Synthesis and Biological Evaluation of Iodine-125 Iodocaramiphen. A Potential M_1 Muscarinic Imaging Agent for SPECT.

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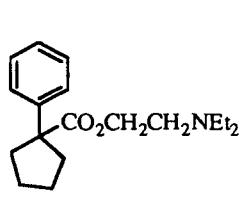
ABSTRACT

Iodocaramiphen (**2**) is a selective muscarinic antagonist which binds *in vitro* with high affinity and selectivity to the M_1 subtype of the muscarinic receptor. We report the synthesis of iodine-125 labeled iodocaramiphen ([^{125}I]-**2**) via a tributylstannyl intermediate (**3**) in 50 % radiochemical yield with a specific activity greater than 1000 mCi/μmol. Biodistribution studies in female Fischer rats demonstrated that [^{125}I]-**2** had significant cerebral localization (0.7 % injected dose / gram) at 60 minutes post injection. The uptake of activity washed out rapidly from the brain, however, and did not demonstrate specific uptake in those cerebral regions rich in muscarinic receptors. In addition, preinjection with (±)-QNB (5 mg/kg) blocked uptake of approximately 25% of the injected radiolabel in the brain at 2 hours. The non-selectivity of **2** toward muscarinic receptors *in vivo* may result from the metabolism of **2** by various esterases or the affinity of **2** for sigma sites in the brain.

KEYWORDS: Iodine-125, M_1 muscarinic receptor, Iodocaramiphen

INTRODUCTION

The antimuscarinic drug caramiphen (**1**) was originally described as an antispasmodic and anti-Parkinson agent and is an effective antidote for the treatment of organophosphate poisoning due to its ability to cross the blood-brain barrier (1). Recent *in vitro* binding studies have demonstrated that **1** is a selective muscarinic M_1 antagonist (2), with a 27-fold selectivity for the M_1 receptor subtype. An



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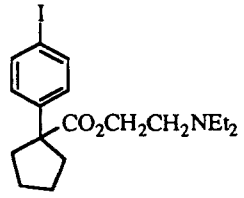

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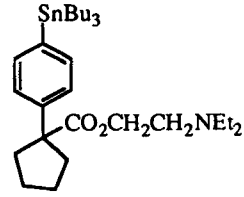

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examination of the effect of aromatic substituent on the *in vitro* binding affinity and receptor subtype selectivity of para-substituted caramiphen analogs led to the synthesis and identification of iodocaramiphen, which demonstrated a K_i of 2.1 nM and a 51-fold selectivity for the muscarinic M_1 receptor over the M_2 receptor subtype (3). The ability of caramiphen to penetrate the blood brain barrier and antagonize responses mediated by muscarinic receptors *in vivo* suggests that compounds from this series with greater M_1 selectivity would be useful agents for the study of muscarinic subtypes in the central nervous system (CNS).

Five gene products for the cloned muscarinic receptor have been identified in the CNS (4) , and although pharmacological tools have been developed to study the muscarinic M_1, M_2, and M_3 receptors (5), many of these agents have liabilities which render them less than ideal for conducting *in vivo* labeling studies in brain. For example, although pirenzepine is a selective ligand for the M_1 subtype *in vitro*, its ability to cross the blood-brain barrier is limited due to its hydrophilic nature. Other compounds which are selective for the M_1 receptor in binding assays and are able to enter the brain, such as dicyclomine and trihexyphenidyl, lack a sufficient separation of M_1 and M_2 affinity to render them useful for labeling studies.

