

*Appl. Radiat. Isot.* Vol. 39, No. 5, pp. 441-444, 1988  
*Int. J. Radiat. Appl. Instrum. Part A*  
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 0883-2889/88 \$3.00 + 0.00

## A Captive Solvent Method for Rapid N-<sup>11</sup>C Methylation of Secondary Amides: Application to the Benzodiazepine, 4'-Chlorodiazepam (RO5-4864)

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(Received 12 November 1987)

[<sup>11</sup>C]4'-Chlorodiazepam (RO5-4864), for PET studies of peripheral benzodiazepine receptors, was synthesized by alkylation of 1-desmethyl-4'-chlorodiazepam, in a small volume of acetone adsorbed on acrylic yarn, with [<sup>11</sup>C]methyl iodide in the injection loop of a liquid chromatograph. The reaction mixture was introduced directly onto a small, disposable alumina chromatographic column. Elution with pentane:ethanol gave a product of high chemical and radiochemical purity. A simple heating and cooling device for the injection loop is described.

### Introduction

The radiosynthesis of receptor ligands for PET requires preparative procedures that take place on a scale that has traditionally been associated with microanalytical chemistry. Accordingly, it is often possible to purify a radiopharmaceutical for clinical studies by HPLC while simultaneously determining the chemical and radiochemical purity, and specific activity of the product. A natural extension of this is to make use of the variety of commercially available devices for automatic sample handling in HPLC for the remote handling of radioactive material at all steps in a radiosynthesis. In the system described below, N-[<sup>11</sup>C]methylation of secondary amides with [<sup>11</sup>C]methyl iodide is performed in the injection loop of a liquid chromatograph. The products of the reaction are then transferred directly onto a column for final purification. Modifications of this method should be generally applicable to secondary amines as well as amides, which together include a large number of radiopharmaceuticals of interest for PET.

We have selected the peripheral benzodiazepine receptor ligand, 4'-chlorodiazepam (Fig. 1; 7-chloro-5-[4-chlorophenyl]-1,3-dihydro-1-methyl-2H-1,4-benzodiazepin-2-one; RO5-4864; Weissman *et al.*, 1983) to demonstrate the utility of the method. The synthesis of 1-[<sup>11</sup>C]4'-chlorodiazepam from [<sup>11</sup>C]methyl iodide has been reported (Turton *et al.*, 1984). Its potential for imaging regional cerebral gliosis by PET was suggested.

Three innovations contribute to the simplicity and general applicability of this system for N-alkylation with [<sup>11</sup>C]methyl iodide. Firstly, a captive solvent method (Jewett *et al.*, 1985) allows trapping and subsequent reaction of

[<sup>11</sup>C]methyl iodide in only 75 μL of acetone in the injection loop of a liquid chromatograph. This volume of solvent can be introduced directly onto the chromatographic column without significant loss of resolution. Secondly, a simple device is used for the rapid cooling and heating of the injection loop over the range of -60 to 220°C to permit trapping of the [<sup>11</sup>C]methyl iodide at a low temperature and subsequent pressurization and heating of the reactants at any desired temperature. While the pressures during the reaction and chromatography in the present method do not exceed 4 atm, the approach is a general one, which will allow reactions under pressure and subsequent purification on an HPLC column where necessary. Thirdly, low pressure chromatography on small alumina columns with a pentane-ethanol mixture permits rapid, efficient separation of <sup>11</sup>C-labeled radiopharmaceuticals from their secondary amide or amine precursors. The solvent is non-toxic and can be removed simultaneously with elution of the product from the column by simply warming in a stream of N<sub>2</sub>.

