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High efficiency preparation of L-[*S*-methyl-^{11}C]methionine by on-column [^{11}C]methylation on C18 Sep-Pak

Claudio Pascali[a], Anna Bogni[a], Ren Iwata[b], Donatella Decise[a],

Flavio Crippa[a], and Emilio Bombardieri[a, *]

[a]National Cancer Institute, Nuclear Medicine Department – V. Venezian,1 – 20133 Milan, Italy

[b]CYRIC, Tohoku University – Aramaki, Aoba-ku, Sendai 980-77, Japan

Summary

A novel approach to the synthesis of L-[*S*-methyl-^{11}C]methionine ([^{11}C]MET) is described which involves a commercial C18 Sep-Pak Plus as a solid-phase support material for the [^{11}C]methylation step. The present method, which uses [^{11}C]CH_3I produced by the classical $LiAlH_4$/HI route, supplies [^{11}C]MET ready for injection within 11 min from the end of bombardment (EOB) with a radiochemical yield, decay corrected at EOB, of 78% and a radiochemical purity at the end of synthesis (EOS) higher than 99%.

The required setup is extremely simple and easy to automate and can be reset for a further synthesis within few minutes. Moreover, due to its versatility, the method can be utilized for the production of other radiopharmaceuticals requiring a simple [^{11}C]methylation.

Key words: [^{11}C]methionine, PET, on-column reaction

Introduction

[^{11}C]Methionine is a useful tracer for tumor imaging with PET: its uptake in brain, head (1), neck, lung (2) and breast tumors (3), as well as lymphomas (4), makes it an attractive alternative or

*Author for correspondence. Ph. +39-02-2390220, FAX +39-02-2367874

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complementary radiopharmaceutical to [^{18}F]FDG. The focus of some PET Centers on tumor studies results in a high clinical demand for the routine synthesis of this radiopharmaceutical. That in turn places a heavy burden on the laboratory because of the need for repeated batch productions during the day. As an alternative, we have looked for a reliable and simple to perform synthesis able to yield enough [^{11}C]MET to fill the daily requirements.

On-column [^{11}C]methylation on a normethyl precursor using [^{11}C]CH_3I is probably the most convenient way to achieve this since it has many advantageous features, such as small amounts of reagents, short reaction time and easy applicability to an automated system. This approach had been successfully applied to the preparation of several radiopharmaceuticals (5,6) as well as [^{11}C]MET itself (7). The latter synthesis utilized a guard column filled with a mixture of PorapakQ-FlusinT on which L-homocysteine thiolactone had been previously adsorbed. After trapping of [^{11}C]CH_3I at –40 °C on PorapakQ, a solution of sodium hydroxide in aqueous ethanol was loaded and the temperature raised to 80 °C in order the [^{11}C]methylation to proceed.

Using this work as starting point we tried to further simplify the setup and to reduce the preparation time by a) replacing the elaborated mixture of resins-precursor with a commercial C18 Sep-Pak pre-loaded with the reagents, and b) performing the [^{11}C]CH_3I trapping and alkylation steps at room temperature.

Experimental

Materials and methods

Reagents and solvents were used as purchased without further purification, unless specified. D-Methionine was obtained from Sigma and L-methionine from BDH. NaH_2PO_4 and H_3PO_4 were from Aldrich. Absolute EtOH was purchased from Carlo Erba. L-Homocysteine thiolactone hydrochloride, L-proline, $Cu(OAc)_2$ and NaOAc were obtained from Fluka. Sep-Pak C18 Plus cartridges were obtained from Waters, 10 mL sterile vials from Sorin and Millex-GS 0.22 μm filter units from Millipore.

HI (57%, Merck) was purified by distillation under nitrogen in the dark and then split into 0.8 mL aliquots for single use and stored in the dark in 1 mL microvials (Wheaton) at 4 °C. 0.05M $LiAlH_4$ in

dry THF was prepared by diluting in a glove-box under nitrogen atmosphere a 1M solution (Sure-Seal bottle, Aldrich) with dry THF (Sure-Seal bottle, Aldrich) and storing it, for single use, in amber-colored 5 mL microvials at 4 °C. The HI and $LiAlH_4$ solutions were used up to 3 months after their preparation without any problem.

[^{11}C]Carbon dioxide was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction (17 MeV, 40 μA) on a nitrogen + 1% oxygen mixture (N60 and N55 purity grade, respectively; Air Liquide). [^{11}C]Carbon dioxide was then converted into [^{11}C]CH_3I according to the classical reduction with $LiAlH_4$/HI followed by a purification on a column filled with Sicapent-Ascarite to remove any [^{11}C]CH_3OH and unreacted [^{11}C]CO_2.

