Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXXVIII, No. 10

Remotely-controlled Production of the 5-HT_{1A} Receptor Radioligand, [*carbonyl*-^{11}C]WAY-100635, via ^{11}C-Carboxylation of an Immobilized Grignard Reagent

Julie A. McCarron, David R. Turton, Victor W. Pike and Keith G. Poole

Cyclotron Unit, MRC Clinical Sciences Centre, Royal Postgraduate Medical School, Hammersmith Hospital, Ducane Road, London W12 ONN, U.K.

WAY-100635 [*N*-(2-(1-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl))-*N*-(2-pyridyl)-cyclohexanecarboxamide], when labelled in its carbonyl position with carbon-11 ($t_{1/2}$ = 20.4 min), is an effective radioligand for the study of 5-HT_{1A} receptors in human brain with positron emission tomography (PET). A simple remotely-controlled procedure was developed for the routine synthesis of [*carbonyl*-^{11}C]WAY-100635. Thus, cyclohexylmagnesium chloride is coated onto the inner surface of a narrow polypropylene tube. Cyclotron-produced [^{11}C]carbon dioxide is passed into the tube in a nitrogen stream. A solution of thionyl chloride in tetrahydrofuran is then passed through the tube to convert the trapped radioactive adduct into [*carbonyl*-^{11}C]cyclohexanecarbonyl chloride and to release this labelling agent into a vial containing 1-(2-methoxyphenyl)-4-(2-(2-pyridylamino)ethyl)piperazine plus triethylamine. The vial is sealed and heated to 70°C for 5 min. [*carbonyl*-^{11}C]WAY-100635 is isolated by sample-enrichment and reverse phase HPLC and formulated for human intravenous injection by evaporation of solvent and dissolution in 'saline for injection'. The novel use of the immobilized Grignard reagent has the advantages that only small quantities of all reagents are required so simplifying product purification. Moreover, the procedure was readily adapted for operation in a shielded hot-cell with remote control for radiation safety. The remotely-controlled radiosynthesis takes 45 min and gives high radioactivities (2.96–5.92 GBq) of formulated [*carbonyl*-^{11}C]WAY-100635 in > 99% radiochemical purity and high specific radioactivity (average, 192 GBq/μmol).

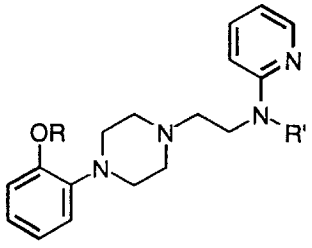
Key words: [*carbonyl*-^{11}C]WAY-100635, ^{11}C-carboxylation, immobilized Grignard reagent, radioligand, PET, 5-HT_{1A} receptors.

CCC 0362–4803/96/010941–13

Received 24 April 1996
Revised 29 April 1996

Introduction

WAY-100635 [*N*-(2-(1-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl))-*N*-(2-pyridyl)-cyclohexanecarboxamide] (I) is a highly potent and selective antagonist at 5-HT_{1A} receptors (1–3). The labelling of WAY-100635 with carbon-11 ($t_{1/2}$ = 20.4 min) in its *O*-methyl group recently provided the first effective radioligand for the delineation of 5-HT_{1A} receptors in rat (4–6), monkey (6) and human brain (7) with positron emission tomography (PET). However, it is now known that the use of [*O-methyl*-^{11}C]WAY-100635 (II) in humans *in vivo* is complicated by the formation of the descyclohexanecarbonyl analogue, [*O-methyl*-^{11}C]WAY-100634 (III), as a metabolite in plasma (8–10). This radioactive metabolite is able to penetrate the blood-brain barrier and contribute to nonspecific and specific binding in primate brain, thereby hampering the development of a biomathematical model for interpretation of the acquired PET data in terms of useful radioligand binding parameters (9–11). More recently we have reported that the labelling of WAY-100635 with carbon-11 in its carbonyl position (5) provides a superior radioligand for studies of 5-HT_{1A} receptors in human brain (12). This radioligand gives only very polar metabolites in plasma, reduced nonspecific binding in all brain regions and higher signal contrast. Consequently, it is possible to apply conventional biomathematical models to estimate binding parameters, such as regional values for binding potential in humans (13) and K_D and B_{max} in monkey (14).



(I) R = Me, R' = cyclohexanecarbonyl
(II) R = $^{11}CH_3$, R' = cyclohexanecarbonyl
(III) R = $^{11}CH_3$, R' = H
(IV) R = Me, R' = [*carbonyl*-^{11}C]cyclohexanecarbonyl
(V) R = Me, R' = H

Here, we report a simple remotely-controlled synthesis of [*carbonyl*-^{11}C]WAY-100635 (IV), based on the preparation of [*carbonyl*-^{11}C]cyclohexanecarbonyl chloride, via ^{11}C-carboxylation of an immobilized Grignard reagent, and ^{11}C-acylation of WAY-100634 [1-(2-methoxyphenyl)-4-(2-(pyridylamino)ethyl)piperazine] (V) (Figure 1). High radioactivities of [*carbonyl*-^{11}C]WAY-100635 at high specific radioactivity are now routinely prepared by this procedure for PET studies in humans.

