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A Single-Mode Microwave Cavity for Reducing Radiolabelling Reaction Times, Demonstrated by Alkylations with [^{11}C]Alkyl Halides

Sharon A. Stone-Elander *[1,2], Nils Elander [3], Jan-Olov Thorell [1,2], Gunnar Solås [4] and Jan Svennebrink [4]

[1] Karolinska Pharmacy, Box 160, S-17176 Stockholm, Sweden
[2] Clinical Neurophysiology, Karolinska Hospital, Box 130, S-17176 Stockholm, Sweden
[3] Atomic Physics, University of Stockholm, Frescativägen 24, S-10405 Stockholm, Sweden
[4] Industrial Microelectronics Center, P.O. Box 1084, S-16421 Kista, Sweden

Summary

A single-mode microwave cavity was constructed for microscale reactions with consideration taken to the space limitations typical for shielded working spaces used with positron emitting radionuclides. The effect of the microwave field in this cavity on typical reaction media is demonstrated. Reaction times for radiolabelling neuroreceptor ligands with [^{11}C]methyl iodide and [2-^{11}C]isopropyl iodide were shown to be considerably reduced compared with the times reported in the literature for the corresponding thermal procedures.

Key words: microwaves, carbon-11, [^{11}C]methyl iodide, [2-^{11}C]isopropyl iodide, Flumazenil, nimodipine, positron emission tomography

*Author for correspondence

Introduction

The first successful use of microwave techniques to speed up syntheses of radiopharmaceuticals for positron emission tomography (PET) was reported in 1987 (1). A number of radiolabelling procedures using commercially-available microwave ovens have subsequently been reported (see references in 2). We have previously demonstrated (3) that a coaxial resonance microwave cavity can be used to generate a well-defined, intense electromagnetic field in small samples. In nucleophilic aromatic [^{18}F]radiofluorinations of substrates with varying leaving groups and degree of activation, yields comparable to or better than thermal methods could be obtained with low microwave input power and reaction times ≤ 0.5 min (2). Though time gains are important in synthetic procedures with all of the positron-emitting radionuclides, the shorter the half-life, the greater the impact on the success of the procedure will be. This was demonstrated by speeding up reactions based on the radiolabelling precursor [^{11}C]CN$^-$ which were performed in aqueous as well as alcoholic media (4-6). In one study (4), comparisons with thermal procedures in the literature

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indicated that the labelling times were reduced to 1/15 - 1/20th by microwave treatment with otherwise identical conditions, which would have increased the radioactivity at the end-of-synthesis (E.O.S.) by nearly a factor of 2.

Experiences with the coaxial cavity indicated that reaction times for radiofluorinations were even much shorter than those previously reported for methods using microwave ovens (2). However, the equipment used had a number of features that were not optimal. The open construction of the cylindrical cavity required careful shielding from the microwaves leaking during operation. A coaxial cable delivered the microwaves to the cavity from the magnetron, thereby keeping bulky electronics and power supplies outside the working space (an advantage with respect to the space limitations though considerable heat losses (*ca.* 50%) were observed with the coaxial cable). Extremely fast reactions were observed with reaction volumes of the order of 200-300 μL, but best results were obtained if the diameter of the sample vessel was kept as small as possible. The access to the cavity was easier than to an oven (no doors to be opened and closed, etc.) but placement of the vessel in the cavity opening required manual adjustments of the apparatus. For a number of radiolabelling procedures, it might be desirable to use the cavity for different reaction vessels or to move the vessel for connections and disconnections to flow lines, etc. A more flexible means of placing the apparatus in the microwave field was therefore desirable.

A new microwave cavity has therefore been constructed with the aim of retaining the positive features and improving some of the technical limitations of the first apparatus. Its use in speeding up alkylations with [^{11}C]methyl iodide and [2-^{11}C]isopropyl iodide (previously reported in preliminary communications 7 and 8) is demonstrated.

