

Synthesis of a Nonpeptide Carbon-11 Labeled Substance P Antagonist for PET Studies

RENATO B. DEL ROSARIO¹, THOMAS J. MANGNER¹, DAVID L. GILDERSLEEVE¹, PAUL D. SHREVE¹, DONALD M. WIELAND^{1*}, JOHN A. LOWE III², SUSAN E. DROZDA² and R. MICHAEL SNIDER²

¹Division of Nuclear Medicine, University of Michigan Medical Center, Ann Arbor, MI 48109-0028 and

²Central Research Division, Pfizer Inc., Groton, CT 06340, U.S.A.

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CP 96,345 is a nonpeptide high affinity antagonist of the substance P (NK₁) receptor. The radiosynthesis of [¹¹C]CP 96,345 suitable for Positron Emission Tomography (PET) applications is described. [¹¹C]CP 96,345 was prepared by O-methylation of a desmethyl precursor via *in situ* generation of its phenolate salt. The *in vivo* tissue distribution of [¹¹C]CP 96,345 in guinea pigs (*n* = 2) at 5 and 30 min was determined. Uptake was low in brain (≈ 0.04% dose/g) and highest (≈ 1-2% dose/g) in the spleen and lungs. The present findings indicate that the use of [¹¹C]CP 96,345 in PET might be more applicable to the study of substance P receptors in peripheral tissues involved with inflammatory disease and arthritis.

Introduction

The compound CP 96,345 has recently been reported to be the first potent nonpeptide antagonist of substance P (Lowe *et al.*, 1991, 1992; McLean *et al.*, 1991; Snider *et al.*, 1991). CP 96,345 binds stereoselectively and with high affinity ($K_d = 0.2$ nM) to the substance P (NK-1) receptor (Lowe *et al.*, 1992; Snider *et al.*, 1991). Radiolabeled CP 96,345 has the potential for *in vitro* and *in vivo* localization of substance P receptor sites. Successful mapping of substance P receptor sites may yield useful diagnostic information in the study of arthritis, inflammatory diseases and carcinoid tumor (Payan, 1989). A preliminary account of the characterization of substance P receptor sites in guinea pig brain using [³H]CP 96,345 (Lowe *et al.*, 1991) has been published (McLean, 1991). The present work describes the synthesis of carbon-11 analogue CP 96,345 suitable for Positron Emission Tomography applications and its *in vivo* tissue distribution in guinea pigs.