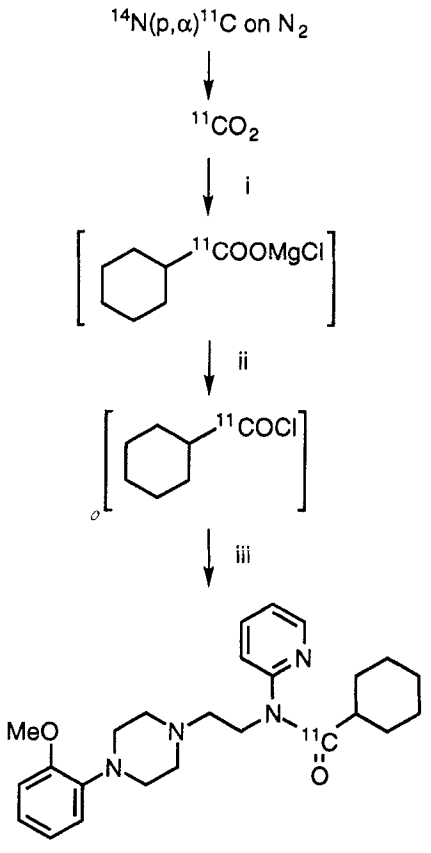


Figure 1. The radiosynthesis of [*carbonyl*-^{11}C]WAY-100635 (IV) from cyclotron-produced [^{11}C]carbon dioxide via dissolved cyclohexylmagnesium chloride (method A) or immobilized cyclohexylmagnesium chloride (method B), i, cyclohexylmagnesium chloride solution (method A) or immobilized cyclohexylmagnesium chloride (method B); ii, thionyl chloride; iii, WAY-100634 (V) plus triethylamine.

Results and Discussion

The approach that we have taken to labelling WAY-100635 in its carbonyl position with carbon-11 at high specific radioactivity is to acylate the amine, WAY-100634, with no-carrier-added (NCA) [*carbonyl*-^{11}C]cyclohexanecarbonyl chloride (5). Lower aliphatic ^{11}C-labelled acid chlorides are easily prepared from cyclotron-produced [^{11}C]carbon dioxide (15,16) and, because they may be isolated by simple distillation, have proved especially useful for the preparation of NCA ^{11}C-labelled amides. Preceding this work the preparation of involatile [*carbonyl*-^{11}C]cyclohexanecarbonyl chloride (b.p. 184°C) was unknown, though the next lower homologue had been reported (17).

Initially we tried to prepare [*carbonyl*-^{11}C]cyclohexanecarbonyl chloride according to the technique originally described for the preparation of lower ^{11}C-labelled acid chlorides, namely carboxylation of a Grignard reagent with [^{11}C]carbon dioxide followed by treatment with phthaloyl dichloride plus 2,6-*di-tert*-butyl-pyridine (DTBP) and separation of product by distillation (15,16). Attempts to isolate the radioactive acid chloride by distillation at 200°C proved impractical because of the co-distillation of base. This problem persisted even when a less volatile sterically-hindered base, 2,6-*di-tert*-butyl-4-methylpyridine, was used. We next explored the use of an involatile polymeric amine, poly(4-vinyl-pyridine), in place of DTBP as base. This enabled the radioactive acid chloride to be isolated but in a radiochemical yield (14%, decay-corrected from [^{11}C]carbon dioxide) that was insufficient to merit further pursuit of this approach. We therefore sought a means to prepare the radioactive acid chloride in good radiochemical yield, and in a useful form for reaction with WAY-100634, without the need for isolation by distillation.

A one-pot procedure has been reported for the preparation of involatile [*carbonyl*-^{11}C](4-benzofuranyl)carbonyl chloride and its successful use to label an amine (18). We explored this type of procedure for the preparation of [*carbonyl*-^{11}C]WAY-100635. Cyclotron-produced [^{11}C]carbon dioxide was dispensed into a vial containing cyclohexylmagnesium chloride dissolved in a mixture of diethyl ether and THF and the carboxylation adduct was treated with thionyl chloride. Addition of triethylamine plus, in the same mole quantity as the Grignard reagent, WAY-100634 (60 mg), followed by heating of the sealed reaction mixture at 70°C for 5 min gave [*carbonyl*-^{11}C]WAY-100635 in 60% radiochemical yield (decay-corrected from trapped [^{11}C]carbon dioxide) (5). However, the large quantity of precursor used in this procedure resulted in difficult separation of the radioligand. Though, it was possible to purify the radioligand from a fraction of the reaction mixture (ca 1/3 rd) by normal phase HPLC on a semi-preparative column, this approach proved impractical for purification of the whole reaction mixture.