Results and Discussion

Design of the microwave equipment

The microwave equipment, shown schematically in Figure 1, consists of a small powerhead connected to the power supply (**P**) unit by electrical cables (**E**). The wave-guide type cavity (**C**) is the central part of the powerhead which also contains a magnetron (power range 0 - 900 W, frequency 2450 MHz) and necessary additional electrical devices. The dimensions of (**C**) were set so that an intensity maximum of the single mode standing wave is positioned at the sample which is inserted into the cavity at a well-defined location. Metal adapters (**S**) of suitable length and positioned from the top of the cavity are used to further increase the microwave amplitude in the sample. The degree of adjustment required is a function of the reaction components, the sample volume, vessel used, etc. The separate power supply (**P**) includes an electric stopwatch for monitoring and steering

the time of treatment of the sample as well as controlling the input power to the magnetron. This design allows the placement of only the powerhead (**M**, **C** and **S**) in the shielded area. By disconnecting (**P**) from (**E**), one power supply may also potentially be used to drive several powerheads permanently installed at different locations.

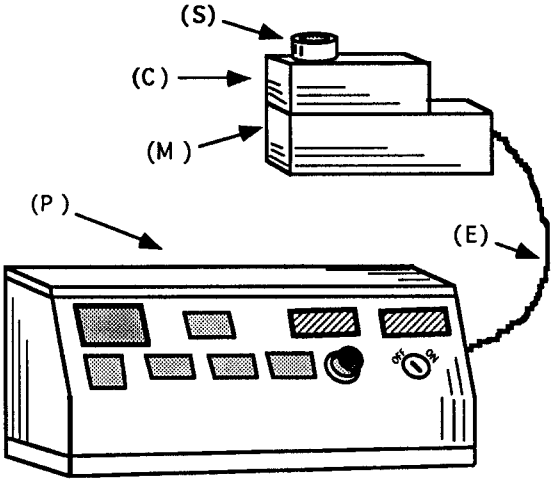


Figure 1
Schematic representation of the separate components of the single-mode microwave cavity.

P = power supply unit
M = ventilated box in which the magnetron is mounted
C = cavity
S = metal adapters that also function as sample holder
E = electrical cables

Design of reaction vessel

The construction of the powerhead places some constraints but also offers a certain flexibility in reaction vessels compared to those typically used with microwave ovens. A reaction mixture can be reproducibly placed in the electromagnetic field via (**S**), though it should be confirmed that the sample has been lowered sufficiently when the vessels used are shorter than the combined depth of (**C**) and (**S**). Here we have used Pyrex tubes (length = 10 cm) fitted with a screw cap and a teflon-backed silicone septum (Figure 2). The total volume is *ca.* 10 mL. For reactions in closed vessels, they are usually not filled more than 1/10th the total volume to allow ample head space for solvent reflux and for containing the pressure developed during the microwave treatment (see Figures 3, 4 and 5 below). Depending on the rate at which the individual samples are heated, the concomitant pressure increases may be adjusted, to some extent, by varying either the size of the reaction vessel or sample volume. The septum may function as a safety release in case the pressure accidentally exceeds that confinable by the vessel. To minimize the risk of explosions, the pressure can be monitored by a pressure gauge connected to the vessel by lines inserted through the septum and the microwave treatment continually adjusted to maintain an acceptable pressure level, which has recently been found to be necessary for even such small sample volumes (5, 9).

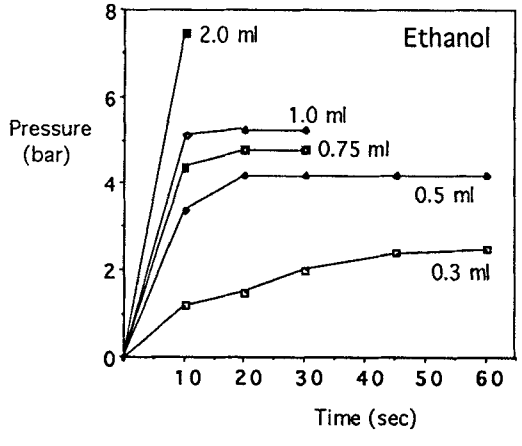
Figure 2

For multi-step procedures, the tube can be lifted from the cavity and connected to flow and reagent lines in the synthesis set-up using needles to pierce the septum. For reactions carried out under gas flows, teflon tubing can be used for leading the gas in and out of the sample. For reactions to be conducted under reflux conditions, the reaction vessel can be attached to a condenser placed above the powerhead by means of a connector for outer-threaded glassware. Such a design minimizes the risk of leakage of microwaves from the apparatus, which is a considerable concern with reflux glassware assembled in microwave ovens (10).

