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SYNTHESIS OF N-{N-[4-(4-{*N*-[^{11}C]METHYLAMINO}PHENYL)BUTYRYL]-L-PROLYL}PYRROLIDINE: A POTENTIAL RADIOTRACER FOR PROLYL ENDOPEPTIDASE

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SUMMARY

The synthesis of the 4-[^{11}C]methylamino derivative of N-{N-[4-(4-Aminophenyl)butyryl]-L-prolyl}pyrrolidine (SUAM-1221), is described as a potential marker for prolyl endopeptidase for *in vivo* positron emission tomography studies. Direct methylation of the 4-amino derivative of SUAM-1221 (1) with methyl iodide provided a mixture of the 4-monomethyl (2) and 4-dimethylamino (3) derivatives which were separated by chromatography. Methylation of 1 with [^{11}C]methyl iodide provided the 4-[^{11}C]methylamino derivative of SUAM-1221, ([^{11}C]2), in 18 - 30% decay corrected radiochemical yield after HPLC purification, with a specific activity >1700 Ci/mmol and a 40 minute synthesis time from end of bombardment.

Key Words: SUAM-1221, Prolyl endopeptidase inhibitor, Carbon-11, PET.

INTRODUCTION

Prolyl endopeptidase ([E.C.3.4.21.26]; PEP) is a serine protease widely distributed among various tissues throughout the body including the central nervous system (1). Many neuropeptides including neurotensin, Substance P, angiotensin II, bradykinin and vasopressin have been shown to be substrates of PEP *in vitro* lending strong support for an important role for this enzyme in the biological regulation of these neuropeptides (2,3). Recent studies by Terwel (4) and Ishiura (5) also suggest a role for PEP in the etiology of Alzheimers disease. The development of suitable *in vivo* markers for PEP that cross the blood-brain-barrier could therefore be useful for understanding the physiological role of this enzyme in the brain in health and disease using positron emission tomography (PET).

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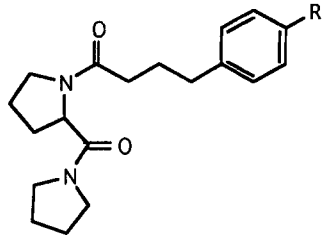
Atack and coworkers (6) recently reported on a series of high affinity (IC_{50} of mouse brain enzyme inhibition of 2 to 5 nM), competitive, nonpeptide PEP inhibitors based on the prototypical PEP inhibitor SUAM-1221 (Figure 1). These compounds also displayed *in vivo* inhibition of brain PEP indicating that they cross the blood-brain-barrier. Furthermore, their studies revealed that the 4 position of the aromatic ring could tolerate both small as well as large substituents with no significant decrease in inhibitor activity.



R = H ;	**SUAM-1221**
R = NH_2 ;	1
R = $NHCH_3$;	2
R = $N(CH_3)_2$;	3

Figure 1. Structure of SUAM-1221 derivatives

SUAM-1221 and its derivatives all contain a proline residue in the L configuration, which presumably is critical to biological activity as observed in the Z-Pro-prolinal class of PEP inhibitors (7). We report here the synthesis of a carbon-11 labeled derivative of SUAM-1221, ([^{11}C]2), substituted at the 4 position of the aromatic ring (Figure 1) as a potential PET radiotracer for PEP.

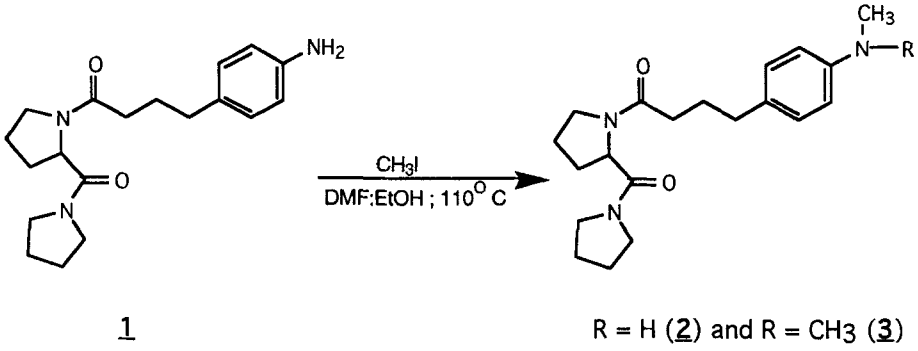
RESULTS AND DISCUSSION

The chemical structure of SUAM-1221 does not allow for a straightforward, convenient synthesis of the corresponding carbon-11 labeled analog. In view of the reported high *in vitro* PEP inhibitory activity of 1 (IC_{50} for mouse brain PEP = 3.1 nM) and its ability to inhibit mouse and rat brain PEP *in vivo* (6), the synthesis of the corresponding [^{11}C]methylamino analog [^{11}C]2 was therefore pursued.

