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SYNTHESIS OF (N-[^{11}C]METHYL)Y-29794, A COMPETITIVE INHIBITOR OF PROLYL ENDOPEPTIDASE

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SUMMARY

Prolyl endopeptidase (PEP: [E.C.3.4.21.26]) is a widely distributed serine peptidase that cleaves a variety of oligopeptides in the brain and peripheral tissues. Y-29794 ((2-(8-dimethylaminooctylthio)-6-isopropyl-3-pyridyl-2-thienyl ketone) is a potent competitive reversible inhibitor of this enzyme. In order to study the biodistribution of PEP *in vivo* we have synthesized (N-[^{11}C]methyl)Y-29794, by [^{11}C]alkylation of the N-desmethyl precursor. The radiotracer was purified by silica gel Sep-Pak and was obtained in 10-17% yields (EOB: synthesis times shorter than 45 min) with >98% radiochemical purities and specific activities >550 Ci/mmol (EOS).

Key-words: (N-[^{11}C]methyl)Y-29794, prolyl endopeptidase, protease inhibitors

INTRODUCTION

Prolyl endopeptidase (PEP: [E.C.3.4.21.26]) is a cytosolic serine protease that hydrolyzes peptide bonds at the carboxylic end of proline and alanine residues of oligopeptides[1,2]. It is a widely distributed enzyme found in many species including man[1,2], and is located in a variety of tissues such as uterus, liver, kidney and brain[1-3]. PEP degrades a variety of oligopeptides including substance P, oxytoxin, angiotensin II, vasopressin and TRH[1,2], and more recently, it has been implicated in the processing of the amyloid precursor protein (APP)[4,5].

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A possible role of PEP in the biochemical mechanism of memory has been suggested by *in vivo* studies, where PEP inhibitors were shown to produce anti-amnesic effects in rodents previously treated with scopolamine[6-8]. Y-29794 (2-(8-dimethylaminooctylthio)-6-isopropyl-3-pyridyl-2-thienyl ketone)[9] (Figure 1) is one of the most potent competitive reversible inhibitors of PEP (IC_{50}=3 nM) and has been shown, in *ex vivo* experiments, to cross the blood brain barrier and inhibit brain PEP in rodents[9].

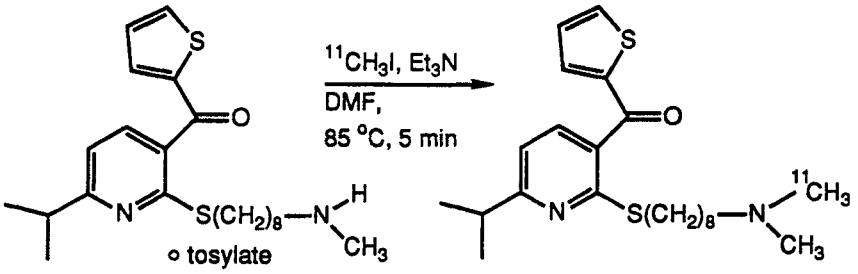
The study of biodistribution and activity of brain proteases using radiolabeled enzyme inhibitors and Positron Emission Tomography (PET) might provide a new and important approach to the evaluation of the roles of peptides and protein degradative processes in normal and diseased human brain. With this goal in mind, we report here the synthesis of (N-[^{11}C]methyl)Y-29794. The results from the biological experiments will be presented elsewhere.

RESULTS AND DISCUSSION

The typical procedure for the synthesis of (N-[^{11}C]methyl)Y-29794 (**2**) is shown in Figure 1. A solution of desmethylY-29794 tosylate **1** in dimethylformamide was neutralized with triethylamine and the free base was reacted with no-carrier-added [^{11}C]CH_3I (5 min at 85 °C). The desired product **2** was purified by silica gel chromatography, using the following procedure: the reaction mixture was adsorbed onto the first of two serial dry silica gel Sep-Paks which were washed with ethyl acetate to remove unreacted [^{11}C]CH_3I and DMF. The product **2** was then selectively eluted from the silica gel using ethyl acetate/triethylamine (99/1); under these conditions, unreacted precursor is not eluted from the silica gel (see below). The organic solvents were evaporated using a stream of nitrogen at 120 °C. Because Y29794 is a very lipophilic compound (calculated logP=6.1), formulation required collection of the solvent fraction carrying the radiotracer in a vial previously washed with aqueous hydrochloric acid, in order to protonate the tertiary amine, followed by addition of sterile water and adjustment of the solution to slightly acidic pH with 1 N sodium bicarbonate. Syntheses times, including the formulation step, were typically less than 45 min from end-of-bombardment (EOB), radiochemical yields ranged between 10-17% (decay corrected to EOB) and radiochemical purities were >98% as determined by HPLC (reaction conditions and yields

have not been optimized). Specific activities were >550 Ci/mmol at end of synthesis (EOS). Isolated **2** was shown to co-elute with authentic Y-29794 by analytical HPLC.