By reducing the mass of precursor in this radiosynthesis to 13 mg adequate radioactivities of pure [*carbonyl*-^{11}C]WAY-100635 for PET studies in humans could be obtained by sample-enrichment and reverse-phase HPLC. This radiosynthesis was adapted to remote control. In 13 consecutive radiosyntheses, each starting with *ca* 110 GBq (3 Ci) of [^{11}C]carbon dioxide, the remotely-controlled apparatus gave an average of 0.78 GBq (21 mCi) of radioligand with an average specific radioactivity of 150 GBq/μmol (4.05 Ci/μmol) at 50 min from the end of radionuclide production. The low yields of isolated radioactive product were attributed to a combination of less efficient ^{11}C-carboxylation and physical losses in the remotely-controlled apparatus.

The specific radioactivity of the radioligand was high, even though commercial Grignard reagent was used in its preparation with no guarantee against minor contamination by atmospheric carbon dioxide (if present, this contaminant would dilute the specific radioactivity of the cyclotron-

produced [^{11}C]carbon dioxide and of the derived radioligand). In one experiment, [*carbonyl*-^{11}C]WAY-100635 was prepared from cyclohexylmagnesium chloride that had been prepared freshly "in house" with precautions against contamination by atmospheric carbon dioxide, using essentially a technique described previously (19). The specific radioactivity of this product was comparable to that prepared from commercial Grignard reagent. This indicated that the commercial Grignard reagent introduced very little carrier carbon dioxide and that the major source of carrier was in the cyclotron-produced [^{11}C]carbon dioxide. For convenience we have continued to use the commercial Grignard reagent.

The rather large quantity of precursor needed to provide an adequate radioactivity of [*carbonyl*-^{11}C]WAY-100635 for routine PET studies led us to develop a new approach whereby the amounts of all reagents and precursor could be reduced. We considered that the inside surface of a polypropylene tube would be effective for the 'immobilization' of a small quantity of Grignard reagent through hydrophobic interactions and that efficient ^{11}C-carboxylation could then be achieved by simply passing gaseous NCA [^{11}C]carbon dioxide over this surface. Chlorination of this adduct with a small quantity of thionyl chloride might then be expected to release the NCA ^{11}C-labelled acid chloride for reaction with amine, in the absence of gross amounts of Grignard reagent and byproducts.

Thus, commercially available polypropylene tubing was connected between two 3-way valves (Figure 2), flushed with nitrogen, cooled to between -5 and 0°C, coated on the inside with a film of cyclohexylmagnesium chloride solution in diethyl ether-THF and then flushed with cyclotron-produced [^{11}C]carbon dioxide in a stream of nitrogen. This procedure trapped over 95% of the [^{11}C]carbon dioxide. A dilute solution of thionyl chloride in THF was washed through the tubing into a vial containing WAY-100634 plus triethylamine. Less than 5% of the radioactivity was left in the polypropylene tube. The vial was sealed and the reaction mixture was stirred for 5 min at 70°C. Analysis of the product by reverse phase HPLC demonstrated the formation of [*carbonyl*-^{11}C]WAY-100635. Further experiments demonstrated that control of the ^{11}C-carboxylation conditions (amount of reagent, time and temperature) and of the relative amounts of thionyl chloride, precursor and triethylamine was important to achieve a high radiochemical yield. Under the preferred conditions, which are detailed in the Experimental, [*carbonyl*-^{11}C]WAY–100635 was obtained in 50–70% radiochemical yield (decay-corrected) from the radioactive acid chloride.

The above procedure was readily incorporated into a lead-shielded hot-cell with remote control of all operations (Figure 2). By coupling this apparatus to sample-enrichment and semi-preparative reverse phase HPLC (Figure 3), purified radioligand could be obtained at 45 min from the end of radionuclide production. Though a somewhat higher yield (6.7 GBq; 181 mCi from a 15 μAh irradiation) could be obtained by omitting sample-enrichment, its routine use was found to result in chemically purer product and also helped to preserve the performance of the main HPLC column.