### Experimental

#### Teflon reaction loop

Details of the construction of the reaction loop are shown in Fig. 2. A 40 mm length (24 mg) of white acrylic yarn (4-ply knitting worsted; Bernat Yarn Co., Inc.) is drawn into the Teflon tube (4.76 mm o.d. × 3.18 mm i.d. × 40 mm long). Forcing the end-fittings into the tube compresses the yarn to 20 mm. The end-fittings are made by pulling 1.59 mm o.d. Teflon tubing snugly into a 20 mm length of 3.18 mm o.d. × 1.59 mm i.d. Teflon tubing. The fittings do not leak at a nitrogen pressure of 4 atm, however they must be restrained from being pushed out by the gas pressure. Using temporary end-fittings, approximately 200 μL of MeOH containing 3% NaOH is passed through the tube containing the yarn. A slow flow of N<sub>2</sub> is passed through the tube for 5 min to force out the excess solution and evaporate the MeOH. This leaves the yarn impregnated with a small amount of NaOH, necessary as a base in the alkylation reaction. The tubes are sealed and stored at -20°C.

Just before the radiosynthesis a tube prepared as described above is warmed to room temperature. A solution of 1 mg of the secondary amide precursor in 75 μL of 95% aqueous acetone is pipetted onto the inlet side of the yarn in the tube, which is then installed in the apparatus. The injection valve is kept in the closed position, and the loop is maintained at about -50°C by occasional pulses of liquid CO<sub>2</sub> to the cooling device.

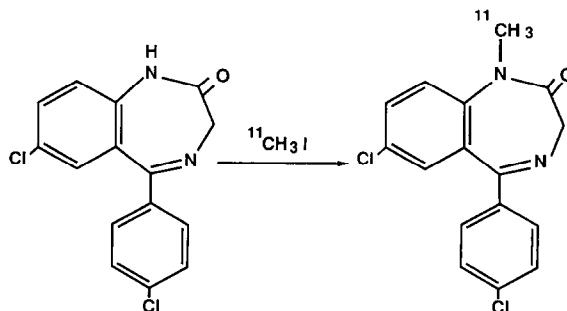


Fig. 1. Reaction scheme for the alkylation of 1-desmethyl-4'-chlorodiazepam with [<sup>11</sup>C]methyl iodide to give [<sup>11</sup>C]4'-chlorodiazepam.

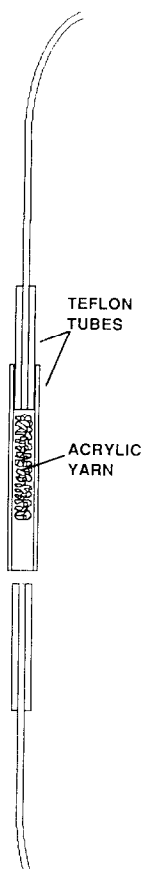


Fig. 2. Schematic diagram of the Teflon reaction loop showing one end-fitting removed.

#### Disposable alumina columns for chromatography

Neutral alumina (J. T. Baker) is washed several times by decantation with water to remove all fines, filtered, washed with 95% ethanol and dried for 10 h at 140°C. The Teflon column (4.76 mm o.d.  $\times$  3.18 mm i.d.  $\times$  165 mm long) is dry-packed with 1.1 g of alumina. Small plugs of polyester fiber retain the packing at both ends. The end fittings are the same as those used for the reaction loop above. A new packing is used for each synthesis.

#### Device for cooling and heating the reaction loop

A schematic diagram of the device for cooling and heating the reaction loop is shown in Fig. 3. The Teflon reaction loop passes through an aluminum spool on which are wound 56 m (12 ohm) of 28 AWG polyimide insulated magnet wire (Hudson Wire Co.). A close fit between the Teflon and aluminum facilitates heat transfer. A heavy-walled Teflon tube (3.18 mm o.d.  $\times$  1.59 mm i.d.) passes along the length of the spool beneath the winding. This tube is perforated with 20 holes made with a 28 gauge needle. A thermistor is embedded in the wire winding.

For cooling, brief pulses of liquid CO<sub>2</sub> are admitted to the Teflon tube. The wire winding acts as a heat exchanger and a matrix for the expansion of the CO<sub>2</sub>. The desired degree of cooling is indicated by the resistance of the thermistor. For heating, brief pulses of 115 VAC are applied to the wire winding. Cooling of the device to -50°C and subsequent heating to 60°C or higher require only a few seconds. The contents of the reaction loop itself require a little longer time to reach thermal equilibrium with the aluminum spool. A temperature controller (Model 70A, RFL Industries, Inc.) has been used, but is slower than remote manual operation.