Synthesis of the monomethylamino and dimethylamino analogs 2 and 3, respectively, was achieved in a combined yield of 70% by direct N-methylation of 1 with 0.75 equivalents of CH_3I in refluxing DMF : EtOH as shown in Figure 2. Integration of the methyl singlets in the ^{1}H NMR of the

crude product mixture revealed a 1:1 mixture of these two products which were separated by flash chromatography.

Direct N-[^{11}C]methylation of 1 with [^{11}C]CH_3I provided [^{11}C]2 in high radiochemical yields (18 - 30%; decay corrected based on [^{11}C]CO_2) and in high specific activity (>1700 Ci/mmol; end of synthesis). No radioactive products corresponding to [^{11}C]3 were detected by either TLC or HPLC analysis of the crude reaction mixture.



1 R = H (2) and R = CH_3 (3)

Figure 2. Synthesis of SUAM-1221 derivatives

Compounds 1 and 2 showed baseline resolution (retention times for 1 and 2 were 6.7 and 12.1 min respectively) under analytical reverse-phase HPLC conditions. There was concern however that on a preparative scale, HPLC purified [^{11}C]2 could contain traces of the first eluting precursor 1 due to tailing since the latter is present in large molar excess. Purification was therefore done using normal-phase conditions as described which provided [^{11}C]2 in high radiochemical and chemical purity (>95%). The identity of [^{11}C]2 was confirmed by TLC and HPLC analysis. In a typical production run an average of 67 mCi (N = 5), of formulated [^{11}C]2 were obtained from an estimated average of 1.26 Ci of [^{11}C]CO_2 in a 40 minute synthesis time.

In summary, we have described a convenient and efficient synthesis of a carbon-11 labeled derivative of the prolyl endopeptidase inhibitor SUAM-1221 as a potential marker for PEP in the central nervous system. Evaluation of this radiotracer in small animals is currently underway and will be reported shortly.

MATERIALS AND METHODS

Infrared spectra were recorded on a Perkin-Elmer Model 727B spectrometer. ^{1}H-NMR spectra were obtained on a Bruker WM 360 MHz spectrometer with tetramethylsilane (TMS) as internal standard. Mass

spectra were obtained on a Finnigan 4021 GCMS/DS (low resolution) or a UG70-250-S (high resolution) instrument.

N-{N-[4-(4-Aminophenyl)butyryl]-L-prolyl}pyrrolidine (1) was synthesized according to the published method (6). All chemical reagents were of reagent grade and purchased from Aldrich Chemical Company, Milwaukee, WI.

Flash chromatography was performed by the method of Still *et al.*(8). Thin-layer chromatography was performed on either Analtech silica gel GF or Whatman reversed phase (C-18) glass-backed plates (10 cm, 250 μm). TLC was performed by co-spotting the labeled compound with the authentic unlabeled compound prior to development. Radiochemical purity was monitored using the following TLC systems: 1) Silica; $CHCl_3$: CH_3OH: NH_4OH (98:2:1); R_f of 1, 2 and 3 were 0.18, 0.31 and 0.41 respectively;
2) C-18; 95 % EtOH: 0.1 M NH_4OAc (1:1); R_f of 1, 2 and 3 were 0.67, 0.44 and 0.28 respectively. TLC plates were scanned for radioactivity using a Berthold Model LB 2832 TLC-linear analyzer equipped with a Model LB 500 data acquisition system.

HPLC analysis was performed on a Phenomenex Ultremex 5 ODS column (5μ, 250 x 4.6 mm) eluted with 95% EtOH: 0.1 M NH_4OAc (40:60) at a flow rate of 1 mL per minute. UV absorbance was monitored at 254 nm. The retention times of 1 , 2, and 3 were 6.7, 12.1 and 29.8 min respectively under these assay conditions. HPLC purification of the radiolabeled product was performed on a Phenomenex Partisil 10 silica gel column (10μ, 250 x 9.4 mm) eluted with CH_2Cl_2: isopropanol: diethylamine (975:25:1) at a flow rate of 7 mL per minute. The retention time of [^{11}C]2 was 5.8 min; retention times for 1 and 3 were 10.5 and 3.7 mins respectively under these assay conditions. UV absorbance (254 nm) and radioactivity were monitored by an in-line UV absorbance and gamma-detector.

Specific activity determinations were made by collection of the radioactive peak corresponding to [^{11}C]2, measurement of the U.V. absorbance peak area of carrier 2 by an automated integrator and comparison with the area of a standard sample of 2.

Production of [^{11}C]CH_3I

[^{11}C]CO_2 was produced with a biomedical cyclotron (CS-30 accelerator; Cyclotron Corporation) by the $^{14}N(p,\alpha)^{11}C$ reaction using nitrogen in an all aluminum target. [^{11}C]CO_2 was converted to [^{11}C]CH_3OH by $LiAlH_4$ reduction, and treated with aqueous HI at reflux (9,10) to generate [^{11}C]CH_3I.