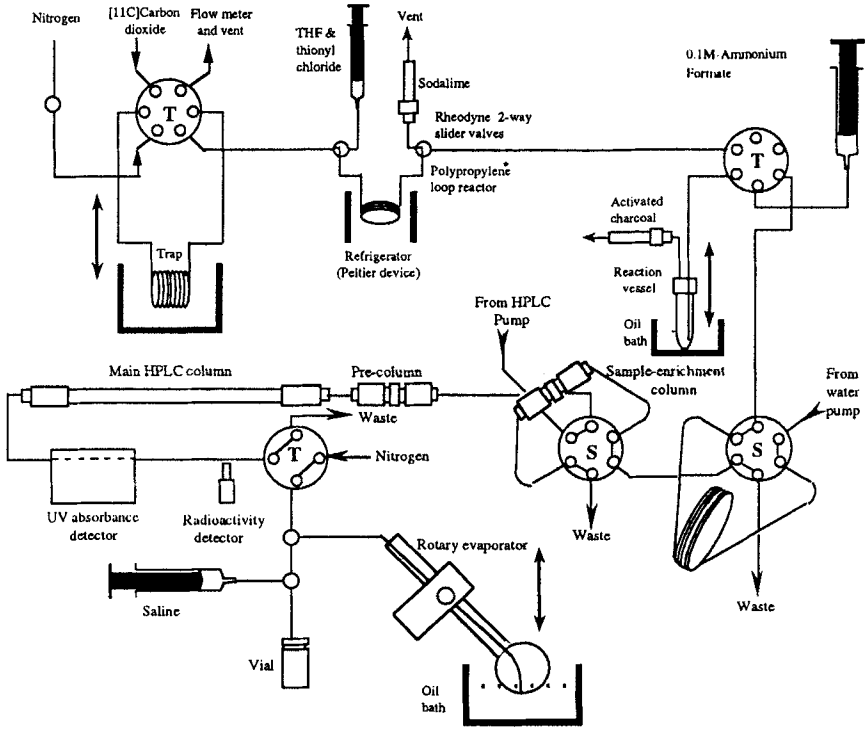


Figure 2. Remotely-controlled apparatus for the production of [*carbonyl*-^{11}C]WAY-100635 via ^{11}C-carboxylation of an immobilized Grignard reagent. T and S represent Teflon and stainless steeel multi-way valves (Rheodyne), respectively. The principles of operation and control of the various components are described in the text.

Preparations of [*carbonyl*-^{11}C]WAY-100635 were analyzed using a variety of normal and reverse-phase HPLC systems. All these methods resolved WAY-100635, WAY-100634 and desmethyl-WAY-100635 and demonstrated that the radioligand had the same chromatographic mobility as authentic WAY-100635. Preparations of [*carbonyl*-^{11}C]WAY-100635 were routinely analyzed using an Ultracarb 5 ODS 30 column, which was found to have durable performance and high column to column reproducibility (Figure 4). For preparations of [*carbonyl*-^{11}C]WAY-100635, radiochemical purities averaged more than 99% and specific radioactivities averaged 192 GBq/μmol (5.2 Ci/μmol; n = 7) at the end of radiosynthesis. The radioligand was found to be radiochemically stable for at least 2 h. WAY-100634 was found to be difficult to exclude entirely from preparations of [*carbonyl*-^{11}C]WAY-100635. Levels in formulated radioligand have ranged from 2.4–3.1 nmol/mL (0.76–0.97 μg/mL) in a total volume of 10 mL. However, the affinity of WAY-100634 for 5-HT$_{1A}$ receptors is lower than that of WAY-100635 and previous experiments in rats have established that an intravenous injection of less than 0.20 μg of WAY-100634 per kg body weight will have no significant effect on the receptor

binding of [*carbonyl*-^{11}C]WAY-100635 *in vivo* (20). The low absolute level of contamination of the radioligand by WAY-100634 is not therefore a problem for its application in PET, especially when only a portion (185–370 MBq; 5–10 mCi) of the high radioactivity product (range, 2.96–5.92 GBq; 80–131 mCi; n = 9, from a 15 μAh irradiation) is required for single administration to a human subject. Thus, typically less than 3 nmol (1 μg) of WAY-100634 will be coadministered with the radioligand to human subjects. After administration of the radioligand to humans a fraction is in any case metabolised to non-radioactive WAY-100634 (8–10).