Synthesis and quality control

Figure 1 shows a schematic diagram of the automated system for the [^{11}C]methylation of L-homocysteine thiolactone hydrochloride and the annexed remote-controlled apparatus for the withdrawal and injection of an aliquot of final solution into an HPLC system for quality control.

For routine production the following conditions were used. L-homocysteine thiolactone hydrochloride (15.4 mg) was freshly dissolved in a stock solution of 0.5M NaOH in EtOH-H_2O 50:50 (1 mL) and a 210 μL aliquot loaded onto the cartridge by syringe just a few minutes before releasing the target charge to the apparatus.

The distilled [^{11}C]CH_3I was directed to the loaded cartridge using a nitrogen flow (15 mL/min, 170 s) and the fraction breacking through was trapped in a charcoal trap. Typically, this step was completed within 8 min from the end of bombardment (EOB)*, after which the Sep-Pak Plus was eluted with 0.05M NaH_2PO_4 (5.8 mL). This solution was then eluted through a further C18 Sep-Pak Plus and a 0.22 μm sterile filter and collected into a vented sterile vial containing 4.2 mL of saline. Then, nitrogen was bubbled for one minute using an additional Teflon tubing to homogenize the final solution and help remove any residual volatile impurity. The final pH was 6-7.

The duration of synthesis including the one-minute nitrogen bubbling was 11 min from EOB and the radiochemical yield from [^{11}C]CO_2, decay corrected at EOB, *ca.* 78% (Table 1), which corresponds to *ca.* 86% from [^{11}C]CH_3I.

* This time includes 1.5 minutes needed for the cryogenic trapping of [^{11}C]CO_2 when the target is emptied and a further 1 minute for its release to the apparatus into the hot-cell.

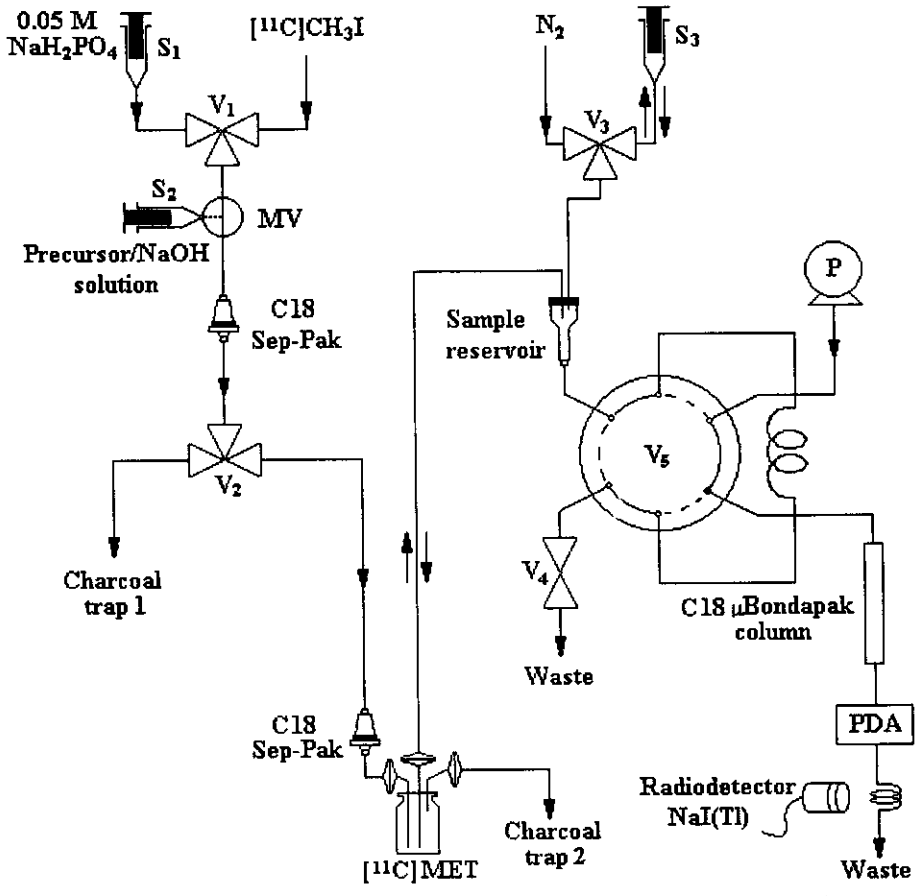

Figure 1. Diagram of the automated system for the production of L-[*S*-methyl-^{11}C]methionine ([^{11}C]MET). Also depicted is the remote-controlled apparatus for quality control by HPLC. **MV**: 3-way manual valve. **V1-V3**: 3-way slider valve (Rheodyne 5301) with pneumatic actuator (Rheodyne 5300). **V4**: 2-way solenoid valve (Series 9, General Valve). **V5**: pneumatically-operated 6-way PEEK injector valve (Rheodyne 9010P). **S1**: motorized syringe. **S2-S3**: manually-operated syringe.