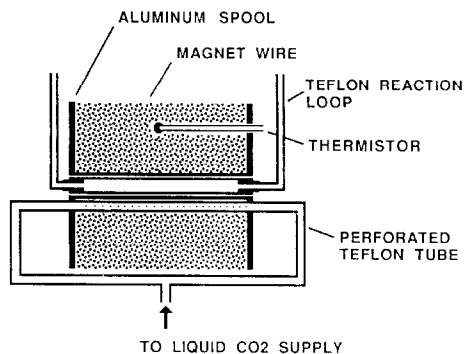


Fig. 3. Schematic diagram of the device for heating and cooling the reaction loop.

#### Coaxial ionization detectors

Small coaxial ionization detectors similar to those described by Sipilä *et al.* (1985) are installed at two points in the system (Fig. 4) to monitor transfer of [<sup>14</sup>C]methyl iodide to the reaction loop and to measure the radioactivity in the effluent from the chromatographic column. The detectors are shielded internally with lead to reduce the background from the large amounts of radioactivity in the hot cell. The amplified signals from the detectors are recorded continuously during the synthesis.

#### Radiosynthesis

The apparatus for the alkylation reaction is shown schematically in Fig. 4. [<sup>14</sup>C]Methyl iodide, specific activity greater than 1000 Ci/mmol, produced by a method similar to that of Marazano *et al.* (1977), is passed in a stream of N<sub>2</sub> at 40 mL/min through a short column of soda lime to remove traces of HI, and then through the reaction loop maintained at about -50°C. About 95% of the methyl iodide is trapped. The loop is then sealed off by rotation of the injection valve (Model HVP-4-4, Hamilton) and pressurized with N<sub>2</sub> at 4 atm to retard evaporation of the acetone and methyl iodide during heating. The tube is rapidly heated to 60°C and maintained at that temperature for 5 min. The reaction loop is cooled to room temperature by a brief pulse of liquid CO<sub>2</sub>, and nitrogen pressurization is stopped. The injection valve is again rotated and elution with 98:2 pentane:ethanol (1.2 mL/min) is begun. The effluent from the chromatographic column is monitored for radioactivity (Fig. 5). Unreacted [<sup>14</sup>C]methyl iodide elutes in 1.5 min followed by [<sup>14</sup>C]4'-chlorodiazepam at 4 min. When the product begins to elute, the effluent flow is temporarily switched to pass through a sterilized filter (ACRO LC13, fluoropolymer membrane, Gelman Instrument Co.) into a sterile 5 mL septum-stoppered vial in an aluminum heating block maintained at 70°C. A flow of filtered N<sub>2</sub> at 200 mL/min is maintained in and out of the vial during collection of the product to evaporate the solvent. After evaporation of the pentane, physiological saline containing 5% ethanol is added to the vial, which is then sonicated to dissolve the product.

#### Analytical systems

The radiochemical and chemical purity of the product are determined by two TLC systems and one HPLC system:

**TLC system I.** Silica gel-coated plastic sheets (Kieselgel 60 F<sub>254</sub>, E. Merck), developed with 1:1:1 dichloro-methane:ethyl acetate:hexane. *R<sub>f</sub>* of 4'-chlorodiazepam is 0.26; *R<sub>f</sub>* of 1-desmethyl-4'-chlorodiazepam is 0.16.

**TLC system II.** Neutral alumina-coated plastic sheets (Aluminum oxide 60 F<sub>254</sub>, type E, E. Merck), developed with chloroform stabilized with 0.75% ethanol. 4'-Chlorodiazepam (*R<sub>f</sub>* 0.54) elutes well ahead of the 1-desmethyl