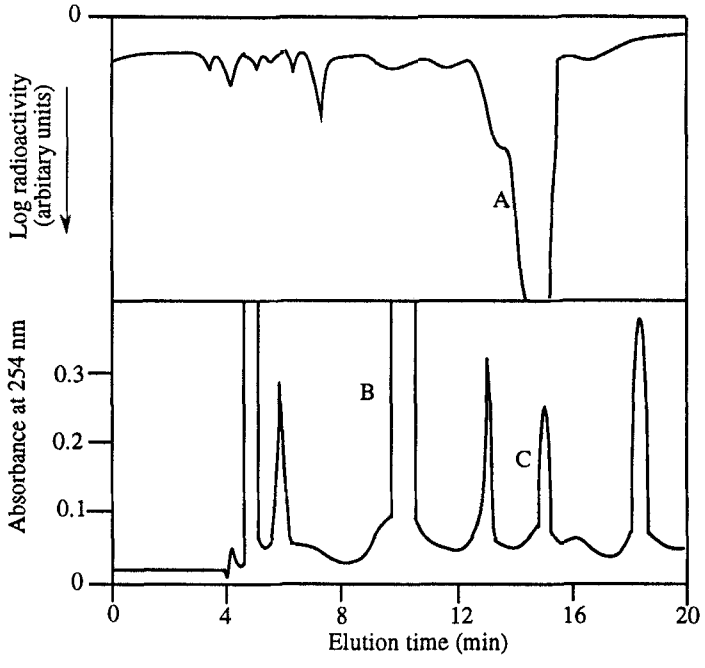


Figure 3. Chromatograms from the preparative separation of [*carbonyl*-^{11}C]WAY-100635 by reverse phase HPLC. Compounds A, B and C are [*carbonyl*-^{11}C]WAY-100635, WAY-100634 and WAY-100635, respectively. Elution conditions are described in the text.

The use of an immobilised Grignard reagent has proved extremely effective for the simple remotely-controlled production of involatile [*carbonyl*-^{11}C]cyclohexanecarbonyl chloride for labelling WAY-100635. The quantity of precursor required (11 μmol; 3.5 mg) for acylation is far less than in our previous procedures (5), the radiosynthesis takes less time, product separation is easier and a higher yield and specific radioactivity are obtained. Moreover, the simplicity of the required operations facilitated automation and remote control. We have already found that this method is applicable to the production of other involatile ^{11}C-labelled acid chlorides, including [*carbonyl*-^{11}C]benzoyl chloride, and should therefore prove valuable for producing a variety of radiopharmaceuticals for PET.

This simplified and efficient method for the production of [*carbonyl*-^{11}C]WAY-100635 will enable this excellent radioligand to be applied extensively with PET for the study of 5-HT_{1A} receptors in human brain, especially in relation to neuropsychiatric disorders and the binding of psychotropic drugs.

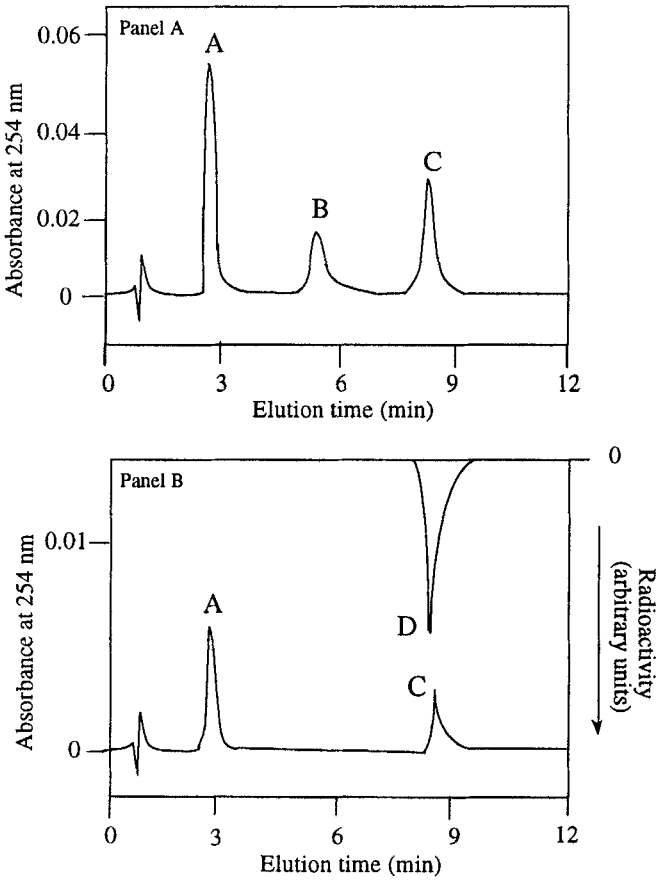


Figure 4. Chromatograms from for the routine quality control of [*carbonyl*-^{11}C]WAY-100635 by reverse phase HPLC on an Ultracarb 5 ODS column. Panel A shows the separation of an injected mixture of reference compounds, namely WAY-100634 (A), desmethyl-WAY-100634 (B) and WAY-100635 (C). Panel B shows a radiochromatogram from the analysis of a production of [*carbonyl*-^{11}C]WAY-100635 (D). Elution conditions are described in the text.

Experimental

Chemicals

WAY-100635 [*N* - (2-(1-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl))-*N*-(2-pyridyl)-cyclohexane-carboxamide trihydrochloride] and WAY-100634 [1-(2-methoxyphenyl)-4-(2-(2-pyridylamino)ethyl)piperazine trihydrochloride (1/3 hydrate)] were donated by Wyeth Research (U.K.)

