Synthesis and Biodistribution of Iodine-125-Labeled 1-Azabicyclo[2.2.2]oct-3-yl α-Hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate. A New Ligand for the Potential Imaging of Muscarinic Receptors by Single Photon Emission Computed Tomography

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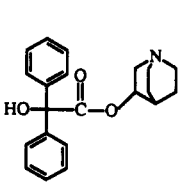
1-Azabicyclo[2.2.2]oct-3-yl α-hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate (IQNP, **3**), an analogue of QNB in which a phenyl ring has been replaced with an iodopropenyl substituent, was prepared and evaluated in vitro and in vivo for m-AChR selectivity and specificity. High specific activity [^{125}I]IQNP ([^{125}I]-**3**) was synthesized in greater than 60% yield utilizing an electrophilic iododestannylation reaction with hydrogen peroxide for the oxidation of iodide. In in vitro receptor binding studies, **3** demonstrated high affinity for M_1 (K_i = 0.78 nM), M_2 (K_i = 1.06 nM), and M_3 (K_i = 0.27 nM) subtypes. In vivo biodistribution studies in female rats [^{125}I]-**3** demonstrated high uptake in areas rich in muscarinic receptors such as the brain (cortex and striatum) and the heart. Blocking studies were performed with a series of receptor specific agents and demonstrated that the uptake of [^{125}I]-**3** was selective and specific for cerebral muscarinic receptor rich areas and that the binding to m-AChR is reversible. The high-yield preparation and specificity and selectivity of high specific activity [^{125}I]IQNP for muscarinic receptors suggest that this is an attractive new agent for potential imaging of cerebral receptors using single photon tomographic imaging (SPECT).

Introduction

Variations in the density (population) or activity of muscarinic cholinergic receptors (m-AChR) play an essential role in many physiological and behavioral responses. Changes in m-AChR have been implicated in various disease states such as Parkinson's disease, Huntington's chorea, and Alzheimer's dementia.[1-6] These observations have stimulated interest in the ability to image the distribution of cerebral m-AChR binding sites noninvasively in vivo with external imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT). 1-Azabicyclo-[2.2.2]oct-3-yl α-hydroxy-α-(4-iodophenyl)-α-phenylacetate (4IQNB) (**2**) was developed as an analogue of the highly potent muscarinic antagonist, 1-azabicyclo[2.2.2]-oct-3-yl α-hydroxy-α,α-phenylacetate (QNB) (**1**), for imaging of m-AChR in the central nervous system.[7-10] When labeled in high specific activity with iodine-125 the uptake of this ligand was highly selective for m-AChR.[7] The (*R*,*R*)-4IQNB isomer, when labeled in high specificity with iodine-123, has been used to obtain images of the distribution of m-AChR in both healthy individuals and patients with dementias.[11-14] It has also been demonstrated in both rats and humans that there is a positive correlation between the concentration of m-AChR and 4IQNB in various cerebral structures.[9,13,15]

The reported low radiochemical yield, however, is one of the major disadvantages for the routine use of **2** for the imaging of m-AChR in human studies.[7] The radiolabeling method which was developed for the preparation of [^{125}I]- and [^{123}I]-4IQNB involves the acid-induced triazene decomposition method which proceeds in low yield (~18%). Since the cost for large amounts of iodine-123 with high specific activity for patient studies is relatively high, reproducibility high yields are essential for extensive clinical studies to be feasible. Other radiolabeling methods of **2** that have recently been reported in efforts to overcome the low radiochemical yield include either the addition of carrier iodine[16] or the copper(I)-assisted nucleophile exchange of 4IQNB.[14] These alternative approaches, however, may result in relatively low specific activity of the final radiopharmaceutical.



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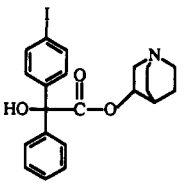

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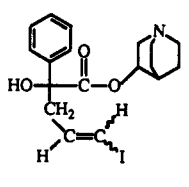

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In this paper we report the synthesis, in vitro, and in vivo evaluation of a new QNB analogue, 1-azabicyclo[2.2.2]-oct-3-yl α-hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate (IQNP, **3**), in which one of the phenyl rings of QNB is replaced by an iodopropenyl group. Earlier reports of various analogues of **1** have indicated that changes could be made in place of one of the phenyl rings without greatly affecting the affinity for the m-AChR receptor.[17] These studies have shown that analogues in which a phenyl ring is replaced with a cyclopentyl, cyclohexyl, or *n*-butyl group are almost equipotent with QNB. Space-filling models show that the carbon backbone of the allyl group closely mimics the phenyl ring. The replacement of a phenyl ring with an iodopropenyl group would also afford a ligand in which the iodine moiety could readily be introduced by a variety of well-established, high-yield approaches.[18,19] In addition, the vinyl iodide group allows incorporation of the iodine in a metabolically stable position,[18] as we have previously demonstrated with a variety of radiopharmaceuticals including fatty acids,[20-24] barbiturates,[25,26] phosphonium cation analogues,[27,28] carbohydrates,[29] and

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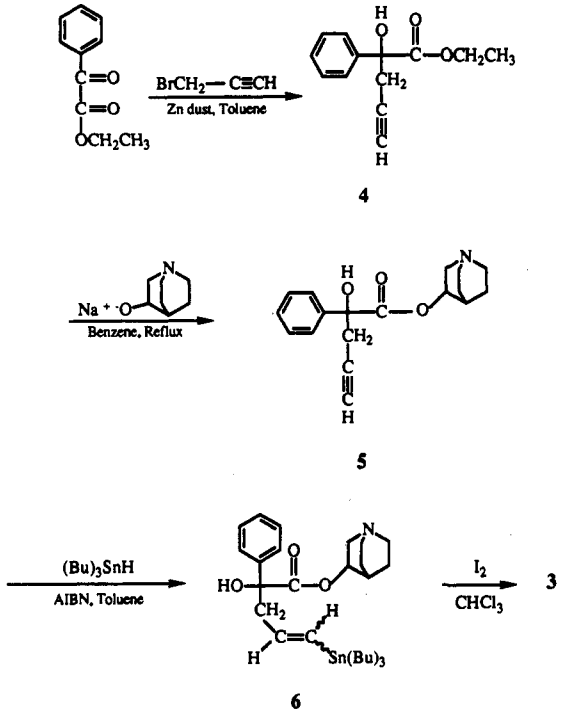
Scheme I



most recently, spiroperidol analogues.[30] We report the synthesis, in vitro evaluation, and the radiolabeling of IQNP (3) with iodine-125, as well as the biodistribution, in vivo displacement studies, and competitive binding studies with this new ligand.

