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Radiosynthesis of a Potent Endothelin Receptor Antagonist: [^{11}C] L-753,037

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SUMMARY

[^{11}C]-L-753,037, [(+)-(5S,6R,7R)-2-butyl-7-[2-((2S)-2-carboxypropyl)-4-[^{11}C]-methoxyphenyl]-5-(3,4-methylenedioxyphenyl)-cyclopenteno-[1,2-b]pyridine-6-carboxylate], a potent mixed antagonist of endothelin receptor subtypes ET_A and ET_B, was synthesized by [^{11}C]-methylation of a phenolic precursor. The time for synthesis, purification, and formulation was 17 minutes with an average specific radioactivity of 2535 mCi/μmol (EOS) and average decay corrected radiochemical yield of 3% based on [^{11}C]-methyl iodide.

Key Words: L-753,037, endothelin, carbon-11, positron emission tomography

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INTRODUCTION

Endothelin (ET), a potent vasoconstrictive peptide, has three isoforms (ET-1, ET-2 and ET-3) that mediate a number of physiologic actions in several organ systems and play a role in vascular function regulation (1,2,3). Two high affinity receptor subtypes have been identified (ET_A and ET_B), and a third (ET_C) has been proposed (2,4). Endothelin has been implicated in cardiovascular, pulmonary, and renal diseases such as hypertension, arterioscleroses, heart failure, myocardial infarction, and ischemia (5). High affinity peptides - ET-1, an endothelin hormone; BQ 3020, an ET_B receptor agonist; and ZK 167054, an endothelin derivative - have been radiolabeled and studied *in vitro* and *in vivo* in animals (6,7,8). However, to date no useful endothelin receptor radioligand has been utilized for *in vivo* human positron emission tomography (PET) imaging.

L-753,037 (Figure 1), also referred to as J-104,132, is a highly selective nonpeptide mixed ET_A and ET_B receptor antagonist with an *in vitro* K_i = 0.034 and 0.104 nM, respectively, to cloned human receptors (9). In this study, [^{11}C]-L-753,037 was synthesized by reacting [^{11}C]-methyl iodide with the phenolic precursor, L-843,974 (Figure 1). The synthetic procedure, purification, formulation, and characterization are described.

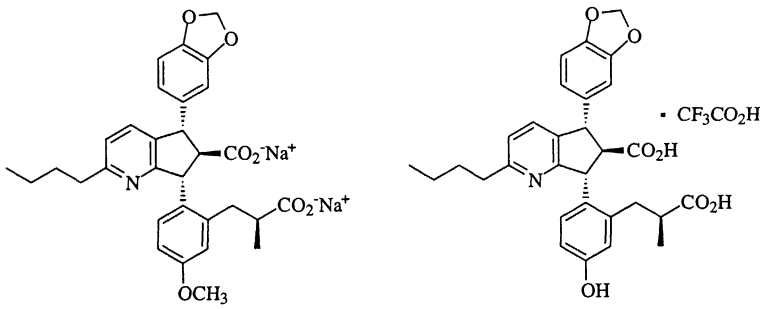

Figure 1: Structures of L-753,037 and L-843,974.

RESULTS

[^{11}C]-L-753,037 was synthesized by alkylation of the phenolate precursor L-843,974 with [^{11}C]-methyl iodide in the presence of tetrabutylammonium hydroxide. The [^{11}C]-methyl iodide was synthesized by standard methods. Sufficient base must be added to the precursor to neutralize the trifluoroacetate salt and create the phenolate ion. The precursor also contains two carboxylic acid groups that are also deprotonated by base. Initial experiments indicated that a molar ratio of about 3 base to 1 precursor gave a modest yield of [^{11}C]-L-753,037. With this ratio, the decay corrected yield (n=5) varied from 0.3 to 5.0% from [^{11}C]-methyl iodide. A lower ratio of base to precursor reduced the yield significantly, and a higher ratio increased the production of radiolabeled hydrophilic compounds. Figure 2 is a typical preparative chromatogram. The radioactive trace shows hydrophilic peaks that elute prior to the unlabeled precursor, and possibly two peaks directly before and one peak after the [^{11}C]-L-753,037 product peak. Aqueous 0.1 M NaOH added to the reaction mixture and heated at 80°C for

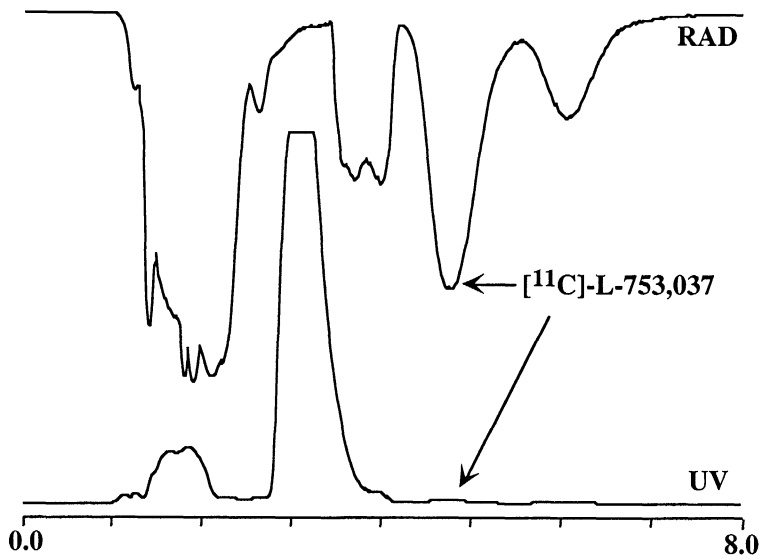

Figure 2: Preparative ultraviolet and radioactive chromatograms of the [^{11}C]-L-753,037 reaction mixture.