Ltd. WAY-100634 was dried in a vacuum oven at 50°C for 2 h before use. Methylmagnesium chloride solution in diethyl ether (2 M), diethyl ether and anhydrous THF were purchased in Sure/Seal™ bottles from Aldrich Chemical Co. Ltd. HPLC solvents were purchased from Fisons Ltd. All other chemicals were purchased from Aldrich Chemical Co. Ltd and were of the highest grade.

Preparation of cyclohexylmagnesium chloride

Cyclohexyl chloride (1.19 g; 0.1 mol) was added to magnesium turnings (0.243 g; 0.1 mol) and a few crystals of iodine in dry diethyl ether (5 mL) under dry nitrogen (19). The reaction mixture was stirred for 1 h at room temperature under nitrogen, during which time the colour of iodine disappeared. The reaction mixture was stirred for a further 5 h during which time most of the magnesium dissolved. The reagent was stored in a sealed vial under nitrogen and used the following day.

Production of no-carrier-added [^{11}C]carbon dioxide

No-carrier-added [^{11}C]carbon dioxide was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction on nitrogen gas containing 0.1% oxygen (15 bar) using a beam (30 μA) of 19 MeV protons from a Scanditronix MC40 (Mark II) cyclotron. The [^{11}C]carbon dioxide was trapped from the irradiated gas in a coil (2 turns, ca 1.5 cm diameter) of stainless steel tube (1/16"o.d.) immersed in liquid argon. The yield from a 30 or 45 min irradiation was about 93 or 110 GBq (2.5 or 3 Ci), respectively.

Construction of remotely-controlled apparatus for radiosyntheses

Radiosyntheses were performed in a lead-shielded hot-cell with remote control of all operations using a programmable logic controller (Toshiba Ltd) for the initial trapping and dispensing of [^{11}C]carbon dioxide, and wafer-switch control for the remainder of operations. The lowering and raising of the stainless steel trap for [^{11}C]carbon dioxide, in and out of liquid argon, was achieved using a pneumatically-driven piston. A pneumatically-driven Teflon multi-way valve (Rheodyne Ltd) was used to direct the flow of gases through the trap. The same type of valve and control were used to transfer reaction mixtures between reaction vessels. A Peltier device (Thermoelectric Devices Ltd, Moreton-in-Marsh, U.K.) was used to cool a polypropylene coil reactor. This reactor was equipped with a pneumatically-controlled 2-way 3-port slider valve (Rheodyne Ltd) at each end. Additions of reagents and solutions were made from glass syringes with Teflon plungers (Hamilton Ltd), driven by lead screws with electric motors (Model 440-335, Radiospares Ltd). Reaction oil baths were thermostatically controlled with a Cal 9000 device (Radiospares Ltd). Two pneumatically-controlled stainless steel multiway valves were used in tandem (Models 7010 and 7000; Rheodyne Ltd) for sample-enrichment and injection onto preparative HPLC. HPLC was equipped with a UV absorbance detector (Model SA

6506; Severn Analytical Ltd) and a radioactivity detector (G-M. tube; Minalarm Ltd) linked to a dual-pen chart recorder. A microrotatory evaporator (Heidolph Ltd), adapted to use a pneumatically-operated piston for remotely-controlled raising and lowering of the sample, was used for evaporation of HPLC solvent and radioligand formulation. Figure 2 depicts the configuration of the apparatus for the production of [*carbonyl*-^{11}C]WAY-100635 when using a polypropylene tube reactor (see below).

Remotely-controlled synthesis of NCA [*carbonyl*-^{11}C]WAY-100635 via ^{11}C-carboxylation of dissolved Grignard reagent

Dry cyclotron-produced [^{11}C]carbon dioxide from a 17.5 μAh irradiation was dispensed for 2 min in a stream of dry nitrogen (6 mL/min) from the warmed stainless steel trap into a solution of commercial cyclohexylmagnesium chloride (0.2 mmol) in diethyl ether (250 μL) plus THF (250 μL) under nitrogen. A solution of thionyl chloride (0.28 mmol; 20 μL) in THF (100 μL) was added to the solution which was then sealed and heated at 70°C for 2 min. The reaction vessel was vented and heated while flushed with nitrogen for a further 3 min to evaporate off excess thionyl chloride and diethyl ether. The residual radioactive product was re-dissolved in THF (500 μL) and transferred to a septum-sealed vial (volume, 1 mL) containing WAY-100634 (0.04 mmol; 13 mg) and triethylamine (0.287 mmol; 40 μL). The reaction mixture was stirred in the sealed vial at 70°C for 5 min and then quenched with water (5 mL). The resultant solution was injected onto a sample-enrichment column (Partisil 10 ODS-2; 50 mm x 4.6 mm i.d.; Whatman Ltd) that had been pre-washed with water. The column was washed with water (2.5 mL/min for 3 min) and then back-eluted at 6.0 mL/min with methanol-0.1M-ammonium formate-triethylamine (65: 35: 0.3 by vol.) into a reverse phase column (Nucleosil C18; 250 mm x 10 mm i.d.; particle size, 5 μ; Technicol Ltd). Elution of the HPLC column was continued at 6 mL/min. The radioactive product that eluted with a retention time of 17 min was rotary evaporated to dryness under reduced pressure and then dissolved in 'saline for injections' (0.9% w/v; 10 mL). Finally, within a particle-free aseptic environment, the product was filtered through a sterile filter (0.22 μ pore size; Acrodisc; Gelman Ltd) into a sterile septum-sealed vial.