Chemistry

IQNP (3) was prepared as shown in Scheme I. Ethyl α-hydroxy-α-phenyl-α-(1-propyn-3-yl)acetate (4) was prepared by the Reformatsky reaction of ethyl benzoylformate with propargyl bromide and zinc dust in anhydrous toluene. When the reaction was performed neat, the decomposition of propargyl bromide resulted in a low yield of 4 due to the violent exothermic nature of the reaction which was difficult to control. Transesterification of 4 with the sodium salt of 3-quinuclidinol afforded 1-azabicyclo[2.2.2]octyl-3-yl α-hydroxy-α-phenyl-α-(1-propyn-3-yl)acetate (5).

Treatment of 5 with excess tributyltin hydride in the presence of a slight molar excess of the 2,2-azobis(2-methylpropionitrile) (AIBN), a radical initiator, at 80 °C afforded a mixture of (*E*)- and (*Z*)-1-azabicyclo[2.2.2]octyl-3-yl α-hydroxy-α-(1-(tributylstannyl)-1-propen-3-yl)-α-phenylacetate (6) in an approximately 1:1 ratio as determined by thin-layer chromatographic (TLC) and nuclear magnetic resonance (NMR) analysis. Since complete separation of the two isomers by flash column chromatography could not be achieved, the isomeric mixture was thus carried to the next step without further separation. When temperatures higher than 80 °C were employed in the stannylation sequence the product yield was decreased due to the subsequent decomposition of 6.

Compound 6 was then treated with iodine in chloroform to afford an approximately 1:1 mixture of (*E*)- and (*Z*)-1-azabicyclo[2.2.2]octyl-3-yl α-hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate (3). Although TLC analysis indicated the presence of a single isomer, high-pressure liquid chromatographic (HPLC) and NMR analysis of 3 indicated an approximate equal mixture of the (*E*)- and (*Z*)-isomers. As above, the (*E*)- and (*Z*)-isomers of 3 could not be separated by flash column chromatography.

Scheme II

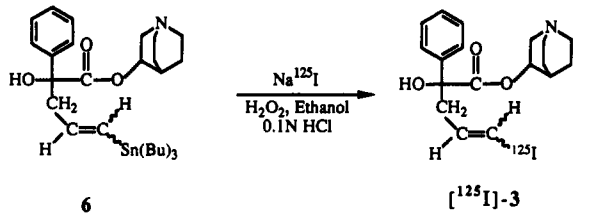


Table I. Muscarinic Binding Affinity of 3 and Various Muscarinic Antagonists[a]

compound	M_1 rat cortex K_i, nM	M_2 rat heart K_i, nM	M_3 rat submaxillary gland K_i, nM
atropine	0.26 ± 0.01[b]	0.76 ± 0.03[b]	0.12 ± 0.01
pirenzepine	5.21 ± 1.13[b]	267 ± 25[b]	28 ± 0.5
(*R*)-(−)-QNB	0.15 ± 0.021[b]	0.03 ± 0.01[b]	0.16 ± 0.01
IQNP (3)	0.78 ± 0.10	1.06 ± 0.17	0.27 ± 0.02

[a] Data are the mean ± SEM (n = 3). [b] Reference 41.

Radiochemistry

IQNP (3) was radioiodinated by the procedure shown in Scheme II, which involves iododestannylation of 6 by treatment with sodium [^{125}I]iodide and a 3.0% solution of hydrogen peroxide at room temperature followed by HPLC purification. [^{125}I]-3 was obtained in an overall radiochemical yield of ~60% with a specific activity of ~1500 mCi/μmol. Specific activity was determined from the mass observed from the HPLC UV trace.

We initially investigated the use of *N*-chlorosuccinimide as the oxidizing agent, but this led to a low radiochemical yield (10–15%) with most of the activity remaining as unreacted [^{125}I]ICl as determined by TLC analysis. We therefore investigated the use of hydrogen peroxide as the oxidizing agent. The use of hydrogen peroxide would avoid the formation of potential chlorinated byproducts and make the final purification of the product less complicated. We observed that the use of a 30% hydrogen peroxide solution resulted in an unacceptable low yield of [^{125}I]-3 (<5.0%) with the formation of several undetermined radioiodinated byproducts as determined by HPLC analysis. The use of a 3.0% hydrogen peroxide solution resulted in greater than 80% crude radiochemical yield and greater than 60% radiochemical yield after HPLC purification.

Biological Results

Radioligand binding data for IQNP (3) and reference muscarinic agents are shown in Table I. Compounds were evaluated for binding by displacement of [^{3}H]pirenzepine (PZ) from M_1 sites in rat cortex homogenates, [^{3}H]-(−)-quinuclidinyl benzilate (QNB) from M_2 sites in rat heart homogenates, and [^{3}H]-*N*-methylscopolamine (NMS) from M_3 sites in rat submaxillary gland homogenates. The results show that 3 binds with high affinity to the M_1, M_2, and M_3 subtype muscarinic receptor; however, 3 does not appear to demonstrate selectivity between the subtypes of m-AChR.

The results of biodistribution studies of [^{125}I]-3 in female rats over a 6-h time period are shown in Table II. These data demonstrate that those cerebral regions which are rich in m-AChR (striatum and cortex)[31] have the highest accumulation of radioactivity over the time period studied. The cerebellum, which contains a low concen-

Table II. Biodistribution of [^{125}I]QNP ([^{125}I]-**3**) in Female Fisher VAF Rats[a]

organ	mean percent injected dose/gram ± SD at time (min)					
	15	30	60	120	240	360
blood	0.85 ± 0.15	0.62 ± 0.05	0.38 ± 0.05	0.38 ± 0.03	0.27 ± 0.04	0.21 ± 0.02
liver	1.46 ± 0.18	1.10 ± 0.05	0.80 ± 0.06	0.95 ± 0.04	0.75 ± 0.08	0.65 ± 0.07
kidney	5.09 ± 0.66	2.95 ± 0.27	1.40 ± 0.22	0.67 ± 0.07	0.30 ± 0.04	0.20 ± 0.02
heart	1.44 ± 0.14	1.11 ± 0.06	0.77 ± 0.05	0.68 ± 0.05	0.28 ± 0.03	0.17 ± 0.02
lung	4.19 ± 0.48	2.94 ± 0.17	1.60 ± 0.15	0.96 ± 0.13	0.36 ± 0.04	0.22 ± 0.02
cortex	0.99 ± 0.09	0.80 ± 0.09	0.86 ± 0.05	0.64 ± 0.02	0.54 ± 0.04	0.47 ± 0.06
striatum	0.82 ± 0.10	0.69 ± 0.11	0.86 ± 0.11	0.93 ± 0.12	0.52 ± 0.04	0.41 ± 0.07
cerebellum	0.54 ± 0.05	0.36 ± 0.02	0.30 ± 0.02	0.24 ± 0.01	0.09 ± 0.01	0.06 ± 0.01
rest of brain	0.72 ± 0.06	0.53 ± 0.04	0.58 ± 0.07	0.41 ± 0.03	0.32 ± 0.02	0.27 ± 0.03

[a] Groups of 5 rats/time point.

tration of m-AChR,[32] had only low uptake of radioactivity which continued to decrease over the course of the study. Although radioactivity which initially accumulated in the striatum and cortex decreased over the time period studied, the striatum to cerebellum and the cortex to cerebellum ratios increased after 6 h to values to 6.8:1 and 7.8:1, respectively. This increase resulted from the washout of nonspecifically bound activity which occurs more rapidly than the washout of activity in the striatum and cortex.