Pyrex is not completely transparent to microwaves. Part of the microwaves will therefore be absorbed by the vessel. However, for most potential applications this loss of energy is probably insignificant since the absorption of Pyrex is considerably smaller than that of most samples. As shown for ethanol (>99%) treated with 160 W in this apparatus (Figure 3), increasing the volume from 0.3 to 2.0 mL continually increased the rate that the pressure built up in the vessel which is related to the increase in temperature of the medium. Such studies previously conducted in microwave ovens have shown that the volume of a given sample can be increased up to an "optimal volume" which exhibits a maximum rate of heating for those conditions. Increases above this volume (which varies with the heat capacity for different solvents), will not be accompanied by an increase in the rate of heating to the same temperature (10, 11). The observation here that such an "optimal volume" was not reached for volumes up to 2 mL, which are typical for small scale radiolabelling reactions, indicates that all the available microwave energy is not being absorbed. Therefore, any possible absorption by the Pyrex vessel is not a significant concern here and even larger volumes can probably be used with suitable vessels.

Figure 3

The effect of increasing volumes of ethanol on the pressure generated inside the reaction vessel. Sample volume was varied from 0.3-2.0 mL (3 to 20% of the vessel volume) and was treated with 160 W up to 60 sec, depending on the magnitude of the pressure increases.



Effects of the composition of the reaction media

The extent to which a given transformation will be accelerated by microwave treatment will be affected by not only the microwave power and uniformity of the field but also by the size of the load and its thermal and dielectric properties (see, for example, the discussion in (12)). In thermal procedures heat is applied from the boundaries and the rate of diffusion through the vessel and the media will determine the rate of heating. In microwave treatment heat is generated by the micro-motion of molecules in the sample in response to the rapidly reversing electric field and is transported from the sample bulk to the boundaries. Consequently, polar solvents are heated by the reversing electromagnetic field while nonpolar substances are generally affected only indirectly by the heat generated by the motion of other polar components in the sample. It has been shown that solvents in open vessels in a microwave oven "superheat", i.e. boil at temperatures much higher than their conventional boiling points (11, 13). In closed reaction vessels the pressure will increase, thereby raising the boiling points even more (10). As shown in Figure 4, the pressures generated in closed vessels using this equipment are highest with solvents of high dielectric loss tangents and low boiling points, which is in good agreement with results obtained using a thick-walled Pyrex vessel in a microwave oven (10). Conducting reactions at temperatures much higher than those usually attained under reflux conditions by conventional heating can explain most reaction accelerations with microwave treatment.

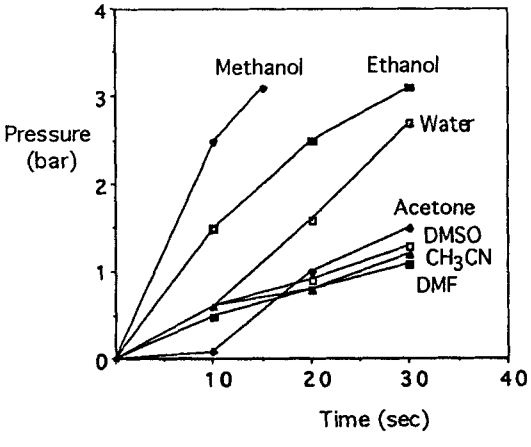


Figure 4

Pressure increases observed on treatment of a fixed volume (0.4 mL, *ca.* 4% of total reaction vessel) of typical solvents with a microwave power of 60 W.

In the microwave field ionic species added to a sample may not only participate in the chemical transformations but will also magnify the microwave heating effects by their interaction with the rapidly oscillating electric field. In PET radiochemistry, this phenomenon has been exploited to drive

cyano-dehalogenation reactions using [^{11}C]CN^- (4) and *N*-alkylations of apomorphines and tetralins with [^{18}F]alkylhalides (9). Even substrates with very slow reaction kinetics could thus sometimes be used to obtain reasonable radiochemical yields. Under corresponding thermal conditions these salt additions to the reaction mixture did not usually affect the speed of conversions to the same extent.

The microwave treatment of mineral acids and bases in closed vessels should be performed with caution due to the extremely high pressures generated under relatively moderate conditions (Figure 5). Acid digestion of samples with microwave ovens are performed with sealed bombs equipped with pressure gauges so that the microwave field can be pulsed at intervals which maintain a controllable pressure level in the vessel. Lack of such monitoring can result in rather violent explosions as we previously experienced when attempting to effect the cyclization of phenylenediamine and diethyl [1-^{11}C]oxalate to [2-^{11}C]-2,3-dihydroxyquinoxalines with mineral acids (5) at microwave power ≥ 150 W.