Remotely controlled NCA [*carbonyl*-^{11}C]WAY-100635 via ^{11}C-carboxylation of an immobilized Grignard reagent.

The following radiosynthesis was performed in remotely-controlled apparatus configured as depicted in Figure 2.

A coil of polypropylene tube (40 cm length; 1/16"o.d.; 1/32" i.d.; New Age Industries, Inc. Willow Grove, PA, USA) was flushed with nitrogen. The tube was cooled to between -5 and 0°C. Cyclohexylmagnesium chloride solution (2.0 M in diethyl ether, 100 μL) was diluted with THF (100 μL) and passed through the polypropylene tube to leave a thin film of reagent on the inner surface.

[^{11}C]Carbon dioxide was dispensed from the warmed stainless steel coil and passed through the polypropylene tube in a stream of nitrogen (4.0 mL/min) for 2 min. Thionyl chloride (69 μmol; 5 μL) in THF (400 μL) was then washed through the tube into a septum-sealed vial (volume 1 mL) containing pre-dried WAY-100634 (11 μmol; 3.5 mg) plus triethylamine (0.14 mmol; 20 μL) under nitrogen. The reaction mixture was stirred in the sealed vial at 70°C for 5 min and then taken up in 0.1M-ammonium formate (5 mL) and passed into a sample-enrichment column (Partisil 10 ODS-2; 50 mm x 4.6 mm i.d.; Whatman Ltd) that had been pre-washed with 0.1M-ammonium formate solution. The partially purified reaction mixture was then back-eluted onto a reverse phase column (Ultracarb 7 ODS 30; 250 mm x 10 mm i.d.; 5 μ particle size; Phenomenex Ltd) eluted with methanol-0.1M-ammonium formate-triethylamine (80: 20: 0.2 by vol.) at 3.0 mL/min. Eluate was monitored for absorbance at 254 nm and radioactivity. [*Carbonyl*-^{11}C]WAY-100635 (retention time, 16 min) was collected, rotary evaporated under reduced pressure to dryness and formulated in 10 mL (0.9% w/v) saline ready for intravenous injection. Finally, within a particle-free and aseptic environment, the product was filtered through a sterile filter (0.22 μ pore size; Acrodisc; Gelman Ltd) into a sterile septum-sealed vial.

Identification and Routine Analysis of [*carbonyl*-^{11}C]WAY-100635

Product was identified by analytical HPLC using a variety of reverse-phase and normal phase columns, including *i)* a Nucleosil-C18 column (particle size 5 μ; 250 x 3.9 mm i.d.; Technicol Ltd) eluted at 1.4 mL/min with 0.02M-potassium dihydrogen phosphate-acetonitrile (35: 65 v/v) containing *N,N*-dimethyl-octylamine (0.05% v/v) as modifier and adjusted to pH 4.5 with orthophosphoric acid, *ii)* a Symmetry C18 column (particle size 5 μ; 150 x 3.9 mm i.d.; Waters Associates Inc.) eluted at 1.0 mL/min with 0.02M-potassium dihydrogen phosphate-acetonitrile (60: 40 v/v), *iii)* a Ultracarb 5 ODS 30 column (particle size 5 μ, 150 x 3.9 mm i.d.; Phenomenex Ltd) eluted at 1.4 mL/min with 0.02M-potassium dihydrogen phosphate-acetonitrile (60: 40 v/v), *iv)* a μ–Bondapak C18 column (300 x 7.8 mm i.d.; Waters Associates Inc.) eluted with methanol-0.1M-ammonium formate-triethylamine (65: 35: 0.3 by vol.) at 4.5 mL/min, and *v)* a μ–Porasil column (300 x 7.9 mm i.d.; Waters Associates Inc.) eluted with dichloromethane-ethyl acetate-triethylamine acetate (100: 0.1: 0.028 by vol.). Eluates were monitored for absorbance at 254 nm and radioactivity.