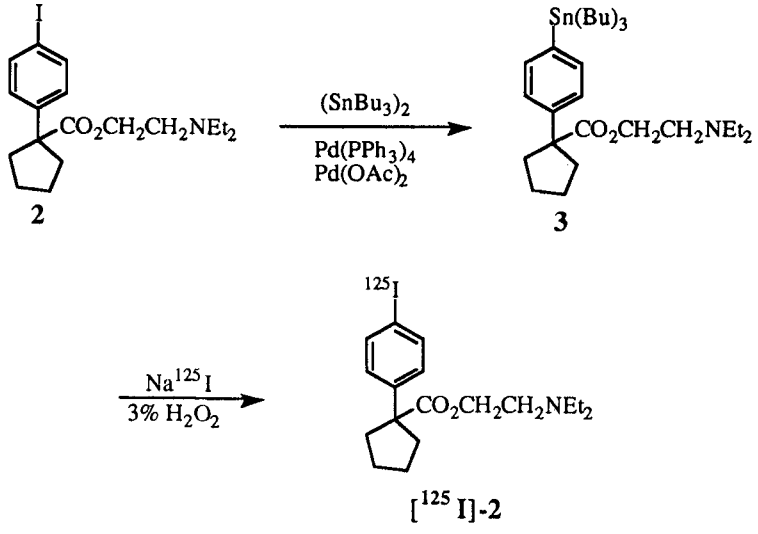
Several studies have shown that muscarinic receptors are altered in Alzheimer's disease (AD). Differences in the distribution of muscarinic receptors labeled with the SPECT agent [^{123}I]-3-quinuclidinyl-4-iodobenzilate (IQNB) have been reported in patients with AD (6). Studies of binding in brain homogenates prepared from AD patients have shown that M_1 sites labeled by [^{3}H]pirenzepine are increased in the striatum (7), and sites labeled with [^{3}H]QNB are increased in the cortex (8). The high-affinity component of agonist binding is reduced in the frontal cortex of patients with AD (9), and sites labeled by [^{3}H]acetylcholine are decreased in the cortex (10). Collectively the data suggest a loss of agonist binding sites and an up-regulation of remaining antagonist binding sites in areas known to be affected in AD. The identification of an M_1 selective muscarinic antagonist would be useful for labeling specific muscarinic receptor populations in the brain, and the availability of such a ligand would permit a greater understanding of the effects of AD on specific muscarinic receptor populations in the brain, possibly serving as a potential diagnostic agent for Alzheimer's and other dementias.

We report the synthesis of [^{125}I]-labeled iodocaramiphen ([^{125}I]-**2**) via a tributylstannyl intermediate (**3**) and its biodistribution in rats to evaluate its potential use as an M_1 selective muscarinic ligand for use in SPECT studies.

RESULTS AND DISCUSSION

Iodocaramiphen (2) was initially prepared via a triazine decomposition method (3). Although this approach has been successfully used for the preparation of a variety of radioiodinated compounds (11), we decided not to pursue this methodology due to the low radiochemical yields that have often been reported. Iododemetallation reactions involving the tributylstannyl group are well known, easily conducted, and result in high yield incorporation of radioactive iodine into molecules of biological interest. This method was therefore investigated for the preparation of [^{125}I]-2. p-(Tributylstannyl)-caramiphen (3) was prepared by treatment of 2 with bis(tributyltin) in the presence of palladium (II) acetate and tetrakis(triphenylphosphine) palladium (0) in triethylamine (Scheme 1). Flash column chromatography afforded 3 free of 2 as determined by thin layer chromatographic (TLC), nuclear magnetic resonance (NMR), and analytical high performance liquid chromatographic (HPLC) analyses. HPLC analysis indicated 3 was greater than 99.9% pure with no trace of 2 detected.

The reaction of 3 with sodium iodide-125 utilizing a 3% solution of hydrogen peroxide as the oxidant afforded [^{125}I]-iodocaramiphen ([^{125}I]-2) (Scheme 1) in 50% radiochemical yield after HPLC purification with a specific activity greater than 1000 mCi/μmol. Specific activity was determined from the mass observed by HPLC purification of the crude reaction mixture and comparing the mass associated with the UV absorbance at 254 nM of a standard curve derived from the HPLC trace of known solution concentrations of 2. By this technique the lowest level of detection of 2 that could be observed



2 3 [^{125}I]-2

Scheme 1

from the standard curve was approximately 2 nmol. No mass was observed to be associated with [^{125}I]-**2** from the UV-HPLC purification trace. In addition, [^{125}I]-**2** had analogous chromatographic properties as **2** when analyzed by TLC and HPLC.

The results of biodistribution studies in female rats with [^{125}I]-**2** over a four hour time period are shown in Table 1. Initial thyroid activity levels were low demonstrating an apparent *in vivo* stability of radioiodinated **2**. However since the activity washed quickly from the brain, a more detailed analysis of the metabolic stability was not performed. Both the brain and heart, which are rich in muscarinic receptors (12, 13), demonstrated high levels of activity post-injection of [^{125}I]-**2**. The maximum uptake of activity occurred approximately one hour post-injection and by four hours the activity had washed-out to approximately 15% of the level observed at one hour. In addition, the levels of activity were similar in areas of the brain rich in M_1 muscarinic receptors (cortex and striatum) compared to regions low in M_1 receptor concentrations (cerebellum). Blockage of muscarinic receptor sites by the preinjection of 5 mg/

Table 1. Biodistribution of [^{125}I]-Iodocaramiphen ([^{125}I]-**2**) in Female Fischer Rats (n=5).