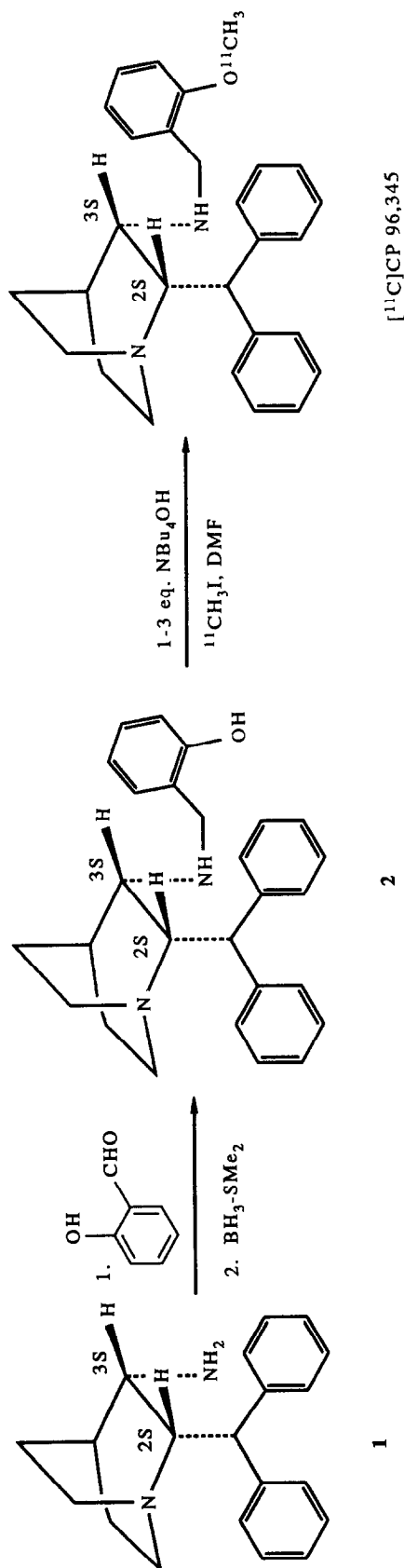
Experimental

Radiosynthesis

[¹¹C]CP 96,345, **3** (Fig. 1), was prepared by reaction of (2S,3S)-*cis*-2-(diphenylmethyl)-*N*-[(2-hydroxy-

phenyl)methyl]-1-azabicyclo[2.2.2]-octan-3-amine, **2**, with [¹¹C]methyl iodide. The desmethyl precursor **2** was prepared from the previously reported optically active compound **1** (Lowe *et al.*, 1992) by imine formation with salicylaldehyde (Fig. 1) followed by reduction with borane/methyl sulfide. Compound **2** (1-2 mg) was dissolved in 8-9 μL of 1 M tetrabutylammonium hydroxide in methanol (Aldrich Chemical Co.) in a septum-capped vial. The methanolic solution was evaporated under a gentle stream of nitrogen for 1-2 h and the residue of phenolate salt was dissolved in 150-200 μL of dimethyl formamide. The [¹¹C]methyl iodide was trapped in the solution at -40°C and the reaction mixture was subsequently heated at 85-90°C for 5 min. [¹¹C]CP 96,345 was purified by reverse phase HPLC using a Waters Bondapak CN column (3.9 × 300 mm) eluted with ethanol/0.1 M ammonium acetate (1:4). [¹¹C]CP 96,345 eluted at 19-20 min at a flow rate of 2.3 mL/min. Radiochemical yields of 30-50% (decay corrected) were obtained in a total synthesis time of 45-60 min to 1 h from end-of-beam. Analytical HPLC of the HPLC purified **3** showed a high radiochemical purity (< 95%) and a specific activity > 1000 Ci/mmol (end-of-synthesis). An authentic sample of CP 96,345 provided by Pfizer, was used as a reference standard. Radiochromatographic analysis was performed with a 4.6 × 250 mm Phenomenex Ultremex C18 IP using acetonitrile/0.1 M ammonium acetate (95:5, 3.0 mL/min) as solvent.

*Author for correspondence.

Fig. 1. Synthesis of 2 and [¹¹C]CP 96,345.Table 1. Brain uptake of [¹¹C]CP 96,345 in guinea pigs (*n* = 2)

Tissue	%Dose/g	
	5 min	30 min
Striatum	0.053	0.048
Cortex	0.038	0.046
Cerebellum	0.038	0.048
Hippocampus	0.032	0.045
Hypothalamus	0.039	0.042
Olfactory bulb	0.030	0.033

Animal studies

[¹¹C]CP 96,345 was formulated for animal injection by a nine-fold dilution of the HPLC purified product with physiologic saline. Guinea pigs were anesthetized with ether, administered 50–100 μ Ci via femoral vein injection of the above formulation and sacrificed at 5 and 30 min. Biodistribution data for two guinea pigs per each time point in the brain and peripheral tissues are given in Tables 1 and 2.