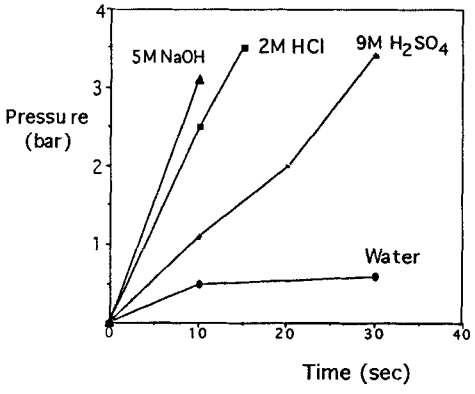


Figure 5
Pressure increases observed on treatment of a fixed volume (0.2 mL, *ca.* 2% of total reaction vessel) of aqueous acids and base with a microwave power of 60 W.

Radiolabelling with [^{11}C]alkyl halides

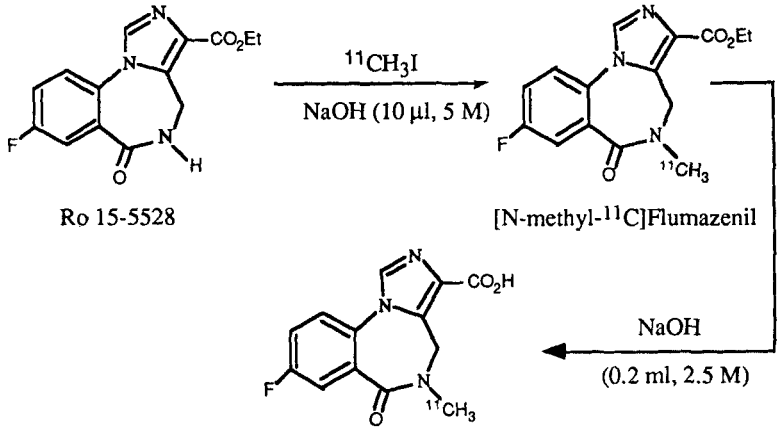
In the first communication of this equipment (7), a preliminary report was also given of its use in the typical *N*- and *O*-alkylations of model compounds with [^{11}C]CH_3I and [2-^{11}C]$(CH_3)_2CHI$. Briefly, conditions could be found for conversions of the alkyl halide ≥ 85% in ≤ 1 min. Only DMF was used as the solvent for reactions with [2-^{11}C]$(CH_3)_2CHI$, while DMF, acetone and sometimes EtOH could be used in the reactions with [^{11}C]CH_3I. While rapid (≤ 5 min) radiolabellings can often be performed thermally with [^{11}C]CH_3I with good conversions, reactions with longer chain halides are typically performed for ≥ 10 min at high temperatures, giving low to moderate yields at E.O.S.

A concern often expressed about using microwave techniques is that molecules may decompose in the electromagnetic field. This misconception originates from experiences of gas phase reactions in microwave plasmas. However, the energy of microwaves is not high enough to break

chemical bonds. Many transformations have now been achieved in microwave ovens (see review in 14). In fact, less degradation has been observed than with thermal procedures (1, 9), presumably because the substrates are exposed to high temperatures for much shorter times.

Two radioligands were synthesized here to illustrate that this cavity may be used in labelling complex molecules. *N*-Alkylation of an amide with [^{11}C]CH_3I to synthesize [*N*-methyl-^{11}C]Flumazenil and subsequent alkaline hydrolysis to its main metabolite [*N*-methyl-^{11}C]Ro 15-3890 (Figure 6) were reported (15) to proceed with good yields thermally (5 + 2 min at 70 °C). Similar yields could be obtained using the same solvent (acetone) with microwaves at considerably reduced times (0.5 + 0.5 min with 100 W). Reduction in reaction times for the two-step procedure would have increased the E.O.S. yields by ~20%. However, by exploiting the fact that DMF is more susceptible to microwave fields than acetone, conversions could be increased by an additional ~25% and ~60% in step 1 and 2, respectively, using less microwave power (0.5 + 0.5 min with 50 W).

