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TECHNICAL NOTE

Radiotracer Synthesis from [^{11}C]-Iodomethane: A Remarkably Simple Captive Solvent Method

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ABSTRACT. A new method of [^{11}C]-methylation is described, which attains the goals of simplicity, high radiochemical yields, speed, versatility, and automation. A standard high performance liquid chromatography (HPLC) injection loop on a standard HPLC injection valve is loaded with a solution (80 μL) of precursor (0.3–1.0 mg) in dimethyl formamide (DMF) or dimethyl sulfoxide (DMSO) (+ base if required). At ambient temperature [^{11}C]-iodomethane is passed through the loop for 3–4 min with >90% trapping of activity. After a further 1–5 min, the contents of the loop are quantitatively injected onto the HPLC column for purification. Radiochemical yields are equal to or superior to conventional solution methods in all cases, even though no heat is applied. [^{11}C]-labeled radiotracers that have been prepared by this method for human or animal studies include Raclopride, N-methylspiperone, Ro 15-1788, FLB 457, RTI-32, Rolipram, SCH 23390, and SKF 82957. Since no vials, transfer lines, cooling, heating, or sealing valves are required, no transfer losses occur, yields are high, and cleanup is minimal, this "loop method" is ideal for most radiopharmaceuticals prepared from [^{11}C]-iodomethane. NUCL MED BIOL 27;6:529–532, 2000. **© 2000 Elsevier Science Inc. All rights reserved.**

KEY WORDS: Positron emission tomography, Radiosynthesis, Methylation, Carbon-11, Iodomethane

INTRODUCTION

Methylation of suitable precursors with [^{11}C]-iodomethane continues to be the workhorse method of synthesis of a majority of positron emission tomography (PET) radiotracers, especially in the area of receptor imaging. Many improvements have been made in the production of [^{11}C]-iodomethane, most recently with the introduction of "gas-phase methods," which are highly automated and produce a product with very high specific activity (1, 9, 10). Efforts to simplify and automate the [^{11}C]-methylation reaction and purification have been less spectacular. Many attempts to streamline the process have revolved around the idea of eliminating the traditional "solution in reaction vial" whereby [^{11}C]-iodomethane is distilled into a vessel containing solvent, precursor, and base/catalyst if required (1, 8, 11). In this common scenario, cooling of the vessel for trapping [^{11}C]-iodomethane is usually required, followed by sealing of the vessel, heating to effect reaction, quenching of the reaction, and transferring of the vessel contents to a high performance liquid chromatography (HPLC) system for purification. The streamlining and automation of this sequence of events, and especially the last, have proven onerous.

An attractive technique to surmount some of these problems was described in the pioneering papers of Jewett and Watkins on captive solvent chemistry (4, 6, 17). Their goal was to develop a solid support to trap reagents and iodomethane together, eliminating the need for a reaction vessel with its septa and needles and their associated problems. Similar efforts have been forthcoming from other groups, where the technique has been named on-line, solid-phase, or immobilized techniques (3, 14, 16, 19). When the solid support is plumbed in to take the place of the HPLC sample loading loop, then transfer losses from reaction vessel to loop can also be eliminated and the process simplified.

Such captive solvent techniques have not been widely adopted in the field, perhaps because no one method fulfills all the requirements of ease of use, reproducibility, and versatility. Some methods have not been fully integrated as part of the HPLC system (17, 19) and some require nonproprietary solid supports (6, 14), while others can degrade the HPLC purification of the radiotracer (3). In addition, cooling and heating the reaction site are still necessary steps in the synthetic sequence. We report here a new method that attains the goals of simplicity, high radiochemical yields, speed, versatility, and ease of automation. Without using any additional solid support and with no heating or cooling, [^{11}C]-iodomethane is trapped directly in a standard HPLC loop coated with precursor solution, reacted, and directly injected onto the HPLC purification column.

METHODS

Precursors (normethyl) for [^{11}C]-methylation were synthesized in house (RTI-32, rolipram, FLB 457, DASB) or commercially available (SCH 23390, NMS, SKF 82957). Raclopride precursor was a gift from Astra Arcus AB (Sodertalje, Sweden) and Ro 15-1788 precursor was a gift from Hoffman-LaRoche (NJ, USA).

General Method for Performing [^{11}C]-Methylations in an HPLC Injection Loop

SETUP. A commercial HPLC injection valve (Valco #AC6W) is equipped with a commercial stainless steel loop (2 mL, Valco #SL2KCW) and a commercial injection port (Valco #VISF-2). A