Results and Discussion

The chiral phenolic precursor 2 was prepared from the intermediate quinuclidine amine 1 (Lowe *et al.*, 1992) (Fig. 1) via imine formation and borane/methyl sulfide reduction. The radiosynthesis of [¹¹C]CP 96,345 was readily accomplished by O-methylation of 2 via *in situ* generation of its phenolate salt using excess tetrabutylammonium hydroxide in dimethylformamide. Co-injection of HPLC of the radio-labeled product along with authentic CP 96,345 gave a single radioactive peak (retention time = 19–22 min) coincident with the UV peak of [¹¹C]CP 96,345. The highly lipophilic nature of [¹¹C]CP 96,345 resulted in very long HPLC elution times using C-8 or C-18 columns which presented practical problems in product recovery and reformulation for animal studies. The use of a Bondapak CN column made it possible to elute the product in a buffer-ethanol (4:1) solvent system which could be conveniently diluted for subsequent injection into guinea pigs.

In vivo biodistribution of [¹¹C]CP 96,345 was performed in the guinea pig. This choice of animals model allowed a direct comparison between [³H]CP 96,345 and [¹¹C]CP 96,345 since *in vitro* results with the tritiated compounds have been published (McLean *et al.*, 1991). Furthermore, the regional binding of substance P to substance P(NK-1) receptors have been studied in the guinea pig brain (Dam

Table 2. Uptake of [¹¹C]CP 96,345 in peripheral tissues

Tissue	%Dose/g	
	5 min	30 min
Left ventricle	0.45	0.19
Left atrium	0.35	0.18
Lung	1.78	2.06
Liver	0.71	0.47
Spleen	1.51	0.71
Pancreas	0.35	0.27
Blood	0.048	0.084

and Quirion, 1986). Biodistribution data for [¹¹C]CP 96,345 in various brain tissues and peripheral tissues 5 and 30 min post-injection ($n = 2$ guinea pigs) are summarized in Tables 1 and 2. Table 1 shows that the overall brain uptake of [¹¹C]CP 96,345 was minimal, $\approx 0.1\%$ of the injected dose at 30 min. The distribution of activity was nearly uniform ($\approx 0.04\%$ dose/g) and nonselective in various regions of the brain, including the striatum, cerebellum, cortex, hypothalamus, hippocampus and olfactory bulb. The calculated variances for brain regions (Table 1) were very small with a maximum value of 0.0002 for the olfactory bulb. Brain activity levels were slightly lower than blood activity and ≈ 3 times lower than heart activity. Table 2 shows that highest peripheral accumulation of activity occurred in the spleen and lungs. For tissues in Table 1, the calculated variances were higher, ranging from 0.00006 to 0.670.

Regional selective binding of both [³H]substance P (Dam and Quirion, 1986) and [³H]CP 96,345 (Lowe *et al.*, 1991) to substance P receptors sites in the brain have been determined qualitatively. For example, qualitative autoradiographic studies of both [³H]CP 96,345 and [³H]substance P have indicated modest binding in the striatum, hippocampus and hypothalamus and dense binding in the olfactory bulb. By contrast, Table 1 shows that the *in vivo* localization of [¹¹C]CP 96,345 was nearly identical in the striatum, cerebellum, cortex, hypothalamus, hippocampus and olfactory bulb. Since the calculated variances in Table 1 were very small, additional *in vivo* studies with larger groups of animals were not conducted.

Based on the present preliminary evaluation of [¹¹C]CP 96,345, we conclude that CP 96,345 is unsuitable as an *in vivo* marker of substance P receptors in the brain. HPLC data and log P measurements ($\log P = 4.5$) indicate that the free base form of CP 96,345, with its two highly basic nitrogens, may exist

mostly in cationic form, either a monocation or possibly even a dication, thus precluding its passage through the blood-brain barrier. Alternative experiments for the low brain uptake, such as extensive binding to blood proteins, cannot be ruled out. PET studies with [¹¹C]CP 96,345 might be more appropriately applied to the detection of substance P receptors in tissue regions of interest involved with inflammatory disease and arthritis (Payan, 1989). Studies to determine the specific binding of [¹¹C]CP 96,345 to various peripheral tissues and its applications in animal models of such diseases are underway.

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