Figure 6: Synthesis of [*N*-methyl-^{11}C]Flumazenil and [*N*-methyl-^{11}C]Ro 15-3890



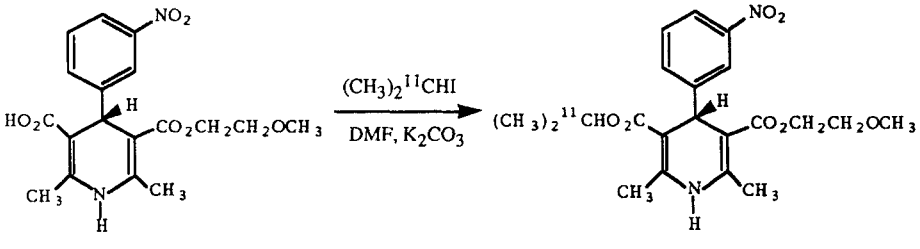
Reaction Step	Thermal Acetone [1]	Microwave Acetone [2]	Microwave DMF [2]
1	5 min, 70 °C, 45%	0.5 min, 100 W, 65%	0.5 min, 50 W, 80%
2	2 min, 70 °C, 40%	0.5 min, 100 W, 60%	0.5 min, 50 W, >95%

[1] Yields reported were decay-corrected, based on [^{11}C]CO_2

[2] Yields are decay-corrected conversions which are based on [^{11}C]CH_3I in step 1 and [N-methyl-^{11}C]flumazenil in step 2

In Figure 7, the results for the alkylation of a carboxylic acid with [2-^{11}C](CH$_3$)$_2$CHI to produce [isopropyl-^{11}C]nimodipine are shown. Typical for "long"-chain [^{11}C]alkyl halides, the thermal procedure required long times (10 min) at high temperature (120 °C) to achieve good yields (16). The ionic carboxylate substrate generated in situ, the base K_2CO_3 and the solvent DMF are all highly polarizable making this type of reaction mixture especially well-suited for microwave treatment. Radiolabelling yields better than those achieved thermally were readily obtained in 1 min with 30 W. Higher conversions combined with the time gains of microwave procedures would increase the E.O.S. yields by nearly a factor of 2, an increase which might help make a large number of such radiolabelling strategies more feasible for ^{11}C chemistry.

Figure 7: Radiolabelling of [isopropyl-^{11}C]nimodipine with [2-^{11}C](CH$_3$)$_2$CHI



W2100 [Isopropyl-^{11}C]nimodipine

Thermal [1]: 10 min, 120 °C, 60-80%	Microwave [1]: 1 min, 30 W, >95%

[1] Yields are decay-corrected conversions which are based on [2-^{11}C](CH$_3$)$_2$CHI

Conclusions

A microwave cavity has been constructed based on our previous experience that a coaxial microwave resonance cavity (3) was very useful in shortening radiolabelling reactions with fluorine-18 (2) and carbon-11 (4). The new apparatus has the following features: easy access by dropping the vessel directly into the cavity via a sample holder, no detectable leakage of the microwaves from the cavity and the bulky power supply is physically separated from the cavity to save space in shielded working areas or to enable one power supply to drive more than one cavity. Higher microwave power was required with this cavity for a given effect on a sample media (e.g. bring to boiling) than for the coaxial resonance cavity, but, on the other hand, a much larger variation in sample size was

tolerated. This is a consequence of differences in the geometry of the two devices. A higher maximum electric amplitude was obtained in the coaxial cavity for a given input power, but this high degree of optimization made it unstable to variations in the polarizability of the sample and geometry of the vessel. This new device has a less well-defined field optimization with a less sharp maximum electric amplitude per input power, but also a more stable mode of operation with respect to variations in the sample.

The microwave cavity was shown here to be useful for speeding up the most common type of labelling in carbon-11 radiochemistry (alkylations with [^{11}C]alkyl halides) and elsewhere for multi-step difunctional precursor syntheses based on [^{11}C]cyanide (5, 6). Reductions in radiolabelling times of this order of magnitude have recently been reported for radiofluorinations with a microwave cavity by Luxen et al. (17). The utility of the microwave cavity for speeding up reactions is, of course, much wider than that of radiolabellings with positron-emitting radionuclides. For example, its use in a flow system for continuous microwave treatment of reaction solutions has also been recently reported for the reaction of carboplatin with N,N-diethyldithiocarbamate as a model for post-HPLC column derivatization reactions (18). A microwave cavity based on the prototype described here is now commercially available (Labwell AB, Uppsala Sweden), which should make these techniques more accessible than has previously been possible.