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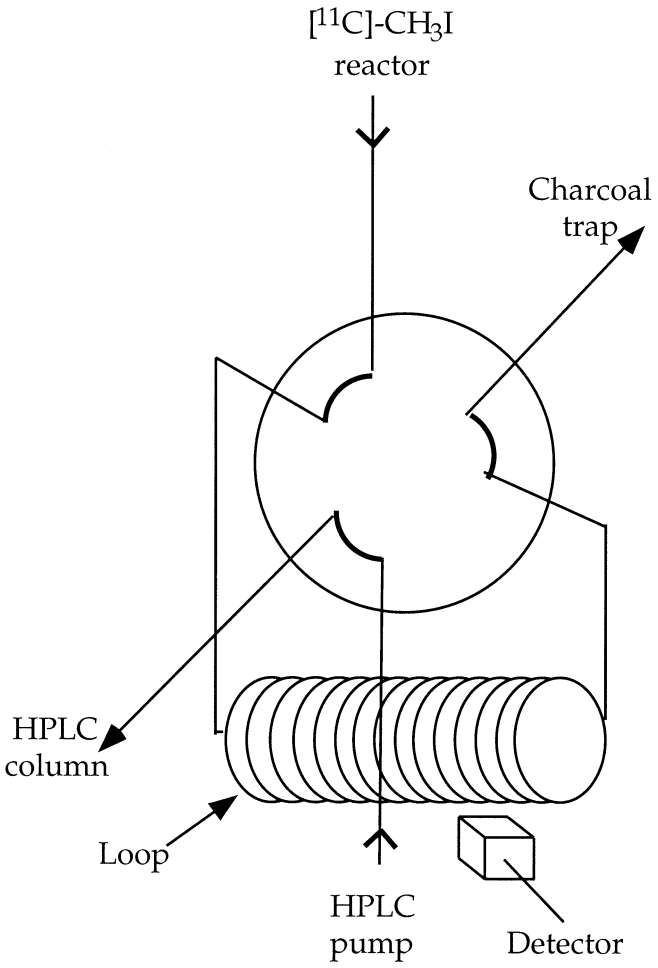

FIG. 1. Apparatus and flow diagram for trapping [^{11}C]-iodomethane in an HPLC loop coated with precursor solution.

charcoal trap is connected to the waste port of the HPLC valve and placed in a well counter to measure untrapped [^{11}C]-iodomethane (Fig. 1). With the valve in the LOAD position, a solution of precursor, solvent, and base (if required; total volume: 80 μL) is injected slowly (5–10 s) into the clean, dry injection loop using a 100 μL gas-tight syringe (Hamilton #1810). The injection port is replaced by the [^{11}C]-iodomethane transfer line, which has a conditioning flow of 8 mL/min N_2 gas passing through it. The loading of the loop with precursor solution can be made at least up to 10 min before end of bombardment (EOB) and no solvent emerges from the waste port. See Table 1 for specific reaction conditions.

TRAPPING AND REACTION OF [^{11}C]-IODOMETHANE. [^{11}C]-iodomethane [produced as previously described (18)] is swept into the HPLC loop coated with precursor solution by a stream of N_2 gas (8 mL/min) at ambient temperature. Radioactivity trapped on the loop is detected by a proximal radiation detector. When activity peaks in the loop (3–4 min), the flow of N_2 is stopped and the reaction allowed to proceed (1–5 min). The contents of the loop are then quantitatively injected onto the HPLC purification column by simply changing the position of the injection valve to INJECT.

CLEANUP. The loop has already been cleaned by the HPLC eluent. Preparation for its next run simply involves switching the valve back to the LOAD position, passing a few mL of ethanol (or water if mobile phase contains phosphate buffer) and acetone through the loop, and blowing dry.

Preparation of Raclopride Precursor (Free Base)

Raclopride precursor (HBr salt, 25.0 mg) was dissolved in 2 mL of methanol and 1 mL of water. Aqueous sodium bicarbonate (1 N, 1.5 mL) was added and the clear solution was evaporated under vacuum to remove the methanol (no heating). The off-white precipitate was collected by filtration under vacuum and dried in a vacuum desiccator (17.87 mg, 89% yield).

TABLE 1. Results of using the "Loop Method" of Radiosynthesis for a Variety of Radiotracers

Compound	Yield (GBq)[a]	Specific activity (GBq/μmole)	Base (μL)	Reaction time (min)	Precursor (mg)
RTI-32	11.1	89	4[b]	1	0.9
SCH 23390	8.4	51	10[c]	5	1.0[d]
FLB 457	4.4	97	2[e]	5	1.2
NMS[f]	10.7	93	2.5[e]	2	1.0
Raclopride	5.1	48	3[g]	5	1.1[h]
Ro 15-1788	13.8	98	4[b]	2	0.6
Rolipram	8.1	81	3[i]	3	0.3
SKF 82957	8.1	84	10[c]	5	1.0[j]
DASB[k]	8.7	57	n/a	5	1.0

All reactions were carried out in DMF except raclopride (DMSO). Solvent and base volume totaled 80 μL. Reported values are for a minimum of three runs for each radiotracer.
[a] Yields are for final formulated product, uncorrected for decay and normalized for a 15-min (40 μA) bombardment that produces 40 GBq of [^{11}C]-CO_2.
[b] Tetrabutylammonium hydroxide (0.5 N in methanol).
[c] Aqueous $NaHCO_3$ (1 N).
[d] HCL salt.
[e] Tetrabutylammonium hydroxide (1 N in methanol).
[f] N-methylspiperone.
[g] 5 N aq. NaOH.
[h] Free base.
[i] Tetrabutylammonium hydroxide (0.17 N in methanol).
[j] HBr salt.
[k] [^{11}C]-3-amino-4-(2-dimethylaminomethyl-phenylsulfanyl)-benzonitrile.