Several key differences are observed when these results are compared to those reported for [^{125}I]-4IQNB[8] and [^{125}I]-(*R*,*S*)-4IQNB.[9] The distribution of activity in the lungs, liver, and kidneys was similar for the two agents over the 6-h study, but differences are observed in a comparison of the cerebral uptake of [^{125}I]-**3** with that reported for [^{125}I]-4IQNB and [^{125}I]-(*R*,*S*)-4IQNB. In the present study uptake of [^{125}I]-**3** in the cortical and striatal regions is higher than values reported for [^{125}I]-(*R*,*S*)-4IQNB, and the radioactivity appears to be retained to a greater degree in these cerebral regions even after 6 h. The radioactivity associated with the cortex and striatum also decreased over the time period of the study by ~50% as compared to ~60% for [^{125}I]-(*R*,*S*)-4IQNB. However, the activity associated with the cerebellum for [^{125}I]-**3** is higher than that reported for [^{125}I]-(*R*,*S*)-4IQNB. Since the cerebellum has been reported to contain a low concentration of m-AChR[32] and global cerebral uptake of [^{125}I]-**3** is higher as compared to 4IQNB (**2**), the activity in the cerebellum may be a result of binding to the receptor sites found in the cerebellum and may not be a true indication of nonspecific binding.

The accumulation of radioactivity in the heart, which contains a large population of m-AChR,[33,34] was also observed to be higher for [^{125}I]-**3** than for **2**. Although radioactivity in the blood was higher for [^{125}I]-**3** as compared to [^{125}I]-(*R*,*S*)-4IQNB,[9] blood activity decreased during this study in contrast to that reported for 4IQNB where the blood level of radioactivity remained constant over a six hour time period. The heart to blood ratio of [^{125}I]-**3** reached a value of 2:1 after 2 h and decreased to 1:1 by 6 h.

These combined results suggest that the cerebral distribution of [^{125}I]-**3** with high uptake in m-AChR-rich areas of the brain is similar to [^{125}I]-(*R*,*S*)-4IQNB. Further studies were thus designed to substantiate that the uptake may result from a receptor-mediated process rather than only blood delivery and nonspecific binding. A series of blocking studies were performed in which a saline solution of (±)-QNB (5 mg/kg) was injected in rats 1 h prior to the intravenous administration of [^{125}I]-**3**. The rats were sacrificed after 3 h, and the heart, brain, and blood were removed and analyzed for radioactivity. In another series

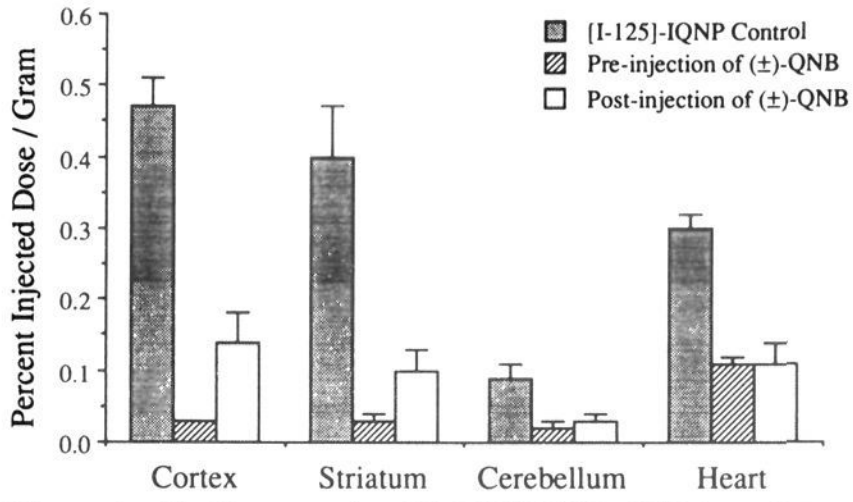


Figure 1. Challenge study with [^{125}I]IQNP ([^{125}I]-**3**) and (±)-QNB.

of rats, [^{125}I]-**3** was injected 1 h prior to the injection of a saline solution of (±)-QNB (5 mg/kg), and the rats were sacrificed 2 h later and the organs removed and assayed for radioactivity. It has been shown that co-injection of 500 nmol/rat of (*R*)-QNB blocks 80% of the uptake of [^{125}I]-4IQNB that localizes in the caudate putamen and cerebellum.[8] Therefore 5 mg/kg (1870 nmol/rat) of (±)-QNB will allow for the saturation of the receptor site. The results of this study are shown in Figure 1. These data clearly indicate that uptake of [^{125}I]-**3** into m-AChR-rich areas of the brain was blocked by greater than 90% by the preinjection of QNB. It was observed that the binding of [^{125}I]-**3** could be displaced by ~80% by the injection of QNB after administration of [^{125}I]-**3**. Although a kinetic evaluation of the uptake of [^{125}I]-**3** has not been performed, these results indicate that the uptake of activity into the brain may be due to a receptor-mediated process and thus is not simply an indication of blood flow, nonspecific, or nonreversible binding to the receptor site.

