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Applied Radiation and Isotopes 63 (2005) 55–61
Applied Radiation and Isotopes
www.elsevier.com/locate/apradiso

Simple automated preparation of *O*-[^{11}C]methyl-L-tyrosine for routine clinical use

Yoichi Ishikawa[a], Ren Iwata[a,*], Shozo Furumoto[b], Claudio Pascali[c], Anna Bogni[c], Kazuo Kubota[d], Kiichi Ishiwata[e]

[a]*CYRIC Tohoku University, Aramaki, Aoba-ku, Sendai 980-8578, Japan*
[b]*TUBERO, Tohoku University, Sendai 980-8575, Japan*
[c]*National Cancer Institute, 20133 Milan, Italy*
[d]*International Medical Center, Tokyo 162-8655, Japan*
[e]*Tokyo Metropolitan Institute of Gerontology, Tokyo 173-0022, Japan*

Received 3 December 2004; received in revised form 15 January 2005; accepted 21 January 2005

Abstract

The previously reported preparation of *O*-[^{11}C]methyl-L-tyrosine ([^{11}C]MT), a promising tumor imaging agent, has been now considerably simplified and automated. Main changes were the use of [^{11}C]methyl iodide ([^{11}C]MeI) in the reaction with L-tyrosine disodium and the use of solid phase extraction on commercially available cartridges instead of HPLC for the final purification. An injectable saline solution of [^{11}C]MT was obtained within 30 min after EOB with radiochemical yield of ca. 60% (decay-corrected, based on [^{11}C]MeI). Radiochemical purity was over 97%. The automated preparation was carried out using a miniature module employing manifold valves.

Keywords: C-11; *O*-methyl-L-tyrosine; Amino acid transport; Tumor imaging; Automated synthesis

1. Introduction

In spite of the increasing number of ^{11}C-labeled amino acids so far developed, [^{11}C]methionine ([^{11}C]MET) is still the most frequently used in clinical practice. One of the reasons is its convenient, fast and highly efficient synthesis based on the reaction of [^{11}C]methyl iodide (Comar et al., 1976) or [^{11}C]methyl triflate (Någren and Haldin, 1998) with homocysteine thiolactone hydrochloride. Recently, this procedure was greatly simplified by introducing solid-phase-supported (on-column) [^{11}C]methylation (Pascali et al., 1999). This tracer has proven useful in imaging brain, head neck, lung and breast cancer, but its high uptake in normal liver, pancreas and intestine interferes with the imaging of the abdominal region (Jager et al., 2001).

In the last few years, some novel positron-emitting tyrosine analogues have been proposed for imaging amino acid transport (Laverman et al., 2002). Among these, the synthetic ^{18}F-labeled amino acid *O*-(2-[^{18}F]fluoroethyl)-L-tyrosine ([^{18}F]FET) (Wester et al., 1999) has gained increased interest in the course of its clinical evaluation(Weber et al., 2000; Pauleit et al., 2003). More recently, other similar tyrosine analogues such as *O*-(3-[^{18}F]fluoropropyl)-L-tyrosine ([^{18}F]FPT) (Tang et al., 2003a) and *O*-[^{18}F]fluoromethyl-L-tyrosine ([^{18}F]FMT) (Iwata et al., 2003) were also introduced. Fluorine-18, owing to its longer half-life (109.8 min), is in general the preferred choice for distribution and

*Corresponding author. Tel./fax: +81 22 217 7798.
E-mail address: rencyric@cyric.tohoku.ac.jp (R. Iwata).

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doi:10.1016/j.apradiso.2005.01.014

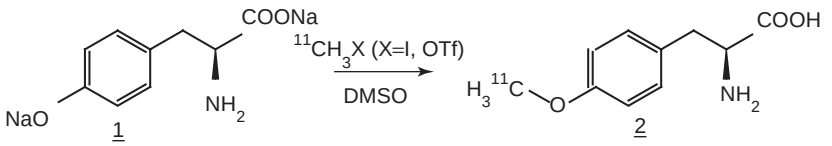

Fig. 1. Synthesis of [^{11}C]MT.

multi-dose production. However, carbon-11 still retains some potential, at least for the latter aspect, if an efficient and simple radiosynthesis from daily multi-run production of [^{11}C]CH_3I (Larsen et al., 1997) is possible. Moreover, quite often, biological information (toxicity, metabolism, etc.) on fluoro-analogues is not available, and thus more workload is needed for introducing the fluoro-tracers in clinical practice. [^{11}C]MT was successfully prepared using [^{11}C]CH_3OTf (Fig. 1) (Iwata et al., 2003) and its biological evaluation in mice showed quite a similar biodistribution to [^{18}F]FET and [^{18}F]FMT (Ishiwata et al., 2004).