Specific radioactivities, and the radiochemical and chemical purities of the formulated radioligand were routinely determined on small samples (100 μL), taken soon after preparation, by analytical HPLC on the Ultracarb 5 ODS 30 column as described above (Figure 4). The analytical method was calibrated by injection of known masses of WAY-100635 and WAY-100634. Peak areas were measured using a Laura integrator (Lablogic Ltd) or Turbochrome integrator (Perkin Elmer Ltd). Some preparations were re-analyzed at up to 2 h after preparation to assess radiochemical stability.

Acknowledgements. The authors are grateful to the MRC for granting a PhD studentship to JAMcC, to Drs Ian A. Cliffe and Allan Fletcher [Wyeth Research (U.K.) Ltd] for the provision of reference materials and to Mrs Diana Bateman and Ms Deborah Fahy for routine quality control measurements.

References

1. Cliffe I.A. *206th National Meeting of the American Chemical Society*, August 22–27, 1993, Chicago, Il, Abstr. MEDI 30, 1993.
2. Fletcher A., Bill D.J., Cliffe I.A., Forster E.A. and Reilly Y.T. *Br. J. Pharmacol.*, **112**, 91P, 1994.
3. Forster E.A., Cliffe I.A., Bill D.J., Dover G.M., Jones D., Reilly Y. and Fletcher A. *Eur J. Pharmacol.*, **281**, 81, 1995.
4. Pike V.W., Hume S.P., Ashworth S., Opacka-Juffry J., McCarron J.A., Cliffe I.A., Fletcher A. and White A.C. *3rd IUPHAR Sat. Meet. Serotonin*, Chicago, 1994, Abstract 73 p. 92.
5. Pike V.W., McCarron J.A., Hume S.P., Ashworth S., Opacka-Juffry J., Osman S., Lammertsma A.A., Poole K.G., Fletcher A., White A.C. and Cliffe I.A. *Med. Chem. Res.*, **5**, 208,1995.
6. Mathis C.A., Simpson N.R., Mahmood K., Kinahan P.E. and Mintun M.A. *Life Sci.*, **55**, 403, 1994.
7. Pike V.W., McCarron J.A, Lammertsma A.A., Hume S.P., Poole K., Grasby P.M., Malizia A., Cliffe I.A., Fletcher A. and Bench C.J. *Eur. J. Pharmacol.*, **283**, R1, 1995.
8. Osman S., Lundkvist C., Pike V.W., Halldin C., McCarron J.A., Hume S.P., Luthra S.K., Bench C.J., Grasby P.M., Swahn C.-G., Wikström H., Barf T., Ginovart N., Farde L., Cliffe I.A. and Fletcher A. *J. Label. Compd. Radiopharm.*, **37**, 283, 1995.
9. Osman S., McCarron J.A., Hume S.P., Ashworth S., Pike V.W., Luthra S.K., Lammertsma A.A., Bench C., Grasby P., Cliffe I.A. and Fletcher A. *Neuroimage*, **2**, S11, 1995.
10. Osman S., Lundkvist C., Pike V.W., Halldin C., McCarron J.A., Swahn C.-G., Ginovart N., Luthra S.K., Bench C.J., Grasby P.M., Wikström H., Barf T., Cliffe I.A., Fletcher A. and Farde L. *Nucl. Med. Biol.*, 1995. In Press.
11. Price J.C., Mathis C.A., Simpson N.R., Mahmood K. and Mintun M.A. *Neuroimage*, **2**, S61, 1995.
12. Pike V.W., McCarron J.A., Lammertsma A.A., Osman S., Bench C.J., Grasby P.M., Cliffe I.A. and Fletcher A. *Eur. J. Pharmacol.* In Press.
13. Lammertsma A.A., Bench C.J., Hume S.P., Osman S., Sargent P., McCarron J.A., Pike V.W. and Grasby P.M. Submitted 1996.
14. Farde L., Halldin C., Pike V.W., Lundkvist C., Ginovart N., McCarron J.A., Swahn C.-G., Osman

S., Cliffe I.A. and Fletcher A. Submitted 1996.

15. Luthra S.K., Pike V.W. and Brady F. *J. Chem. Soc. Chem. Commun.*, 1423, 1985.
16. Luthra S.K., Pike V.W. and Brady F. *Appl. Radiat. Isot.*, **41**, 471, 1990.
17. Yorke J.C., Prenant C. and Crouzel C. *J. Label. Compd. Radiopharm.*, **35**, 262, 1994.
18. Scripko J.G., Huang C.C. and Kilbourn M.R. *J. Label. Compd. Radiopharm.*, **35**, 141, 1994.
19. Pike V.W., Eakins M.N., Allan R.M. and Selwyn A.P. *Int. J. Appl. Radiat. Isot.*, **33**, 505, 1982.
20. Hume S.P., Ashworth S., Opacka-Juffry J., Ahier R.G., Lammertsma A.A., Pike V.W., Cliffe I.A., Fletcher A. and White A.C. *Eur. J. Pharmacol.*, **271**, 515, 1994.