Figure 1. Synthesis of (N-[^{11}C]methyl)Y-29794



1
DesmethylY-29794 tosylate

2
(N-[^{11}C]methyl)Y-29794
10-17% (EOB), <45 min
>98% radiochemical purity
>550 Ci/mmol (EOS)

The purification of **2** described here was developed to specifically avoid the use of HPLC. We have found that small disposable chromatographic columns (custom-packed columns or Sep-Paks) can provide effective separation of radiopharmaceutical products from chemical and radiochemical impurities, and this technique has been used in preparations of such radiotracers as [^{11}C]flumazenil[10], [^{18}F]GBR 12909[11], and [^{11}C]raclopride (unpublished results). In the development of this technique for purification of (N-[^{11}C]methyl)Y-29794, a solution of 2 mg of desmethylY28794 tosylate **1** (twice the amount used in the radiochemical synthesis) and triethylamine in dimethylformamide was loaded onto two dry serial silica gel Sep-Paks which were eluted sequentially with ethyl acetate (this was discarded) and ethyl acetate/triethylamine (99/1) solution; volumes were identical to those utilized in the radiochemical synthesis. The acetate/triethylamine eluant was evaporated using a gentle stream of nitrogen (in order to avoid interference of ethyl acetate with the UV absorption of **1**) and the residue was dissolved in acetonitrile (10 mL). The UV absorption of this solution was then measured (Perkin Elmer Lamda 6 UV/VIS spectrophotometer) and compared to the absorptions of known concentrations of desmethylY-29794 tosylate solutions in acetonitrile. The absorption of the solution (318 nm, λ_{max} of desmethylY-29794) was near the limits of detection and corresponded to a

concentration of 0.33 μg/mL of precursor, demonstrating that the silica gel Sep-Paks were efficient at removing >99% of precursor loaded onto the columns. Also, the amount of **1** eluted from the silica gel was unaffected by the rate of passage of solvents through the Sep-Paks.

In conclusion we have synthesized (N-[^{11}C]methyl)Y-29794, including a simple purification procedure that does not require HPLC, and have obtained the radiotracer in high specific activities and sufficient yields to undertake animal studies.

EXPERIMENTAL

Materials and methods. Triethylamine and dimethyl formamide were purchased from Aldrich Chemical Company and were used without further purification. All solvents used were high pressure liquid chromatography grade. The Sep-Paks used were silica cartridges purchased from Waters Associates. [^{11}C]Carbon dioxide (approx. 2500 mCi), produced by proton irradiation of a nitrogen gas target at 20 μA for 25 min, was converted to [^{11}C]methyl iodide by lithium aluminum hydride reduction followed by treatment with hydroiodic acid. All radiosyntheses were done in small glass vessels (1 mL) in a remote apparatus which was manipulated from outside the hot cell. Determination of specific activities and chemical and radiochemical purities were done using analytical HPLC (0.46 x25 cm, silica gel column, UV 320 nm, CH_2Cl_2: EtOAC: Et_3N 80:18:2, 1 mL/min, R_t of **2** =4.7 min).

Synthesis of (N-[^{11}C]methyl)Y-29794. A glass reaction vessel containing a solution of desmethylY-29794 tosylate (1 mg, 1.7 μmol) and triethylamine (5 μL, 69 μmol) in 250 μL of dimethylformamide was placed in a remote apparatus, and all subsequent steps were done from outside of the closed hot cell. The reaction vessel was cooled to approximately -30 °C, and [^{11}C]CH_3I (carried by a stream of nitrogen) bubbled into the vessel. The reaction vessel was then closed and heated at 85 °C for 5 min. After cooling to room temperature, the mixture was loaded onto two dry silica gel Sep-Paks, connected in series. The reaction vial was washed with 1 mL of ethyl acetate which was also loaded on the Sep-Paks. The Sep-Paks were washed with ethyl acetate (20 mL) which was discarded, and (N-[^{11}C]methyl)Y-29794 was then eluted using 10 mL of a solution of ethyl acetate/triethylamine (99/1). The eluent was collected in a vial previously washed with 1N

hydrochloric acid (but not dried), and the solvent was removed with a stream of nitrogen at 120 °C. To the isolated (N-[^{11}C]methyl)Y-29794 was first added 1.5 mL of 0.3N hydrochloric acid followed by addition of 1.2 mL water and approximately 350 µL of 1.0 N sodium bicarbonate solution, until the solution became slightly acidic (pH approx. 6.5). The radiochemical yield was 31.8 mCi (17%, EOB) in a synthesis of 45 min. The radiochemical purity was >98% and the specific activity was 638 Ci/mmol at EOS.

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