Automation is an essential feature for routine preparations and its implementation is easier when the radiosynthesis is simpler. Unfortunately, our previously reported [^{11}C]MT preparation (Iwata et al., 2003) required an HPLC purification and subsequent removal of the HPLC eluent using a rotary evaporator. As a result, the automation of such a system would have been quite complex to achieve. Recently, it was reported that [^{18}F]FET and [^{18}F]FPT, which were synthesized by the reaction of L-tyrosine disodium with the corresponding [^{18}F]fluoroalkyl tosylates, were successfully purified by solid phase extraction (SPE) (Tang et al., 2003a, b). Inspired by this result, we simplified the method for the preparation of [^{11}C]MT by applying commercial SPE cartridges to the final purification and by developing a miniaturized, automated radio-synthesis module using small manifold valves.

2. Materials and methods

All reagents and solvents were commercially available and used without further purification. Anhydrous dimethysulfoxide (DMSO) was purchased from Aldrich, disodium salt of L-tyrosine from Sigma, and *O*-methyl-L-tyrosine from Bachem. SPE cartridges of Sep-Pak Plus silica, tC18, C18 and AC-2 were obtained from Waters and Bond Elut Jr. SCX cartridges (1000 mg) from Varian.

HPLC analysis of the crude reaction mixture was performed on an ODS column (YMC Pack Pro C18, 4.6×100 mm) with a solvent system of H_2O/EtOH/AcOH (92.5:5:2.5) at a flow rate of 1.5 mL/min. Retention time of *O*-methyl-L-tyrosine was 4.0 min.

2.1. Reaction of [^{11}C]MeI or [^{11}C]MeOTf with L-tyrosine disodium

[^{11}C]Carbon dioxide was produced by bombarding an N_2 target containing 0.5% O_2 with 12 MeV protons. It was then converted into [^{11}C]MeI by gas phase iodination via [^{11}C]CH_4 with a MeI MicroLab system (GE). The [^{11}C]MeOTf was obtained by passing [^{11}C]MeI through a heated AgOTf column (190 °C) (Jewett, 1992).

2.1.1. Bubbling method

A solution of L-tyrosine disodium in DMSO (2 mg/0.2 mL or 3 mg/0.3 mL) in a small vial (Wheaton V vial, 3 or 5 mL) was bubbled with the labeling precursor carried by He flow (50 mL/min) over about 2 min at room temperature through a disposable needle (stainless steel 25G, 0.24 mm I.D.). A short charcoal column (Sep-Pak AC-2) connected to the outlet of the vial adsorbed the non-retained labeling precursor and was used to determine the trapping efficiency. The reaction was quenched immediately after bubbling by adding water (2 mL). An aliquot of the resulting mixture was sampled for HPLC analysis, while the remaining portion was purified by SPE.

2.1.2. On-column method

The same precursor solution (0.2 mL) was loaded onto a Sep-Pak C18 cartridge connected by a three-way valve to a AC-2 Sep-Pak. The labeling precursor ([^{11}C]MeI or [^{11}C]MeOTf) was then passed through at a flow rate of 50 mL/min. After flashing with He for 1 min, the C18 cartridge was eluted with MeCN. The eluate was assayed by HPLC.

2.2. Purification of [^{11}C]MT by SPE

2.2.1. Separation with silica-C18 cartridges

The procedure was adopted from literature (Tang et al., 2003b). After bubbling with the [^{11}C]methylating agent, the DMSO solution was injected onto two combined silica and C18 (activated with EtOH and water) cartridges, which were then eluted with Et_2O (10 mL) and phosphate-buffered saline (PBS, 5 mL). Radiochemical purity of [^{11}C]MT was assayed by HPLC.