RESULTS AND DISCUSSION

The "loop method" described above, which consists of trapping and reacting [^{11}C]-iodomethane directly in the injection loop of the HPLC valve, has been applied to the synthesis of a variety of PET radiopharmaceuticals (Table 1). In all cases the method was successful, with results equal to or superior those using conventional solutions in a vial method. This includes parameters of success such as trapping of [^{11}C]-iodomethane, radiochemical yield, time of synthesis, purity of final product, and specific activity. The one initial exception was [^{11}C]-raclopride, where radiochemical yields were very poor (<10%) when the HBr salt of the precursor was employed. The major product in this case was [^{11}C]-bromomethane (identified by HPLC), which was not reactive enough at ambient temperature for efficient incorporation into product. Using the free base of the precursor completely eliminated this problem. A similar observation in the synthesis of [^{11}C]-raclopride from [^{11}C]-labeled methyl triflate and the HBr salt of the precursor was recently reported (7).

One reported disadvantage of using a solid support as a means to bring together precursor and [^{11}C]-iodomethane is that the reaction mixture is strongly retained by the support, with the result that HPLC separations are less efficient (3). Using only the loop as a support avoids this pitfall, and no differences in chromatographic separations could be discerned as compared to injections of solutions of reaction mixtures.

Two things are surprising about these results at first sight: (1) the efficiency with which [^{11}C]-iodomethane is trapped without cooling or the use of a solid support to increase surface area, and (2) the speed of the methylations without the aid of heat. Trapping in the loop is not simply a consequence of reaction of [^{11}C]-iodomethane with precursor. Experiments showed that loading the loop with DMF (70 μL) with no added base or precursor also resulted in >90% trapping efficiency of the [^{11}C]-iodomethane. The volume of applied solution was designed (by inspection) to coat the entire loop surface; larger volumes resulted in breakthrough of solvent upon purging the loop for several minutes with N_2 gas. Assuming that the entire internal surface of the loop (internal radius of 0.5 mm) is coated, the surface area of the film is 59 cm^2 with a film thickness of 20 μm, a value that is not that much greater than the films used in thick film gas chromatography columns. Breakthrough of [^{11}C]-iodomethane (1–8%) using the loop method was often less than that found using our standard solution conditions (−20°C, 200 μL DMF, 1 mL V-vial, 8 mL/min). The situation is akin to the reported efficient trapping of [^{11}C]CO_2 by a thin film of Grignard reagent supported on thin plastic tubing (13).

Another rather surprising result was the fast rates of reaction of [^{11}C]-iodomethane with precursor at ambient temperature when literature reports call for heating, often to 90°C for 5 min (12). Under the conditions described in Table 1, unreacted [^{11}C]-iodomethane accounted for less than 10% of total activity by HPLC (*i.e.*, reactions were at least 90% complete). A number of factors may contribute to the faster than expected reaction rates. The solvent volume is about one third that normally employed in solution, and thus the concentration of precursor is increased by a factor of three (for the same mass of precursor). Often thick-walled reaction vessels, cooled to −20°C or below, are used for [^{11}C]-iodomethane solution chemistry, and the time required to transfer heat to the reaction mixture is significant compared to the total reaction time employed. We found that upon immersion into an oil bath at 90°C, a 1 mL V-vial containing 250 μL of dimethyl formamide (DMF) that had been cooled to −40°C took 60 s to reach 32°C and 120 sec to reach 61°C. Finally, iodomethane is inherently a very reactive alkylating agent (15), and perhaps the field tends to use longer reaction times than strictly necessary when the decay rate of ^{11}C is considered (2).

The versatility of the method is apparent upon examination of the variety of radiotracers synthesized (Table 1). A number of functional groups have been methylated with [^{11}C]-iodomethane by this method, including phenols, acids, amides, and 2° amines. Since no vials, transfer lines, cooling, heating, or sealing valves are required, no transfer losses occur, yields are high, and cleanup is minimal, this method is ideal for most radiotracers prepared using [^{11}C]-iodomethane. At our PET Centre all [^{11}C]-methylations for human studies, animal studies, or chemistry research are now performed using this "loop method." A few limitations bear mentioning, however. We have not examined trapping at higher N_2 sweep gas flow rates and would presume that this would result in lower trapping efficiencies. Only DMF and dimethyl sulfoxide (DMSO) have been tried as reaction solvents, and all HPLC purifications were done under reverse-phase conditions. Finally, it would be interesting to investigate the method with [^{11}C]-labeled methyl triflate (5), which has even lower volatility and higher reactivity than [^{11}C]-iodomethane.

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