5 minutes greatly reduces the size of the peaks before and after the product without affecting the product yield. These side product peaks are related to ester formations (data not shown). No base hydrolysis of the radiolabeled esters was performed since the preparative chromatography gave good separation of the product from these side products. The synthesis, semipreparative high performance liquid chromatography (HPLC), and formulation was completed in an average time of 17 minutes (n=5) with an average decay corrected radiochemical yield of 3% based on [^{11}C]-methyl iodide. The average specific radioactivity was 2535 mCi/μmole at end of synthesis. The final formulated solution was chemically and radiochemically pure as determined by analytical HPLC. All final solutions (n=5) were found to be sterile and pyrogen free.

EXPERIMENTAL

L-753,037 and L-843,974 were prepared by Merck Research Laboratories, West Point, PA (10). Dimethylformamide (DMF) was stirred over BaO overnight and vacuum distilled prior to use. All other reagents were ACS or HPLC purity. HPLC analysis and purification were performed with two Waters 590EF HPLC pumps, an in-line Waters fixed wavelength (254 nm) detector, and a Ortec single two inch NaI crystal radioactive detector. HPLC chromatograms were recorded by a Rainin Dynamax dual channel control/interface module connected to a Macintosh computer with appropriate program software (Dynamax - version 1.4). HPLC semipreparative purification were completed on an Alltech 10 μm C-18 Econosil column (10 x 250 mm) using a mobile phase of 45% acetonitrile / 55% water (0.1 M ammonium formate) at a flow rate of 10 mL/min. Chemical and radiochemical purity were determined using an Alltech 10 μm C-18 HPLC column (4.6 x 250 mm) with a mobile phase of 60% acetonitrile / 40% water (0.1 M ammonium formate) at a flow rate of 6 mL/min. A dose calibrator (Capintec 12R) was used for all radioactivity measurements. A standard limulus amebocyte lysate assay (Biowhittaker) was performed to test for endotoxins in the final formulated solution. Sterility was determined using a standard aerobic and anaerobic procedure for the Bactec 9050 fluorescence system (Becton Dickinson and Co.).

Radiosynthesis and purification of [^{11}C]-L-753,037.

L-843,974 (2 mg, 3.2 μmoles) was dissolved in 200 μL of DMF followed by the addition of 10 μL of methanolic tetrabutylammonium hydroxide (1.0 M; Aldrich). [^{11}C]-Methyl iodide was synthesized from cyclotron produced [^{11}C]-carbon dioxide by standard methods. The [^{11}C]-methyl iodide was transferred into the sealed precursor vial placed in a dry ice-ethanol bath immediately before bubbling occurs. After the radioactivity reached a plateau, the vial was heated to 80°C for 3 minutes. HPLC solvent (200 μL) was added prior to applying the solution to the semipreparative HPLC column. After collection and vacuum evaporation to dryness, the product was redissolved in 7 mL of sterile normal saline followed by sterile filtration into a sterile evacuated vial. The chemical and radiochemical purity of the final solution were determined as described previously. Sterile 8.4% sodium bicarbonate (3 mL) was added after the determination of purity.

CONCLUSION

The radiosynthesis of [^{11}C]-L-753037 by the method described produces a high specific radioactivity tracer in low but sufficient yield for preliminary biological studies. Development of a higher yield synthesis - i.e., protection of the acid moieties and deprotection after radiolabeling - that creates product suitable for human PET imaging is in progress.

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REFERENCES

1. Hickey, K.A. *et al.* Am. J. Physiol. **248**: C550-C556 (1985)
2. Yamagisawa M. *et al.* Nature **332**: 411-415 (1988)

3. Inoue A. *et al.* Proc. Natl. Acad. Sci. U.S.A. **86**: 2863-2867 (1989)
4. Sokolovsky M. *et al.* J. Biol. Chem. **267**: 20551-20554 (1992)
5. Doherty A.M. J. Med. Chem. **35**: 1493-1508 (1992)
6. Gibson R.E., *et al.* Nuc. Med. Biol. **26**: 193-199 (1999)
7. Johnstrom P, *et al.* J. Labelled Cpd. Radiopharm. **42**: S105-S107 (1999)
8. Dinkelborg L.M., *et al.* J. Nuc. Med. **39**: 1819-1822 (1998)
9. Nishikibe M. *et al.* J. Pharm. Exp. Thera. **289**: 1262-1270 (1999)
10. Song Z.J. *et al.* J. Org. Chem. **64**: 9658-9667 (1999)

