (**I**). S_v was found to be (1.4 GBq/μmol; 38 mCi/μmol) for [*O-methyl*-^{11}C]viqualine (**II**) prepared from all the [^{11}C]carbon dioxide retrieved on molecular sieve from an irradiation of nitrogen with a 10 μA beam of 19 MeV protons for 1 min. For this preparation the product contained 6.5 μg (21 nmol) of stable viqualine (**I**).

No trace of *desmethyl*-viqualine (**III**) was detected by either HPLC system.

2) Thin layer chromatography

A sample of the radioactive fraction from semi-preparative HPLC was analysed by TLC on silica gel using CH_2Cl_2/EtOH/Et_3N (10: 5: 0.2 by vol.) as eluent. The stable compound was visualised under UV-light and radioactivity was detected by autoradiography. The product was found to be chemically and radiochemically pure and to co-migrate with authentic viqualine (**I**).

3) Mass spectrometry

A sample of the radioactive fraction from semi-preparative HPLC was concentrated, allowed to decay and analysed by mass spectrometry (C. I. +ve, 70 eV). The obtained spectrum was identical to that recorded for reference viqualine (**I**), *i.e.* *m/z* = 311 ([M+1]+, 100%).

Further validation of the radiosynthesis

A preparation was carried out using ^{13}C-enriched (90 atom %) iodomethane (1.2 μL) in place of [^{11}C]iodomethane. The organic eluate obtained from the C18 Sep-Pak, containing crude product, was examined by ^{1}H-decoupled Fourier transform ^{13}C-NMR spectroscopy (62.9 MHz; $CDCl_3$) with multiplicity determined by DEPT editing. Two peaks were observed. The major peak (relative peak height, 100%) had the same chemical shift (δ_{TMS} = 55.4 ppm) as the *O*-Me carbon in the corresponding spectrum (*vide supra*) of reference viqualine (**I**), whereas the smaller peak (relative peak height, 38.3%: δ_{TMS} = 23.6 ppm;) remains unidentified. DEPT editing shows that each peak arises from a methyl carbon. The aqueous eluate was also examined and gave only a peak at δ_{TMS} = 21.0 ppm, that was too weak for DEPT editing.

DISCUSSION

The aim of the present work was to label viqualine with the positron-emitting radionuclide, carbon-11, at high specific activity for evaluation as a possible radioligand for PET studies of the 5HT re-uptake site. The *desmethyl* compound (**III**) was available to us as a possible precursor for [^{11}C]methylation in the *O*-methyl group of viqualine (**I**). For the purpose of [^{11}C]methylation, we chose nca [^{11}C]iodomethane as labelling agent because of its relatively easy and well established production (16, 17), compared to that of the alternative, nca [^{11}C]diazomethane (18).

Examples of the direct [^{11}C]methylation of phenols with nca [^{11}C]iodomethane are limited to the recent labelling of members of the benzamide class of neuroleptics, such as [*O-methyl*-^{11}C]raclopride (19), [*O-methyl*-^{11}C]eticlopride (20), [*O-methyl*-^{11}C]remoxipride (21) and [*O-methyl*-^{11}C]clebopride (22). These radiosyntheses utilise the Williamson reaction, which involves the formation of the free phenoxide ion using a suitable base and reaction of the phenoxide ion with the alkylating agent in a dipolar aprotic solvent (23, 24). Use of these conditions discourages solvation of the phenoxide ion, promoting its reactivity as a nucleophile (25, 26). As a corollary, the possibility for methylation on the α–carbon, through the keto form of the phenoxide anion, is reduced.

The presence of a reactive secondary amino group in the available precursor suggested the possibility of *N*-methylation, even to the extent that it could be the main reaction. Indeed, this is the usual result of treating amino-phenols with [^{11}C]iodomethane in the absence of base (27-29). Nevertheless, we first attempted to label viqualine with carbon-11 by the ^{11}C-methylation of *O*-

desmethyl-viqualine (**III**) hydrochloride under Williamson-type conditions supposing that the phenoxide oxygen might prove more reactive than the piperidino-nitrogen.** This attempt was partly encouraged by knowing that the aromatic amino-group is left untouched by [^{11}C]iodomethane in the labelling of [*O-methyl*-^{11}C]clebopride (22), though of course an aromatic amino-group (typical pK_a = 4.6) would not be expected to be so nucleophilic as a piperidino-nitrogen (typical pK_a = 11). Moreover, in the labelling of benzamide neuroleptics (19-22), nca ^{11}C-methylations of phenols are accomplished adequately in the presence of tertiary amino groups. A high proportion of the available nca [^{11}C]iodomethane was found to react with *desmethyl*-viqualine (**III**), but TLC revealed two main radioactive products. ^{1}H-decoupled ^{13}C-NMR spectroscopy, on the reaction mixture obtained from a parallel preparation with ^{13}C-enriched iodomethane, revealed three peaks. By far the most intense of these had a chemical shift (δ_{TMS} = 46.3 ppm) close to that (46.9 ppm) for the *N*-methyl carbon in *N*-methyl-piperidine (30). This evidence, taken into account with the reactivity of the piperidino-nitrogen, suggested *N*-[^{13}C]methyl-*desmethyl*-viqualine (**VI**) to be the main product. A peak at δ_{TMS} = 55.3 ppm indicated viqualine (**I**) to be formed, but only as a minor product.*** In view of the low overall radiochemical yield of [*O-methyl*-^{11}C]viqualine (**II**) to be expected, and the possible difficulty of its separation as a minor radioactive component, development of this route was not pursued. Instead, we considered protecting the piperidino nitrogen in *desmethyl*-viqualine (**III**), in order to avoid its reaction with [^{11}C]iodomethane .