During each routine production the identity of the reaction product was confirmed by withdrawing an aliquot of sample in a remote-controlled fashion (8) into a sample reservoir using the tube previously employed for nitrogen bubbling. The sample was pushed from the reservoir into an HPLC-loop and injected into a C18 μBondapak column (3.9x300 mm, Waters) eluted with 3mM NaH_2PO_4 adjusted at pH 3.1 with H_3PO_4. The elution process was monitored using a UV/VIS photodiode array detector, (PDA type SPD-MDAvp, Shimadzu) in series with a radioactivity detector (NaI(Tl)/PMT, type FC-002 Flow-Count, Bioscan). Tipically, the radiochemical purity found was 99.5%. The enantiomeric

purity of L-[*S*-methyl-^{11}C]methionine was assessed by radio-HPLC according to literature (9) using a reversed-phase column and a chiral mobile phase (30mM NaOAc / 17mM L-proline / 8 mM $Cu(OAc)_2$). Under the reaction conditions described above the percentage of L-form was 89%.

Results and discussion

The previously reported radiosynthesis of [^{11}C]MET through solid-supported [^{11}C]methylation on a mixture of precursor/FlusinT/PorapakQ (7) had several disadvantages, including:

- the need for cooling at –40 °C during the trapping phase and the small leaks at which the reaction column was prone at this temperature;
- the tedious preparation and subsequent loading operation of the resins;
- the variability in outcomes resulting both from slight changes in the packing composition and the lack of homogeneity inside the reaction column (the two resins had a different granulometry).

Recently, this system was made somewhat more efficient by adsorbing also NaOH on FlusinT[#]. However, this solution had the drawback of further worsening the problems related to the preparation and packing of the resin mixture.

The use of C18 Sep-Pak as a solid-phase support material for [^{11}C]methylation has already been successfully reported for the synthesis of [^{11}C]WAY 100635 (10). We decided to test the applicability of this method to the preparation of [^{11}C]MET because of its simplicity, since neither cooling for efficient trapping of [^{11}C]CH_3I nor heating to effect the following reaction are required. In addition, the cartridge is commercially available, requiring no packing, which enhances the reproducibility of the synthesis.

Table 1 shows the alkylation yields as well as the percentages of activity retained on the major components.

In agreement with what was previously reported by Wilson *et al.* (10) trapping of [^{11}C]CH_3I on the cartridge was about 92%, with greater breakthrough being observed when higher flow rates were

[#] The NaOH/EtOH/H_2O solution was particularly affected by the loading speed and low temperature, occasionally causing partial clogging because of freezing. Besides, it required a longer cleaning procedure after synthesis.

applied. Table 2 shows how better trapping efficiency of [^{11}C]CH_3I, and thus a higher radiochemical yield, could be achieved by increasing either the EtOH/H_2O ratio or the amount of precursor loaded.

Table 1. Summary of the results obtained for the solid-phase synthesis of [^{11}C]MET. The reaction conditions are those described for routine production in "Synthesis and quality control".

	% of activity decay corrected at EOB*	Minutes from EOB
Trapping of [^{11}C]CO_2 in liquid nitrogen	100	1.5
[^{11}C]CH_3I at the end of distillation	91	8.1
Loss on reactor	3.9	
Loss on Sicapent-Ascarite trap	5.1	
Final solution of [^{11}C]MET	78.4	11.3
Loss on C18 Sep-Pak Plus reactor	1.8	
Loss on charcoal trap 1	7.2	
Loss on charcoal trap 2	0.1	
Loss on sterile filter	1.3	
Loss on C18 Sep-Pak Plus before vial	2.2	

*Data represent the average of 25 experiments.

As for the latter, it was our choice during clinical production to keep this amount at a minimum, *i.e.* 21 μmol, while still assuring a good radiochemical yield. However, considering the very low toxicological relevance of L-homocysteine, the main side product of the reaction, the amount of L-homocysteine thiolactone hydrochloride loaded could well be increased (11).

We observed that [^{11}C]MET was readily eluted from the solid-phase support, whereas other side-products, notably [^{11}C]CH_3I, were more retained. We therefore paid particular attention to the volume of 0.05M NaH_2PO_4 used in order to avoid the co-elution of side products with [^{11}C]MET. The presence of the final C18 Sep-Pak before the collection vial helped considerably to that end. The small traces of [^{11}C]CH_3I co-eluted with [^{11}C]MET were partly removed by one-minute bubbling of inert gas, leaving the final solution with a radiochemical purity higher than 99%.