To determine the selectivity of [^{125}I]-**3** for m-AChR, an additional series of experiments were performed in which different unlabeled receptor specific ligands (5 mg/kg) were preinjected 1 h prior to the injection of [^{125}I]-**3**. The rats were then sacrificed 3 h following the injection of [^{125}I]-**3** and the blood, heart, and brain regions dissected as described above. The receptor-specific ligands that were employed in these studies were spiperone (D_2 dopamine antagonist), (+)-butaclamol (D_2/D_1 dopamine antagonist), (−)-butaclamol (inactive enantiomer), (*S*)-(+)-dexetimide (muscarinic antagonist), (±)-QNB (muscarinic antagonist), and ketanserin (5-HT_2 antagonist). The dose chosen was sufficient to appreciably block the various receptors over the time course of the experiment.[8,35,36] The results of this study, shown in Figure 2, clearly illustrate that only dexetimide and QNB, both muscarinic antagonists, block the uptake of [^{125}I]-**3** in receptor-rich areas and that this blockage of uptake is not due to the large doses of challenge

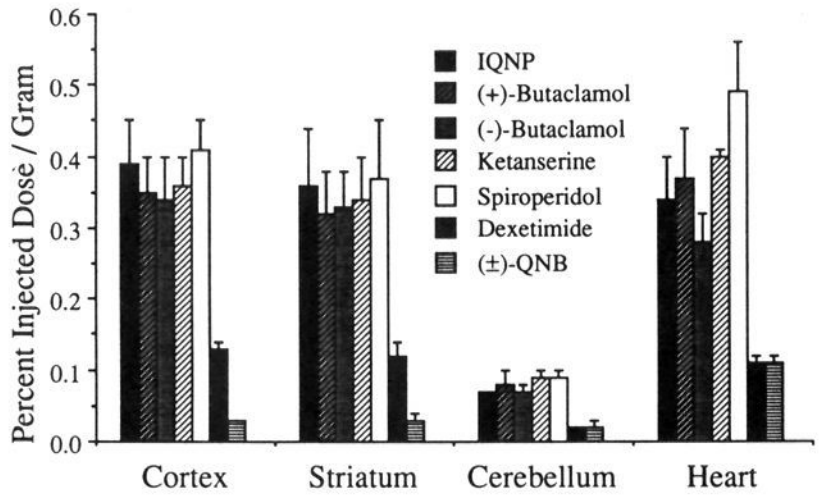


Figure 2. Challenge studies of [125I]IQNP ([125I]-**3**) with various receptor specific ligands.

ligands employed in this study. The other blocking agents showed no effect on the uptake of [125I]-**3**. The results of these experiments also demonstrate that QNB blocked greater than 90% of the uptake of **7** as compared to ~65% for dexetimide. This result may suggest that the half life of dexetimide at the receptor site is not as long as QNB and therefore [125I]-**3** may be useful in studies which require a longer protocol from injection of the tracer to the time of the imaging study. QNB and dexitimide also block the uptake of activity in the cerebellum, which suggests that a majority of activity associated with the cerebellum is not due to nonspecific binding but the activity is bound to the small concentration of m-AChR located in this region. This data also demonstrates that QNB and dexetimide block uptake of [125I]-**3** in the heart by ~60%, which is in agreement with values reported for other radioiodinated m-AChR ligands.

Conclusions

We have prepared 1-azabicyclo[2.2.2]oct-3-yl α-hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate (IQNP, **3**) through a tributylstannyl intermediate (**6**). The radiolabeling of **3** was accomplished in one step utilizing hydrogen peroxide as the oxidizing agent to afford 1-azabicyclo[2.2.2]oct-3-yl α-hydroxy-α-([125I]-1-iodo-1-propen-3-yl)-α-phenylacetate([125I]-**3**) with high specific activity and in high radiochemical yield. Biodistribution studies in female rats demonstrated uptake in m-AChR-rich areas of the brain with an increasing cortex to cerebellum ratio and striatum to cerebellum ratio of 7.8:1 and 6.8:1, respectively, after 6 h. Theoretical cortex to cerebellum and striatum to cerebellum ratios of 15:1 and 11:1, respectively, determined from receptor concentrations have been reported[15] for in vitro studies. Uptake in receptor poor areas of the brain (cerebellum) was low and non-specific binding decreased over time. Activity also accumulated in the heart with a heart to blood ratio of about 2:1 at 2 h, which decreased to about 1:1 over the time frame of the experiment. When these results were compared to the biodistribution data reported for [125I]-4IQNB and [125I]-(*R*,*S*)-4IQNB, [125I]-**3** had the greatest uptake in receptor rich areas. Blocking studies performed with (±)-QNB, a potent muscarinic antagonist, demonstrated that uptake of activity in the striatum and cortex is specific and reversible for m-AChR. In addition, blocking studies performed with a series of receptor specific ligands have demonstrated that the uptake of activity is selective for muscarinic receptors with only dexetimide and QNB blocking the uptake of [125I]-**3**. These results therefore demonstrate that 1-azabicyclo[2.2.2]oct-3-yl α-hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate (IQNP, **3**) is specific and selective for m-AChR, and the replacement of a phenyl ring of QNB with an iodopropenyl group overcomes the problem of a low radiochemical yield. The separation of the various stereoisomers of IQNP is in progress to identify the optimal stereoisomer for further evaluation and future imaging studies. Initial studies have shown that (*E*,*Z*)-(*R*)-azabicyclo[2.2.2]oct-3-yl (*R*,*S*)-α-hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate does have higher uptake in m-AChR-rich areas of the brain in addition to the heart. This is in agreement with that reported for QNB in which the *R* isomer of the quinuclidinyl moiety is the active isomer of the racemic mixture.[37]

Experimental Section

General. Anhydrous toluene was purchased from Aldrich Chemical Co. Triethylamine was distilled from calcium hydride and stored over potassium hydroxide prior to use. Ketanserin tartrate, (+)- and (−)-butaclamol hydrochloride, and (*S*)-(+)-dexetimide hydrochloride were purchased from Research Biochemicals Inc. (Natick, MA). (±)-QNB oxalate was prepared from a literature method[38] and spiroperidol was purchased from Janssen Pharmaceutical (Belgium). [3H]PZ (87 Ci/mmol), [3H]-(−)-QNB (31.9 Ci/mmol) and [3H]NMS (81.9 Ci/mmol) were purchased form New England Nuclear (Boston, MA). All other chemicals and solvents were analytical grade and were used without further purification. Sodium [125I]-iodide was purchased from New England Nuclear (specific activity ca. 2500 Ci/mmol). Sep-Paks (C18) were purchased from Waters, Inc. Thin-layer chromatographic analyses (TLC) were performed with silica gel GF-254 plates. Analytical high-performance liquid chromatographic (HPLC) analysis was performed using a Whatmann Partisil 5 (3.9 mm × 30 cm) column with an on-line Beckman Model 170 Radioisotope detector. Purification of [125I]-**3** was performed using a Waters Uporasil semipreparative (7.8-mm × 30-cm) column. Proton (1H) and carbon (13C) 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). Elemental analyses were determined at Galbraith Laboratories (Knoxville, TN).