The initial activity of the [^{11}C]CO_2 produced at bombardment was estimated from a previously determined standard curve of ^{11}C activity produced versus irradiation times in the same target. Estimates of initial

target activity from this standard curve were used to determine overall radiochemical yields.

N-{N-[4-(4-{*N*-Methylamino}phenyl)butyryl]-L-prolyl}pyrrolidine (2) and N-{N-[4-(4-{*N,N*-Dimethylamino}phenyl)butyryl]-L-prolyl}-pyrrolidine (3)

A solution of 1 (0.248 g, 0.75 mmol) in dry DMF: absolute EtOH (1:1; 10 mL) was stirred and treated dropwise at 23 °C with a solution of CH_3I (0.082 g, 36 µL, 0.58 mmol) in absolute EtOH (1.5 mL). The reaction mixture was heated at reflux at 110 °C for 4 hours and the EtOH removed by rotoevaporation. Residual DMF was then removed at 60 °C by means of a vacuum pump. The crude product was partitioned between 1N NaOH (50 mL) and EtOAc (50 mL) and the organic layer removed. The aqueous layer was extracted once with EtOAc (50 mL) and the combined organic layers washed with H_2O (100 mL) and dried (Na_2SO_4). Removal of volatiles gave a light brown oil which was flash chromatographed with $CHCl_3$: CH_3OH: NH_4OH (98:2:1) to afford 73 mg (37%) of 2 and 68 mg (33%) of 3 as pale yellow oils.

2: IR (KBr) 3350, 1633 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.01 (d, J = 8.4 Hz, 2H), 6.55 (d, J = 8.4 Hz, 2H), 4.63 (dd, 1H), 3.83 (q, 1H), 3.65 - 3.53 (m, 2H), 3.45 - 3.33 (m, 3H), 2.81 (s, 3H), 2.56 (t, J = 7.3 Hz, 2H), 2.32 - 2.19 (m, 3H), 2.10 - 1.80 (m, 9H); MS (EI, 70 eV), m/z(relative intensity): 343 (M^+, 13.4), 176 (76), 134 (15.6), 133 (48.3), 120 (50.6), 112 (100), 70 (79.8), 55 (15.4); High resolution MS (EI, 70 eV) calculated for $C_{20}H_{29}N_3O_2$: 343.2260. Found: 343.2261.

3: IR (KBr) 3450 (broad), 1625 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.07 (d, J = 8.7 Hz, 2H), 6.69 (d, J = 8.7 Hz, 2H), 4.64 (dd, 1H), 3.84 (q, 1H), 3.66 - 3.53 (m, 2H), 3.46 - 3.34 (m, 3H), 2.90 (s, 6H), 2.58 (t, J = 7.4 Hz, 2H), 2.33 - 2.19 (m, 3H), 2.10 - 1.78 (m, 9H); MS (EI, 70 eV), m/z (relative intensity): 357 (M^+, 18.5), 190 (77.6), 147 (100), 134 (72.8), 112 (91.6), 70 (71.3), 55 (15.9); High resolution MS (EI, 70 eV) calculated for $C_{21}H_{31}N_3O_2$: 357.2416. Found : 357.2426.

N-{N-[4-(4-{*N*-[^{11}C]Methylamino}phenyl)butyryl]-L-prolyl} pyrrolidine; ([^{11}C]2)

[^{11}C]Methyl iodide carried by a nitrogen stream was trapped in a reaction vial containing a solution of 1 (1.2 mg , 9 µmol) in DMF (0.15 mL) at -30 to -40 °C. The reaction mixture was heated at 95 °C for 5 min, then purged with nitrogen for 15 seconds to remove unreacted [^{11}C]CH_3I. The reaction mixture was then diluted with water (0.5 mL) and transferred by remote handling onto a short reversed phase column (300 mg C-18; PrepSep; Fisher Scientific) that had been prewashed with methanol (50 mL) followed by

water (100 mL). The extraction column was rinsed with water (1.0 mL) and dried by passing nitrogen through it at 80 psi for 2 minutes. The crude [^{11}C]$_2$ was then eluted off the C-18 guard column with the HPLC mobile phase (CH_2Cl_2: isopropanol: diethylamine [975:25:1]) onto a semi-preparative silica column for purification. The radioactive peak corresponding to [^{11}C]$_2$ was collected in a sterile vial and the solvent removed using low heat (40 °C) and a nitrogen flow. The residue was formulated in phosphate buffered saline (pH = 6.0) and filtered through a 0.2 μm alumina filter (Anotop) into a sterile 10 mL multidose vial. The decay corrected radiochemical yield ranged from 18 - 30% (N = 5); and the specific activity was determined to be >1700 Ci/mmol at end of synthesis.

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