	Mean Percent Injected Dose / Gram ± Standard Deviation					
	Time (minutes)					
Organ	15	30	60	120	120[a]	240
Blood	0.88±0.29	0.63±0.20	0.43±0.02	0.36±0.03	0.20±0.03	0.39±0.02
Heart	0.55±0.20	0.36±0.12	0.44±0.03	0.35±0.02	0.25±0.02	0.21±0.01
Cortex	0.47±0.18	0.35±0.13	0.79±0.06	0.43±0.02	0.31±0.02	0.10±0.01
Striatum	0.45±0.15	0.31±0.11	0.70±0.03	0.38±0.03	0.29±0.03	0.08±0.04
Cerebellum	0.40±0.17	0.32±0.13	0.72±0.08	0.40±0.04	0.33±0.03	0.11±0.02
Thyroid[b]	0.14±0.03	c	0.11±0.01	c	c	c

[a]Pretreatment with (±)-QNB

[b]% dose/organ

[c]not evaluated

kg of (±)-3-quinuclidinyl benzilate [(±)-QNB], a potent muscarinic antagonist, caused only a modest decrease (~25%) in the uptake of activity compared to the non-treated animals. It has been shown previously that the administered dose of 5mg/kg of (±)-QNB is sufficient to block the muscarinic receptor site by 90% (14).

Although iodocaramiphen is highly selective for the M_1 receptor *in vitro*, one explanation for its lack of selectivity for muscarinic receptors *in vivo* may be due to the affinity of iodocaramiphen for sigma sites in the brain (15). For example, high uptake of [^{125}I]-**2** was observed in the cerebellum, a region with a low concentration of muscarinic receptors but high concentrations of sigma sites (16). Alternatively, iodocaramiphen may be metabolized to an iodinated metabolite which lacks muscarinic activity, which would explain the inability of (±)-QNB to displace [^{125}I]-**2** *in vivo*.

These results, therefore, indicate that the use of [^{125}I]-**2** is not a candidate for the *in vivo* detection and imaging of M_1 subtype muscarinic receptors. The reason for the differences *in vitro* and *in vivo* are not known but may result from the metabolism of **2** by various esterases, or may be due to its affinity for sigma sites in brain.

MATERIALS AND METHODS

General. Triethylamine was distilled from calcium hydride and stored over potassium hydroxide prior to use. 2-(Diethylamino)ethyl 1-(p-iodophenyl)cyclopentane carboxylate (**2**) (3) and (±)-QNB oxalate (17) were prepared from literature methods. All other chemicals and solvents were analytical grade and were used without further purification. Sodium iodide-125 was purchased from New England Nuclear (specific activity ca. 2200 Ci/mmol). Sep-Paks (C-18) were purchased from Waters, Inc. and thin layer chromatographic analyses (TLC) were performed with silica gel GF-254 plates. High performance liquid chromatographic (HPLC) analysis was performed using a Uporasil semi-preparative (7.8 mm x 30 cm) column with an on-line Beckman Model 170 Radioisotope detector. Purification of [^{125}I]-**2** was performed using a Waters column. Proton (^{1}H) and carbon (^{13}C) NMR spectra were obtained with a Varian Gemini 200 instrument. Proton spectra are reported using tetramethylsilane as the internal standard and carbon spectra are reported using chloroform as the reference signal (77.0 ppm).

2-(Diethylamino)ethyl 1-(p-tributylstannylphenyl)cyclopentanecarboxylate (3).