Ethyl α-Hydroxy-α-phenyl-α-(1-propyn-3-yl)acetate (4). Zinc dust (8.12 g, 124.2 mmol), anhydrous toluene (20 mL), ethyl benzoylformate (10.54 g, 59.2 mmol), and propargyl bromide (7.08 g, 59.5 mmol) were added to a flask under argon. A water-cooled condenser was attached to the flask and the solution heated in an oil bath at 100 °C for 4.5 h. The solution was then cooled to room temperature, diluted with 200 mL of chloroform, and poured into an ice/1.0 M HCl solution (1:1 v/v). The mixture was transferred to a separatory funnel, and the organic layer was separated. The aqueous layer was washed with 100 mL of chloroform, and the organic fractions were combined and washed with 100 mL of water. The organic solution was then dried over sodium sulfate, filtered, and evaporated to dryness to afford a dark orange-colored oil. The product was purified twice by column chromatography. The first column used a gravity silica column with a mobile phase of hexane/ethyl acetate (9:1). The final purification was performed using a silica flash column with a mobile phase of hexane/ethyl acetate (9:1). The purified product was isolated as a pale yellow liquid (4.35 g, 33.6%); 1H NMR ($CDCl_3$) δ 7.61 (m, 2 H), 7.32 (m, 3 H), 4.26 (m, 2 H), 4.02 (bs, 1 H), 3.21 (d, 1 H, J = 2.6 Hz), 3.12 (d, 1 H, J = 2.6 Hz), 2.86 (d, 1 H, J = 2.6 Hz), 2.78 (d, 1 H, J = 2.6 Hz), 2.03 (t, 1 H, J = 2.6 Hz), 1.27 (t, 3 H); 13C NMR ($CDCl_3$) δ 173.13 (CO), 139.87 (C), 128.12 (CH), 127.97 (CH), 125.19 (CH), 79.38 (C), 77.16 (C), 70.96 (CH), 62.64 (CH_2), 30.82 (CH_2), 13.98 (CH_3); TLC (silica, 10% hexane/ethyl acetate) R_f = 0.25.

1-Azabicyclo[2.2.2]oct-3-yl α-Hydroxy-α-phenyl-α-(1-propyn-3-yl)acetate (5). 3-Quinuclidinol (2.70 g, 21.2 mmol) and benzene (100 mL) were combined in a dry flask. The solution was refluxed for 1 h under an argon atmosphere utilizing a Dean–Stark trap to remove any moisture. Freshly cut pieces of sodium

metal (ca. 1 g, ca. 0.04 mol) were then added, and the solution was refluxed for 1 h. The solution was allowed to cool slightly and was transferred, via double-tipped needle technique to remove unreacted sodium metal, into a flask which contained a solution of 4 (2.15 g, 9.8 mmol) which had been refluxed for 1 h under argon utilizing a Dean–Stark reflux head to remove traces of moisture. The mixture was then refluxed utilizing a Dean–Stark reflux head for 24 h. The solution was evaporated to dryness under vacuum and then diluted with water (100 mL). The resulting solution was then washed twice with 100 mL of chloroform. The organic fractions were combined, washed twice with 200 mL of water, dried over magnesium sulfate, and evaporated to dryness to afford a dark orange oil. The product was purified by silica column chromatography (chloroform/methanol/ammonium hydroxide [9:1:0.1]) to afford **5** as an orange oil (693.2 mg, 23.6%): ^{1}H NMR ($CDCl_3$) δ 7.65–7.50 (m, 2 H), 7.40–7.20 (m, 3 H), 4.86 (m, 1 H), 4.46 (bs, 1 H), 3.30–3.10 (m, 2 H), 2.95–2.60 (m, 6 H), 2.15–1.90 (m, 1 H), 2.07 (t, 1 H), 1.80–1.20 (m, 4 H); ^{13}C NMR ($CDCl_3$) δ 173.04 (CO), 140.19 (C), 128.22 (CH), 127.91 (CH), 125.24 (CH), 79.27 (C), 77.07 (C), 73.96 (CH), 71.16 (CH), 54.86 (CH_2), 47.10 (CH_2), 46.17 (CH_2), 30.87 (CH_2), 25.01 (CH), 24.25 (CH_2), 19.48 (CH_2); TLC (silica, 20% chloroform:methanol) R_f = 0.33.

1-Azabicyclo[2.2.2]oct-3-yl α-Hydroxy-α-phenyl-α-(1-(tributylstannyl)-1-propen-3-yl)acetate (6). To a clean dry flask under an argon atmosphere was added **5** (693.2 mg, 2.3 mmol), anhydrous toluene (15 mL), 2,2-azobis(2-methylpropionitrile) (AIBN) (424.0 mg, 2.6 mmol), and tributyltin hydride (919.7 mg, 3.2 mmol). The solution was stirred under argon at 75 °C for 5 h and then cooled to room temperature. The mixture was diluted with chloroform (100 mL), washed with water (100 mL), and dried over sodium sulfate to afford a yellow oil upon evaporation of the solvent. The product was purified by silica column chromatography by initial elution with chloroform containing 1.0% ammonium hydroxide to remove tributyltin hydride followed by chloroform/methanol/ammonium hydroxide (95:5:1) to afford **6** as a pale yellow oil (550.4 mg, 40.3%): ^{1}H NMR ($CDCl_3$) δ 7.62–7.58 (m, 2 H), 7.37–7.25 (m, 3 H), 6.17–5.89 (m, 2 H), 4.83–4.75 (m, 1 H), 4.10 (bs, 1 H), 3.25–3.03 (m, 2 H), 2.91–2.67 (m, 6 H), 2.03–1.90 (m, 1 H), 1.67–1.17 (m, 16 H), 0.97–0.67 (m, 15 H); ^{13}C NMR ($CDCl_3$) δ 174.25 (CO), 142.22 (CH), 141.55 (C), 133.94 (CH), 127.94 (CH), 127.48 (CH), 125.45 (CH), 77.12 (C), 73.51 (CH), 50.03 (CH_2), 48.01 (CH_2), 47.27 (CH_2), 46.31 (CH_2), 29.02 (CH_2), 27.24 (CH_2), 25.01 (CH), 24.31 (CH_2), 19.41 (CH_2), 13.70 (CH_3), 9.49 (CH_2); TLC (silica, 10% chloroform/methanol) R_f = 0.50.

1-Azabicyclo[2.2.2]oct-3-yl α-Hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate (3). Compound **6** (475.5 mg, 0.80 mmol) was dissolved in 10 mL of chloroform, and a 0.85 M solution of iodide in chloroform was slowly added until the color of iodine persisted. The solution was stirred at room temperature for 18 h. The solution was then diluted with chloroform (50 mL) and washed with a 10% sodium bisulfite solution (50 mL) and water (50 mL). The organic solution was dried over sodium sulfate and evaporated to dryness to afford a pale yellow oil. Purification by column chromatography (silica, chloroform/methanol/ammonium hydroxide [95:5:1]) afforded **3** as a pale yellow oil (163.3 mg, 47.7%): mp 151–2 °C (oxalate, from ether/ethanol); ^{1}H NMR ($CDCl_3$) δ 7.62–7.51 (m, 2 H), 7.40–7.25 (m, 3 H), 6.64–6.45 (m, 1 H), 6.35–6.12 (m, 1 H), 4.87–4.78 (m, 1 H), 4.20 (bs, 1 H), 3.29–2.30 (m, 8 H), 2.00–1.20 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 173.63 (CO), 140.95 (C), 140.00 (CH), 128.19 (CH), 127.86 (CH), 125.18 (CH), 78.87 (CH), 77.22 (C), 73.85 (CH), 54.95 (CH_2), 47.15 (CH_2), 46.20 (CH_2), 45.89 (CH_2), 24.98 (CH), 24.25 (CH_2), 19.39 (CH_2); TLC (silica, 10% chloroform/methanol) R_f = 0.34. Anal. ($C_{20}H_{24}INO_7 \cdot 0.5H_2O$) Calcd: C, 45.64; H, 4.79; N, 2.66. Found: C, 45.66; H, 4.70; N, 2.20.