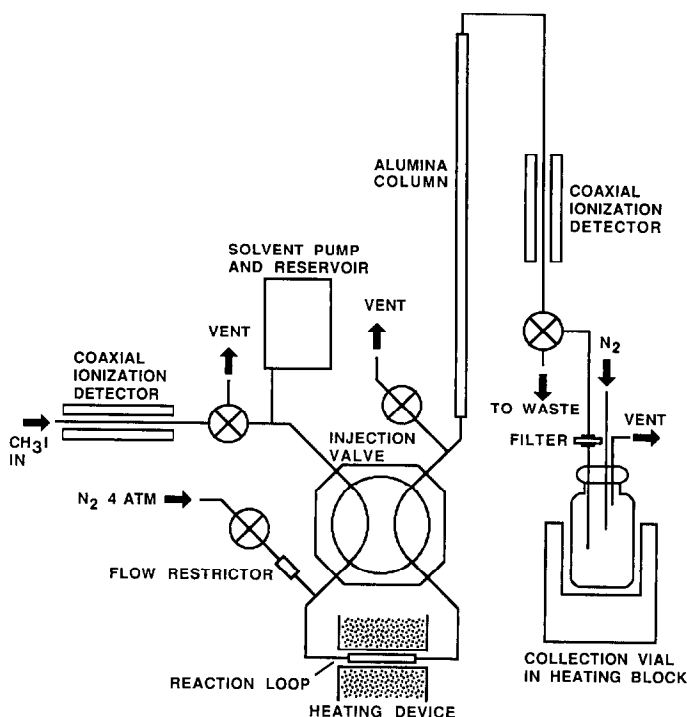


Fig. 4. Schematic diagram of the apparatus for the synthesis of [ $^{11}\text{C}$ ]4'-chlorodiazepam.

precursor ( $R_f$  0.14). Compounds are detected under short-wave u.v. light. The radioactive compounds on the TLC plates are detected by a scanning ionization detector (Berthold, Model LB 2820).

**HPLC.** A C-8 reverse-phase column (150  $\times$  4.6 mm, 5 micron, Maxsil 5C8, Phenomenex) is eluted with 60:40 acetonitrile:water, 1 mL/min. 4'-Chlorodiazepam ( $R_f$  5.53 min) elutes after the desmethyl precursor ( $R_f$  4.27 min). The compounds are detected quantitatively by u.v. absorbance at 238 nm (Model V<sup>4</sup>, ISCO), and then by a flow through NaI(Tl) scintillation detector (Model 170, Beckman Instruments). The radioactive peak corresponding to [ $^{11}\text{C}$ ]4'-chlorodiazepam is collected and the radioactivity measured in a dose calibrator or NaI(Tl) well counter.

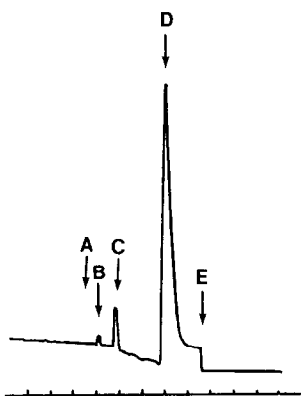


Fig. 5. Response of the coaxial ionization detector to the effluent from the alumina column during purification of [ $^{11}\text{C}$ ]4'-chlorodiazepam. Each division = 1 min. A: reaction mixture transferred to column. B: unidentified radioactive material. C: unreacted [ $^{11}\text{C}$ ]methyl iodide. D: [ $^{11}\text{C}$ ]4'-chlorodiazepam. E: drop in base-line when vial containing the product was removed from the apparatus.

## Results and Discussion

The time from the completion of trapping of [ $^{11}\text{C}$ ]methyl iodide in the reaction loop until delivery of the formulated radiopharmaceutical was approximately 15 min. About 180 mCi of [ $^{11}\text{C}$ ]4'-chlorodiazepam were produced from about 400 mCi of [ $^{11}\text{C}$ ]methyl iodide. Refurbishing the apparatus, including the system for synthesis of [ $^{11}\text{C}$ ]methyl iodide, for another radiosynthesis required about 10 min.

The radiochemical purity of the product was greater than 99% by HPLC (Fig. 6) and greater than 96% in both TLC systems. No u.v.-absorbing chemical contaminants could be detected in the product by HPLC (1% level), nor by either TLC system. Specific activity of the product was 500–1000 Ci/mmol at the completion of formulation for injection, a significant improvement over that reported previously (Turton *et al.*, 1984).