Experimental

The pressure measurements during microwave treatment were performed using the same Pyrex vessel described in Figure 2. Pressure containment was aided by reinforcing the connection of the septum to the screw cap with epoxy glue. A stainless steel tube (i.d. = 0.5 mm, o.d. = 1.6 mm) pierced the septum to connect the vessel to an oil-filled pressure gauge (AGA Gas). The samples to be treated were screwed into the pressure line and lowered into the microwave cavity through (S). This arrangement allowed monitoring of the pressures generated in the vessel without disturbing the field since the lines were well above the microwave cavity. The pressure increases shown in Figures 3-5 are not corrected for possible dead-space in the lines and therefore should be regarded as relative, not absolute, measurements. The microwave powers cited were obtained by calibration of the magnetron input power to the heating of a given volume of water.

All solvents used were commercially available and were of analytical grade. Tetrahydrofuran (Merck) was distilled from sodium/benzophenone before use. Dimethylformamide (DMF, Analar) was stirred over potassium hydroxide pellets, distilled from barium oxide and stored over molecular

sieves (4Å) in the refrigerator. Anhydrous potassium carbonate (K_2CO_3) and lithium aluminum hydride were obtained from Merck and methyl lithium (1.6 M in diethyl ether) from Fluka AG. Flumazenil (Ro 15-1788), Ro 15-5528 and Ro 15-6877 were supplied by W. Hunkeler, Hoffman-La Roche, Basle Switzerland. The desisopropyl acid precursor of nimodipine (W2100) as well as reference samples of nimodipine were supplied by the Department of Chemistry, Bayer AG, Wuppertal, Germany.

Analytical radio-HPLC was performed using a μBondapak C18 column (Waters 300 x 3.9 or x 7.8 mm, 10 μm), a Kontron 420 pump and an LDC Spectromonitor 3000 coupled in series with a Beckman model 170 β-flow radiodetector, to measure the UV-absorption and radioactivity, respectively. The peak areas were integrated by use of a Shimadzu C-R2AX data processor.

[^{11}C]Carbon dioxide was produced at the Karolinska Hospital with a Scanditronix MC16 cyclotron using 17 MeV protons in the $^{14}N(p,\alpha)^{11}C$ reaction. The gas target was irradiated in a batch production with 10-45 μA, depending on the amount of radionuclide desired. [^{11}C]CO_2 was concentrated by rapidly emptying the target and trapping in a stainless steel coil cooled with liquid nitrogen. At the end of the trapping, the coil was warmed to room temperature when the cooling bath was lowered and the [^{11}C]CO_2 was transferred to the one-pot system routinely used for the production of [^{11}C]alkyl halides (19, 20). [^{11}C]CH_3I and [2-^{11}C]$(CH_3)_2CHI$ were synthesized by standard methods (16, 21-23) and distilled through traps of soda lime and phosphorus pentoxide into the reaction solvent (300 μL).

[*N*-Methyl-^{11}C]Flumazenil was synthesized by adding the acetone or DMF solution of [^{11}C]CH_3I to the vessel in Figure 2 containing a mixture of the desmethyl precursor, Ro 15-5528, (1 mg) and sodium hydroxide (5 M, 10 μL) in 100 μL of the solvent. The mixture was treated with various microwave powers for 30 sec intervals. [*N*-Methyl-^{11}C]Ro 15-3890 was subsequently synthesized by adding NaOH (2.5 M, 200 μL) and treating again with microwaves for 30 sec intervals. The progress of the conversions was followed by coelution with reference compounds on radio-HPLC using acetonitrile: phosphoric acid (0.01 M) = 20:80 (15) as the mobile phase (retention times were 3.5, 3, and 6.1 min for [^{11}C]CH_3I, [*N*-methyl-^{11}C]Ro 15-3890 and [*N*-methyl-^{11}C]Flumazenil, respectively).

[Isopropyl-^{11}C]nimodipine was synthesized by adding [2-^{11}C]$(CH_3)_2CHI$ trapped in DMF to the vessel in Figure 2 containing a mixture of the desisopropyl precursor, W2100, (8 mg) and K_2CO_3 (20 mg) in DMF (100 μL). The mixture was treated with various microwave powers for 30 sec intervals. The progress of the conversions was followed by coelution with reference compounds

on radio-HPLC using acetonitrile: phosphoric acid (0.01 M) = 50:50 (16) as the mobile phase (retention times were 8-9 and 12-14 min for [2-^{11}C]$(CH_3)_2$CHI and [isopropyl-^{11}C]nimodipine, respectively).

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