1-Azabicyclo[2.2.2]oct-3-yl α-Hydroxy-α-(1-[^{125}I]-iodo-1-propen-3-yl)-α-phenylacetate ([^{125}I]-3). To a 2.0-mL reactivial was added 50 μL of 3.0% H_2O_2 solution (30% solution diluted with distilled water before use), 100 μL of a 0.1 N HCl solution, and 100 μL of a solution of **6** (1.5 mg/mL in ethanol). A solution of sodium iodide-125 (5.0 mCi) in 50 μL of ethanol was then added, the vial was sealed, and the mixture was stirred at room temperature for 30 min. To this solution was then added 250 μL of a 5% sodium bisulfite solution, 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 was washed with 2 × 5 mL of 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 (mobile phase, methylene chloride/ethanol/triethylamine (97:3:0.06); flow rate, 3.0 mL/min; retention time ~10 min). The fractions containing [^{125}I]-**3** were collected and blown down to dryness which afforded 3.1 mCi (62.2%) of [^{125}I]-**3** with specific activity of ~1500 mCi/μmol. [^{125}I]-**3** was analyzed by comparing the TLC (silica, methylene chloride/methanol (9:1) (R_f = 0.39)) and HPLC mobility to that of the cold compound. In both cases [^{125}I]-**3** cochromatographed with IQNP (**3**). The specific activity was determined by measurement of the UV absorbance peak at 254 nm corresponding to the carrier product and comparing this to a standard curve relating mass to UV absorbance.

Radioligand Binding Assays. Measurement of binding to muscarinic M_1, M_2, and M_3 receptor subtypes was performed by the following procedure. All animal care and use procedures were 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 were approved by the Sterling Winthrop Pharmaceuticals Research Division Institutional Animal Care and Use Committee. Male Sprague–Dawley rats were anesthetized with CO_2 and sacrificed by decapitation. Tissues were dissected and placed in the appropriate assay buffers. The M_1 binding assay used [^{3}H]pirenzepine (PZ) as the ligand, rat cortex as the source of tissue, and 50 mM HEPES–KOH as the assay buffer. The M_2 binding assay used [^{3}H]-(−)-quinuclidinyl benzilate (QNB) as the ligand, whole rat heart as the source of tissue, and 50 mM Tris-HCl as the assay buffer. Finally the M_3 binding assay used [^{3}H]-*N*-methylscopolamine (NMS) as the ligand, rat submaxillary gland as the source of tissue and 50 mM HEPES–KOH as the assay buffer. Tissue were homogenized with a Polytron at setting 6 for 15 s at a concentration of 10 mg/mL ([^{3}H]PZ) or minced and then homogenized at setting 8 for 15 s ([^{3}H]QNB) or at setting 10 for 20 s ([^{3}H]NMS) at a concentration of 8 mg/mL. The homogenates were centrifuged at 48000*g* for 10 min at 4 °C, resuspended, and washed twice with the appropriate buffer. Assay tubes contained 500 μL of tissue, 100 μL of ligand, and sufficient buffer for a final volume of 1 mL for [^{3}H]PZ binding and 2 mL for [^{3}H]QNB or [^{3}H]NMS binding. Nonspecific binding for all assays was defined by 1 μM atropine. Incubations were conducted for 1 h at room temperature for [^{3}H]PZ binding, 1.5 h at room temperature for [^{3}H]NMS binding, and 1 h at 37 °C for [^{3}H]QNB binding. Incubations were terminated by filtration through Whatman GF/B filters that were presoaked with 0.1% poly(ethylenimine) for at least 1 h, followed by three 4-mL washes with ice-cold assay buffer. Following addition of scintillation cocktail, samples were allowed to equilibrate for at least 8 h. The amount of bound radioactivity was determined by liquid scintillation spectrometry using a Beckman LS 5000TA liquid scintillation counter with an efficiency for tritium of approximately 60%.

Displacement experiments at 13 concentrations of nonlabeled drug were performed in triplicate, at final ligand concentrations of approximately 0.5 nM for [^{3}H]PZ, 0.02 nM for [^{3}H]QNB, and 0.1 nM for [^{3}H]NMS. Inhibition constants (K_i values) were calculated using the EBDA/LIGAND program,[39] purchased from Elsevier/Biosoft, Inc.

Biodistribution Studies. Biodistribution studies were performed using female Fisher VAF rats (~125 g). The animal care and use procedures were in accordance with *The Guide for the Care and Use of Laboratory Animals* and the Animal Welfare Act and were reviewed and approved by the Oak Ridge National Laboratory Animal Care and Use Committee. For these studies [^{125}I]-**3** was dissolved in 100 μL of ethanol followed by 50 μL of a 0.1 N HCl solution and diluted to 10 mL with normal saline. Following intravenous injection of [^{125}I]-**3** (1–2 μCi) into a lateral tail vein, the metofane-anesthetized rats were then allowed food and water ad libitum prior to and during the course of the experiment. At various time points the animals were euthanized

by cervical fracture following metofane anesthesia. 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 as described in the literature.[40] 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 γ counter.

For the blocking experiment with (±)-QNB, one group of animals was injected with (±)-QNB (5 mg/kg) 1 h prior to injection of [125I]-3 (1–2 μCi). Another set of animals was injected with (±)-QNB (5 mg/kg) 1 h postinjection of [125I]-3, and a third set of animals was injected with [125I]-3 as the control group. Three hours after the injection of [125I]-3 the animals were killed. The various tissues (striatum, cortex, cerebellum, rest of brain, heart, and blood) were removed, rinsed with saline, blotted dry, and weighed in tared vials as before. The samples were counted in a Packard Minaxi 5000 sodium iodide auto γ counter. For blocking experiments, the various challenge ligands (5 mg/kg) were injected in a group of animals 1 h before injection of [125I]-3 (1–2 μCi). One group of animals were injected with [125I]-3 as a control. The animals were then killed 3 h postinjection of [125I]-3. The various tissues were removed, treated, and analyzed as described above.