Of particular note is the absence of any detectable desmethyl precursor in the product. Neutral or basic alumina selectively retains secondary amides or amines vs the N-methylated analogs. This permits the use of short, disposable alumina columns to achieve complete separation of the desired radiopharmaceutical from the precursor and allows use of a relatively large amount of the precursor, facilitating efficient use of the radiolabel. The radiolabeled product elutes from the column before the precursor, allowing rapid purification. The absence of contamination of the product by the precursor is particularly important for ligands of high specific activity for quantitative receptor studies, since the precursors themselves frequently have a high affinity for the receptors.

The advantages of captive solvent techniques for rapid radiosynthesis have been described (Jewett *et al.*, 1985). The method has been applied recently to advantage for the synthesis of [ $^{18}\text{O}$ ]butanol (Takahashi *et al.*, 1986). It offers a general way to accomplish radiolabeling reactions in chromatographic injection loops, by permitting efficient trapping of a reactant from the gas phase in the presence of a small volume of highly dispersed substrate and solvent. The variety of synthetic fibers and microporous particles

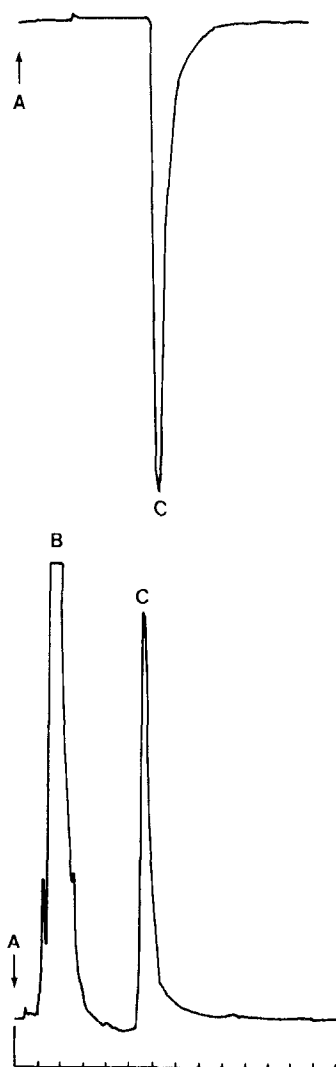


Fig. 6. Response of u.v. (lower trace) and radiochemical (upper trace) detectors in the analytical HPLC of [ $^{11}\text{C}$ ]4-chlorodiazepam formulated for injection. A: inject. B: u.v.-absorbing components of saline solution. C: [ $^{11}\text{C}$ ]4-chlorodiazepam.

available can be adapted to a wide range of reaction conditions. Use of acrylic fiber in the form of a commercially available yarn is inexpensive and convenient. There is no need to use a frit or filter as in the case of a porous

polymer or bonded silica. Impregnation of the fiber with a base such as sodium hydroxide or a quaternary ammonium salt provides a microdispersed aqueous phase, allowing phase-transfer catalysis without mechanical stirring.

The direct transfer of the reaction mixture to the column without removal of solvent has the additional advantage of allowing the unreacted [ $^{11}\text{C}$ ]methyl iodide to be detected quantitatively during the separation (Fig. 5). This should permit rapid optimization of conditions for N-[ $^{11}\text{C}$ ]methylations involving other substrates, solvents and bases.

The ability to carry out the N-alkylation reaction in a chromatographic injection loop results both in mechanical simplicity and miniaturization. The apparatus of Fig. 4 is a single unit with ten 115 VAC control lines, two fluid lines and two coaxial cables for the detectors. Installation or removal from the hot cell require only a minute. This is due in large part to the simplicity of the device for heating and cooling the reaction loop, modifications of which should be applicable to a variety of remote radiolabeling situations.

**Acknowledgements**—This work was supported by Grant PO1 NS 15655 awarded by NINCDS and by Grant 5 T32 CA09015-11 awarded by the National Cancer Institute.

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