A solution of 2-(diethylamino)ethyl 1-(p-iodopheny)cyclopentanecarboxylate (**2**) (154.1 mg, 0.37 mmol), bis(tributyltin) (287.0 mg, 0.49 mmol), palladium (II) acetate (13.7 mg, *0.06 mmol), and*

tetrakis(triphenylphosphine) palladium (0) (39.7 mg, 0.03 mmol) in 15 mL of triethylamine was heated at 80°C for 3.5 hours under an argon atmosphere. The mixture was then cooled, diluted with 100 mL of CH_2Cl_2 and filtered through a celite plug. The solution was evaporated to dryness under vacuum and purified by flash column chromatography. The crude reaction mixture was first applied to a silica column and the column eluted with chloroform followed by chloroform:methanol (96:4). Compound **3** was further purified utilizing another silica column and was eluted with hexane:ethyl acetate (4:1) to afford **3** as a pale yellow oil (80.5 mg, 38.4%). ^{1}H NMR ($CDCl_3$) δ 7.37 (d, 2H, ArH), 7.29 (d, 2H, ArH), 4.09 (t, 2H, -OCH_2-), 2.60 (m, 4H, -CH_2-), 2.46 (q, 4H, -NCH_2CH_3), 1.95-1.10 (m, 15H, -CH_2-), 1.00-083 (m, 15H, -CH_3). ^{13}C NMR ($CDCl_3$) δ 175.79 (CO), 142.78 (C), 139.81 (CSn), 136.23 (CH), 126.30 (CH), 63.15 (CH_2), 59.04 (C), 50.89 (CH_2), 47.45 (CH_2), 36.06 (CH_2) 29.13 (CH_2), 27.43 (CH_2), 23.69 (CH_2), 13.73 (CH_3), 11.96 (CH_3), 9.62 (CH_2). TLC (Silica, 50% Hexane:Ethyl acetate) R_f=0.85.

2-(Diethylamino)ethyl 1-(p-[^{125}I]-iodophenyl)cyclopentanecarboxylate ([^{125}I]-2).

To a 3.0 mL reacti-vial was added 50 μL of 3% H_2O_2 solution (30% solution diluted with distilled water before use), 50 μL of a 0.1 N HCl solution, and 100 μL of a solution of **2** (1.2 mg/mL in ethanol). A solution of sodium iodide-125 (5.0 mCi) in 100 μL of ethanol was then added, the vial sealed, and stirred at room temperature for 30 minutes. To this solution 250 μL of a 5% sodium bisulfite solution was then added followed by the slow addition of 1.0 mL of a saturated sodium bicarbonate solution. The solution was transferred to a syringe containing a C-18 Sep-Pak and diluted to 10 mL with water. The solution was passed through the Sep-Pak and the Sep-Pak washed with 2 X 5 mL water followed by 250 μL of acetonitrile. The product was removed from the Sep-Pak with 3 mL of chloroform. The solution was evaporated to dryness under a stream of argon and taken up in a minimum amount of HPLC solvent and injected into the HPLC using a semi-preparative normal phase column (Mobile phase: hexane:ethyl acetate (75:25); Flow rate: 3.0 mL/min; Retention time 12 minutes). The fractions containing [^{125}I]-**2** were collected and evaporated to dryness under an argon stream, which afforded 2.5 mCi (50.0%) of **2** with a specific activity of >1000 mCi/μmol. [^{125}I]-**2** was analyzed by comparing the TLC (Silica, Hexane:Ethyl acetate (1:1)) (R_f=0.48) and HPLC mobility to that of the cold compound. In both cases [^{125}I]-**2** co-chromatographed with the cold standard and was observed to be >98% radiochemically pure.

Biodistribution Studies

Biodistribution studies were performed using female Fisher VAF rats (150-163 g) housed and cared for in accordance with the "Guide for the Care and Use of Laboratory Animals" (NIH PUBL. No. 86-23, 1985) and the Animal Welfare Act, and the protocol was approved by the ORNL Animal Care and

Use Committee. For these studies [^{125}I]-**2** was dissolved in 100 μL of 0.1 N HCl and diluted to 10 mL with saline containing 10 % ethanol. Following intravenous injection of [^{125}I]-**2** (1 μCi) into the lateral tail vein, the rats were then allowed food and water *ad libitum* during the course of the experiment. At various time points the animals were anesthetized and sacrificed by cervical fracture. The various organs were removed, rinsed with saline, blotted dry and weighed in tared vials. The brains were stored over dry ice prior to dissection (18). Blood samples were obtained from the heart cavity after removal of the heart. The samples were counted in a Packard Minaxi 5000 sodium iodide auto gamma counter.

For the blocking experiment with (±)-QNB, one group of animals was injected intravenously with (±)-QNB (5 mg/kg) one hour prior to the intravenous injection of [^{125}I]-**2** and another set of animals was injected with [^{125}I]-**2** as the control group. Two hours after the injection of [^{125}I]-**2**, the animals were sacrificed and treated as above.

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