Ishiwata *et al.* (12) reported on a L/D ratio decrease of [*S*-methyl-^{11}C]methionine with increasing concentrations of base, the highest percentage of L-enantiomer (97.9%) being obtained when 0.025M NaOH was used (molar ratio precursor/NaOH 1:2.5). The use of such diluted amount of NaOH was possible in their classical setup but was excluded in ours by the effective volume of liquid retained on the Sep-Pak and the need for a high radiochemical yield, which forced us to use larger amount of precursor (Table 2). As a consequence our own results (*ca.* 90% L-form) are not as good in terms of optical purity. Furthermore, even a few experiments run with more diluted NaOH solution (0.05M) failed, in our hands, to yield any notable improvement in enantiomeric purity.

Table 2. Yield and enantiomeric purity* *vs.* reaction parameters.

Precursor loaded (μmol)	(μL)	EtOH/H_2O (vol/vol)	[^{11}C]MeI flow (mL/min)	NaOH/prec (molar ratio)	Charcoal trap 1 *	[^{11}C]MET*	L-form (%)
4.2	210	50/50	15	2.5/1	32.7	7.2	88.5
10.5	210	50/50	15	5/1	16.9	62.6	
21	210	60/40	15	5/1	4.0	91.2	87.6
21	210	50/50	15	3/1	21.0	51.1	89.1
21	210	50/50	15	4/1	22.5	75.1	89.0
21	210	50/50	10	5/1	6.1	86.5	
21	210	50/50	15	5/1	8.6	86.2	89.2
21	210	50/50	15	15/1	5.8	87.7	88.8
21	210	50/50	20	5/1	14.8	66.5	
21	410	50/50	20	5/1	7.5	86.8	
21	210	40/60	15	5/1	34.8	61.6	90.8
21	210	30/70	15	5/1	40.3	57.1	91.9
31.5	210	50/50	20	5/1	6.5	86.5	
42	210	50/50	15	5/1	3.8	93.4	
42	410	50/50	20	2.5/1	1.3	59.5	
42	410	50/50	20	5/1	2.9	92.4	
42	410	50/50	25	5/1	11.1	86.0	
42	410	30/70	15	5/1	16.1	66.2	92.7
42	410	20/80	15	5/1	45.9	44.7	93.2
61	610	20/80	15	5/1	46.8	33.1	
63	210	50/50	15	5/1	1.8	94.9	88.3

* Data represent the average of at least 3 experiments.

* In order to exclude any variation in the preparation of [^{11}C]CH_3I, both percentages refer to the total amount of radioactivity found on charcoal trap 1 + charcoal trap 2 + C18 Sep-Pak + C18 Sep-Pak before vial + sterile filter + [^{11}C]MET, decay corrected at EOB.

However, it must be pointed out that the commercially purchased L-homocysteine thiolactone hydrochloride could well contain a relevant percentage of D-form, as was also assumed by Ishiwata *et al.* (12). This suspicion is supported by the large uncertainty in $[\alpha]_{20}^{D}$ value with which this product is sold. However, the contamination of D-[methyl-^{11}C]methionine is a relevant factor only for protein synthesis rate studies, since its presence does not reduce tumor or brain uptake (13,14).

Our data (Table 2) show that, ratio NaOH/precursor being equal, a slight improvement in enantiomeric purity can be achieved by decreasing the content of EtOH, albeit to the detriment of the radiochemical yield. A similar behavior with regard to the radiochemical yield was previously described for another base-assisted methylation (6). EtOH/H_2O ratios higher than 60 : 40 were not feasible because the basic solution turned cloudy, unless the amount of NaOH was decreased.

All things considered, the above reported conditions for routine production seemed to be a fair compromise between the need for a high radiochemical yield and good enantiomeric purity.

As for the basic precursor solution, no serious effort has been made to investigate its stability. It was simply observed that a 2-hour-old solution did not alter the synthesis performance.

The large amount and considerable specific activity of the [^{11}C]MET produced (up to 1.2 Ci and 2.3 Ci/μmol at EOS, respectively) raised serious doubts as to the stability of the product in terms of radiolysis. We therefore decided to dilute the final solution to 10 mL with a pre-filled amount of saline (4.2 mL). The thus reduced activity concentration was supposed to decrease the extent of this phenomenon, helped in that by the NaH_2PO_4 and the traces of EtOH inevitably accompanying the product which should both scavenge to some extent the radiolytic species produced (15,16). The analyses on such solutions confirmed our expectations, showing only a marginal decrease (*ca.* 2-3%) in radiochemical purity 60 min after EOS.

Conclusions

The method described herein produces L-[*S*-methyl-^{11}C]methionine in 11 min from EOB with a high radiochemical yield and purity. Lately, under our bombardment conditions (17 MeV proton, 30 min, 40 μA) we have been able to produce 1.2 Ci (44.4 GBq) of product ready for use. Furthermore, the remote-controlled setup for quality control allows to shorten this operation and to protect the operator from any radiation exposure.

Work is in progress to apply the system to the synthesis of other [^{11}C]methyl labelled tracers.

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