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References

(1) Reinikainen, K. J.; Riekkinen, P. J.; Halonen, T.; Laakso, M. Decreased Muscarinic Receptor Binding in Cerebral Cortex and Hippocampus in Alzheimer's Disease. *Life Sci.* 1987, *41*, 453–461.
(2) Lange, K. W.; Wells, F. R.; Rossor, M. N.; Jenner, P.; Marsden, C. D. Brain Muscarinic Receptors in Alzheimer's and Parkinson's Disease. *Lancet* 1989, *2*, 1279.
(3) Mizukawa, K.; Ogawa, N.; Sora, Y. H.; Sora, I. Alterations of the Muscarinic Cholinergic (m-AChR) Receptors in the Striatum of the MPTP-Induced Parkinsonian Model in Mice: In Vitro Quantitative Autoradiographical Analysis. *Neurosci. Lett.* 1987, *81*, 105–110.
(4) Perry, E. Acetylcholine and Alzheimer's Disease. *Br. J. Psychiat.* 1988, *152*, 737–740.
(5) Narang, P. K.; Cutler, N. R. Pharmacotherapy in Alzheimer's Disease: Basis and Rationale. *Prog. Neuro-Psychopharmacol. Biol. Psychiat.* 1986, *10*, 519–531.
(6) Sims, N. R.; Bowen, D. M.; Allen, S. J.; Smith, C. C. T.; Neary, D.; Thomas, D. J.; Davison, A. N. Presynaptic Cholinergic Dysfunction in Patients with Dementia. *J. Neurochem.* 1983, *40*, 503–509.
(7) Rzeszotarski, W. J.; Eckelman, W. C.; Francis, B. E.; Simms, D. A.; Gibson, R. E.; Jagoda, E. M.; Grissom, M. P.; Eng, R. R.; Conklin, J. J.; Reba, R. C. Synthesis and Evaluation of Radioiodinated Derivatives of 1-Azabicyclo[2.2.2]oct-3-yl α-Hydroxy-α-(4-iodophenyl)-α-phenylacetate as Potential Radiopharmaceuticals. *J. Med. Chem.* 1984, *27*, 156–159.
(8) Gibson, R. E.; Weckstein, D. J.; Jagoda, E. M.; Rzeszotarski, W. J.; Reba, R. C.; Eckelman, W. C. The Characteristics of I-125 4-IQNB and H-3 QNB *In Vivo* and *In Vitro*. *J. Nucl. Med.* 1984, *25*, 214–222.
(9) Gibson, R. E.; Schneidau, T. A.; Cohen, V. I.; Sood, V.; Ruch, J.; Melograna, J.; Eckelman, W. C.; Reba, R. C. *In Vitro* and *In Vivo* Characteristics of [Iodine-125] 3-(R)-Quinuclidinyl (S)-4-Iodobenzilate. *J. Nucl. Med.* 1989, *30*, 1079–1087.
(10) Cohen, V. I.; Rzeszotarski, W. J.; Gibson, R. E.; Fan, L. H.; Reba, R. C. Preparation and Properties of (R)-1-Azabicyclo[2.2.2]oct-3-yl (R)-α-Hydroxy-α-(4-[125I]iodophenyl)-α-phenylacetate and (R)-1-Azabicyclo[2.2.2]oct-3-yl (S)-α-Hydroxy-α-(4-[125I]iodophenyl)-α-phenylacetate as Potential Radiopharmaceuticals. *J. Pharm. Sci.* 1989, *78*, 833–836.
(11) Eckelman, W. C.; Reba, R. C.; Rzeszotarski, W. J.; Gibson, R. E.; Hill, T.; Holman, B. L.; Budinger, T.; Conklin, J. J.; Eng, R.; Grissom, M. P. External Imaging of Cerebral Muscarinic Acetylcholine Receptors. *Science* 1984, *223*, 291–293.
(12) Holman, B. L.; Gibson, R. E.; Hill, T. C.; Eckelman, W. C.; Albert, M.; Reba, R. C.; Muscarinic Acetylcholine Receptors in Alzheimer's Disease. *In Vivo* Imaging with Iodine 123-Labeled 3-Quinuclidinyl-4-iodobenzilate and Emission Tomography. *JAMA* 1985, *254*, 3063–3066.
(13) Weinberger, D. R.; Gibson, R.; Coppola, R.; Jones, D. W.; Molchan, S.; Sunderland, T.; Berman, K. F.; Reba, R. C. The Distribution of Cerebral Muscarinic Acetylcholine Receptors In Vivo in Patients with Dementia. A Controlled Study with 123IQNB and Single Photon Emission Computed Tomography. *Arch. Neurol.* 1991, *48*, 169–176.
(14) Owens, J.; Murray, T.; McCulloch, J.; Wyper, D. Synthesis of (R,R)123I-QNB, a SPECT Imaging Agent for Cerebral Muscarinic Acetylcholine Receptors In Vivo. *J. Labelled Cmpd. Radiopharm.* 1992, *31*, 45–60.
(15) Gibson, R. E. Muscarinic Acetylcholine Receptors. In *Quantitative Imaging. Neuroreceptors, Neurotransmitters, and Enzymes*; Frost, J. J., Wagner, H. N., Jr., Eds.; Raven Press: New York, 1990; pp 129–152.
(16) Kabalka, G. W.; Gai, Y.-Z.; Mathur, S. Synthesis of Iodine-125 Labeled 3-Quinuclidinyl 4'-Iodobenzilate. *Nucl. Med. Biol.* 1989, *16*, 359–360.
(17) Rzeszotarski, W. J.; Gibson, R. E.; Eckelman, W. C.; Simms, D. A.; Jagoda, E. M.; Ferreira, N. L.; Reba, R. C. Analogues of 3-Quinuclidinyl Benzilate. *J. Med. Chem.* 1982, *25*, 1103–1106.
(18) Seevers, R. H.; Counsell, R. E. Radioiodination Techniques for Small Organic Molecules. *Chem. Rev.* 1982, *82*, 575–590.
(19) Srivastava, P. C.; Knapp, F. F., Jr.; Kabalka, G. W.; Kunda, S. A. Facile-radioiododemetallation Reactions for the Convenient Preparation of Radioiodinated Compounds. *Synth. Commun.* 1985, *15*, 355–364.
(20) Knapp, F. F., Jr.; Goodman, M. M.; Callahan, A. P.; Ferren, L. A.; Kabalka, G. W.; Sastry, K. A. R. New Myocardial Imaging Agents: Stabilization of Radioiodine as a Terminal Vinyl Iodide Moiety on Tellurium Fatty Acids. *J. Med. Chem.* 1983, *26*, 1293–1300.
(21) Knapp, F. F., Jr.; Goodman, M. M.; Kabalka, G. W.; Sastry, K. A. R. Synthesis and Evaluation of Radioiodinated (E)-18-Iodo-17-octadecenoic Acid as a Model Iodoalkenyl Fatty Acid for Myocardial Imaging. *J. Med. Chem.* 1984, *27*, 94–97.
(22) Knapp, F. F., Jr.; Srivastava, P. C.; Callahan, A. P.; Cunningham, E. B.; Kabalka, G. W.; Sastry, K. A. R. Effect of Tellurium Position on the Myocardial Uptake of Radioiodinated 18-Iodotellura-17-octadecenoic Acid Analogues. *J. Med. Chem.* 1984, *27*, 57–63.
(23) Goodman, M. M.; Callahan, A. P.; Knapp, F. F., Jr. Design, Synthesis and Evaluation of ω-Iodovinyl and ω-Iodoalkyl-Substituted Methyl-Branched Long-Chain Fatty Acids. *J. Med. Chem.* 1985, *28*, 807–815.
(24) Goodman, M. M.; Neff, K. H.; Ambrose, K. R.; Knapp, F. F., Jr. Effect of 3-Methyl-branching on the Myocardial Retention of Radioiodinated 19-Iodo-18-nonadecenoic Acid Analogues. *Nucl. Med. Biol.* 1989, *16*, 813–819.
(25) Srivastava, P. C.; Callahan, A. P.; Cunningham, E. B.; Knapp, F. F., Jr. Potential Cerebral Perfusion Agents: Synthesis and Evaluation of a Radioiodinated Vinylalkylbarbituric Acid Analogue. *J. Med. Chem.* 1983, *26*, 742–746.
(26) Srivastava, P. C.; Guyer, C. E.; Knapp, F. F., Jr. Potential Cerebral Perfusion Agents: Synthesis and Evaluation of New Radioiodinated Barbituric Acid Analogs. *J. Heterocycl. Chem.* 1983, *20*, 1081–1084.
(27) Srivastava, P. C.; Knapp, F. F., Jr. (E-1-[123I]Iodo-1-penten-5-yl)-triphenylphosphonium Iodide: Convenient Preparation of a Potentially Useful Myocardial Perfusion Agent. *J. Med. Chem.* 1984, *27*, 978–981.
(28) Srivastava, P. C.; Hay, H. G.; Knapp, F. F., Jr. Effects of Alkyl and Aryl Substitution on the Myocardial Specificity of Radioiodinated Phosphonium, Arsonium, and Ammonium Cations. *J. Med. Chem.* 1985, *28*, 901–904.
(29) Goodman, M. M.; Waterhouse, R. N.; Kabalka, G. W.; Knapp, F. F., Jr. Synthesis and Biological Evaluation of 3-C((E)-2-[125I]Iodoethenyl)-D-Allose: A New Strategy for the Preparation of *In Vivo* Stable Radioiodinated Carbohydrates. *NucCompact* 1990, *21* (2), 64–69.
(30) McPherson, D. W.; Lisic, E.; Knapp, F. F., Jr.; Ambrose, K. R.; Lambert, C. R.; Kung, M.-P.; Kung, H. Synthesis and Evaluation of Two Radioiodinated Iodoalkenyl-Substituted Spiroperidol Analogues. *NucCompact* 1991, *22* (3), 92–98.
(31) Kobayashi, R. M.; Palkovits, M.; Hruska, R. E.; Rothschild, R.; Yamamura, H. I. Regional Distribution of Muscarinic Cholinergic Receptors in Rat Brain. *Brain Res.* 1978, *154*, 13–23.
(32) Frey, K. A.; Ehrenkaufer, R. L. E.; Beaucage, S.; Agranoff, B. W. Quantitative In Vivo Receptor Binding. I. Theory and Application to the Muscarinic Cholinergic Receptor. *J. Neurosci.* 1985, *5*, 421–428.
(33) Gibson, R. E.; Eckelman, W. C.; Vieras, F.; Reba, R. C. The Distribution of the Muscarinic Acetylcholine Receptor Antagonists, Quinuclidinyl Benzilate and Quinuclidinyl Benzilate Methiodide (Both Tritiated), in Rat, Guinea Pig, and Rabbit. *J. Nucl. Med.* 1979, *20*, 865–870.
(34) Fields, J. Z.; Roeske, W. R.; Morkin, E.; Yamamura, H. I. Cardiac Muscarinic Cholinergic Receptors; Biochemical Identification and Characterization. *J. Biol. Chem.* 1978, *253*, 3251–3258.