We selected trityl as a possible protecting group on the basis that it was expected to be i) easy to introduce, ii) resistant to the strong base (NaOH) that would be needed to generate the reactive phenoxide oxygen and iii) rapidly removed by mild acid hydrolysis (1N HCl) without causing *O*-demethylation or generating carbanions that might lead to polymerisation (31 and references therein).

The tritylation of *desmethyl*-viqualine (**III**) under basic conditions gave *O,N*-ditrityl-*desmethyl*-viqualine (**IV**, 27%) and the desired protected precursor, *N*-trityl-*desmethyl*-viqualine (**V**, 19%), as main products. These products were easily separable and distinguished by their NMR and mass spectra. Compound **V** also gave a green blue colour on reaction with sodium hydroxide in DMSO, indicating phenoxide ion formation, thereby affirming that the trityl group was on nitrogen and not on oxygen. In view of our limited demand for the precursor (**V**) no effort was made to improve its preparation. It was found that this precursor (**V**) could be [^{11}C]methylated with nca [^{11}C]iodomethane under Williamson-type conditions in useful radiochemical yield, and that deprotection by treatment with dilute hydrochloric acid gave nca [*O-methyl*-^{11}C]viqualine (**II**) as major product. Complete deprotection could be achieved rapidly. Hence, it was not immediately necessary to consider using any of the various more acid labile 4-methoxy derivatives of trityl (32,33) as protecting groups. That ^{13}C-NMR spectroscopy did not detect any *N*-[^{13}C]methyl-*desmethyl*viqualine in crude product from a parallel reaction with ^{13}C-

The vinyl functions in compounds **I and **III** were not considered to be susceptible to Michael-type *intra-* or *inter*-molecular nucleophilic attack by phenoxide ion to cause unwanted products.

*** The third peak at δ_{TMS} = 45.2 ppm is perhaps due to *N,N*-dimethyl-*desmethyl*-viqualine, arising through sequential methylation of the piperidino nitrogen (9).

enriched iodomethane testifies to the stability of the *N*-trityl group under the described methylation conditions.

In the work up of the radiosynthesis great care must be used in the elution of the C18 Sep-Pak, otherwise [*O-methyl*-^{11}C]viqualine (**II**), which has appreciable water solubility, can be eluted in the first aqueous fraction, rather than as desired in the second ethanol fraction. By analysis of the aqueous fraction by mass spectrometry and, after a synthesis with [^{13}C]iodomethane, by ^{13}C-NMR spectroscopy, it was found that the use of 6 mL of water for washing removed a large proportion of the DMSO and radioactive side products, but allowed all the [*O-methyl*-^{11}C]viqualine (**II**) to be retained on the C18 Sep-Pak for subsequent elution in ethanol. HPLC purification provides radiochemically and chemically pure product (**II**), which is easily formulated for intravenous administration. By using longer irradiations (*e.g.* 30 min) with higher beam currents (*e.g.* 30 μA) of protons to produce the carbon-11 it would be possible to use much higher radioactivity (*ca* 75 GBq; 2 Ci) in the radiosynthesis, giving a corresponding improvement in the activity and specific activity of product. Such improvement has been observed previously in other radiosyntheses, for example that of [*N-methyl*-^{11}C]citalopram (10). Full automation would be required to perform such radiochemistry with safety.

It was not required to sterilise [*O-methyl*-^{11}C]viqualine (**II**) for administration to animals. In view of the future possible need to administer [*O-methyl*-^{11}C]viqualine (**II**) to man intravenously, sterilisation of the formulated product (10 mL) by filtration through a Millex GS filter (pore size 0.22 μm; Millipore Corp.) was tested. Only 23% of the total radioactivity was found to be retained on the filter. The radiosynthesis described here now permits the behaviour of nca [*O-methyl*-^{11}C]viqualine (**VI**) to be evaluated *in vivo*. Furthermore, the radiosynthesis suggests that the trityl protecting group could usefully find wider applicability in rapid carbon-11 radiochemistry.

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