(35) Wilson, A. A.; Dannals, R. F.; Ravert, H. T.; Frost, J. J.; Wagner, H. N., Jr. Synthesis and Biological Evaluation of [^{125}I]-amd [^{123}I]-4-Iododexetimide, a Potent Muscarinic Cholinergic Receptor Antagonist. *J. Med. Chem.* **1989**, *32*, 1057–1062.
(36) Lever, J. R.; Scheffel, U. A.; Stathis, M.; Musachio, J. L.; Wagner, H. N., Jr. In Vitro and In Vivo Binding of (E)- and (Z)-N-(Iodoallyl)-spiperone to Dopamine D_2 and Serotonin 5-HT_2 Neuroreceptors. *Life Sci.* **1990**, *46*, 1967–1976.
(37) Meyerhoffer, A. Absolute Configuration of 3-Quinuclidinyl Benzilate and the Behavioral Effect in the Dog of the Optical Isomers. *J. Med. Chem.* **1972**, *15*, 994–995.
(38) Rzeszotarski, W. J.; McPherson, D. W.; Ferkany, J. W.; Kinnier, W. J.; Noronha-Blob, L.; Kirken-Rzeszotarski, A. Affinity and Selectivity of the Optical Isomers of 3-Quinuclidinyl Benzilate and Related Muscarinic Antagonists. *J. Med. Chem.* **1988**, *31*, 1463–1466.
(39) McPherson, G. A. Analysis of Radioligand Binding Experiments. A Collection of Computer Programs for the IBM PC. *J. Pharmacol. Methods* **1986**, *14*, 213.
(40) Glowinski, J.; Iversen, L. L. Regional Studies of Catecholamines in the Rat Brain I. The Disposition of [^{3}H]Norepinephrine, [^{3}H]-Dopamine and [^{3}H]Dopa in Various Regions of the Brain. *J. Neurochem.* **1966**, *13*, 655–669.
(41) Hudkins, R. L.; DeHaven-Hudkins, D. L.; Stubbins, J. F. Muscarinic Receptor Binding Profile of Para-Substituted Caramiphen Analogues. *J. Med. Chem.* **1991**, *34*, 2984–2989.