Association for Information and Image Management

1100 Wayne Avenue, Suite 1100
Silver Spring, Maryland 20910

301/587-8202

Centimeter

Inches

MANUFACTURED TO AIIM STANDARDS
BY APPLIED IMAGE, INC.

1 of 1

CONF-940301--36

Development of Radiohalogenated Muscarinic Ligands for the *In vivo* Imaging of m-AChR by Nuclear Medicine Techniques.

D. W. McPherson, H. Luo, and F. F. Knapp, Jr.
Nuclear Medicine Group
Oak Ridge National Laboratory
Oak Ridge, TN 37831-6229 USA

Abstract

Alterations in the density of acetylcholinergic muscarinic receptors (m-AChR) have been observed in various dementias. This has spurred interest in the development of radiohalogenated ligands which can be used for the non-invasive *in vivo* detection of m-AChR by nuclear medicine techniques. We have developed a new ligand 1-azabicyclo[2.2.2]oct-3-yl α-hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate (IQNP, **12**) which demonstrates high affinity for the muscarinic receptor. When labeled with radioiodine it has been shown to be selective and specific for m-AChR. Initial studies on the separation and *in vivo* evaluation of the various isomers of IQNP have shown that the stereochemistry of the chiral centers and the configuration around the double bond play an important role in m-AChR subtype specificity. *In vivo* evaluation of these stereoisomers demonstrate that E-(R,R)-IQNP has a high affinity for the M_1 muscarinic subtype while Z-(R,R)-IQNP demonstrate a high affinity for M_1 and M_2 receptor subtypes. These data demonstrate IQNP (**12**) has potential for use in the non-evasive *in vivo* detection of m-AChR by single photon emission computed tomography (SPECT). A brominated analogue, "BrQNP", in which the iodine has been replaced by a bromine atom, has also been prepared and was shown to block the *in vivo* uptake of IQNP in the brain and heart and therefore has potential for positron emission tomographic (PET) studies of m-AChR.

Introduction

It has been demonstrated that excited nerve cells (neurons) communicate with one another chemically (neurotransmitters)[1]. An excited neuron releases the neurotransmitter which then binds to a specific site (receptor) on another nerve cells which in turn causes this neuron to become excited and to effect the uptake or extrusion of certain chemicals or ions. Since the early twentieth century over a fifty neurotransmitters have been identified involving more than a dozen systems such as the dopaminergic, muscarinic, and serotonergic classes of receptors for example.

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

"The submitted manuscript has been authored by a contractor of the U.S. Government under contract No. DE-AC05-84OR21400. Accordingly, the U.S. Government retains a nonexclusive, royalty-free license to publish or reproduce the published form of this contribution, or allow others to do so, for U.S. Government purposes."

The muscarinic acetylcholinergic receptor system (m-AChR) has been implicated in a variety of dementias, such as Alzheimer's and Huntington Chorea, in addition to aging and memory loss[2-9]. These observations have spurred the interest to be able to non-evasively image m-AChR using nuclear medicine techniques such as positron emission tomography (PET) or single photon emission computed tomography (SPECT).

The muscarinic acetylcholinergic receptor has been shown by classical pharmacology to have 3 subclasses, M_1 (high affinity pirenzepine type), M_2 (low affinity pirenzepine subtype) and M_3 (glandular, smooth muscle)[10]. More recently, molecular biological studies have cloned the muscarinic receptor in the central nervous system (CNS) and five distinct subclasses, m1, m2, m3, m4, and m_5 were identified[11-14]. The M_1 class corresponds to the m_1,m_4, and m_5, M_2 to the m_2, and M_3 to the m_3. Although pharmacological tools have been developed to study the muscarinic M_1, M_2, and M_3 receptors (4), 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, 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.

The m-AChR subclasses play an important role in the diagnosis and potential treatment of various dementias. For example, several studies have shown that the density of 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 (4-IQNB, **8**) have been reported in patients with Ad[15-17]. Studies of binding in tissue homogenates prepared from AD patients have shown that M_1 sites labeled by [^{3}H]pirenzepine are increased in striatum[18], and sites labeled with [^{3}H]QNB are increased in cortex[19]. The high-affinity component of agonist binding is reduced in frontal cortex of patients with AD[20], and sites labeled by [^{3}H]acetylcholine are decreased in cortex[21]. 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 selective muscarinic antagonists would be useful for labeling specific muscarinic receptor populations in the brain, and the availability of such ligands would permit a greater understanding of the effects of AD on specific muscarinic receptor populations in the brain, and may serve as a potential diagnostic agent for Alzheimer's and other dementias.

Isotopes which are commonly used in nuclear medicine procedures for the study of neurotransmitter systems include carbon-11 and fluorine-18 for PET and iodine-123 for SPECT. The major advantage of carbon-11 is that it is possible to replace a carbon-12 with carbon-11 enabling the ligand to retain its biological properties. However, the major disadvantage of carbon-11 is its short half-life (20 minutes) which make studies of long duration unfeasible. Fluorine-18 has a longer half-life (110 minutes) but incorporation of the fluorine into the ligand requires the replacement of a hydrogen, hydroxy or methyl group with fluorine which may change the biological properties of the ligand. Another disadvantage in the use of PET isotopes is their relatively high cost since their production by an accelerator (cyclotron) and their short half-lives mandate the preparation site be in close proximity to the imaging center. Iodine-123, however, has a suitable half-life (13.6 hours) which allows for it to be prepared at one site and shipped to the facility in which the ligand can be radiolabeled followed by shipment to a center where patient studies can be performed thereby lowering the cost associated for SPECT studies. This also allows for a greater number of centers to perform this type of study. The major disadvantage in the use of iodine-123 is the iodine molecule is usually replacing a hydrogen, methyl, or hydroxy group and this modification causes a major perturbation to the ligand which can have a major effect on the biological properties of the molecule, such as increasing the lipophilicity of the ligand thereby decreasing the ability of the ligand to pass the blood brain barrier.

Examples of ligands labeled with carbon-11 prepared for the study of m-AChR include 3-quinuclidinyl benzilate (QNB, **1**)[22], 2-α-tropanyl benzilate (**2**)[23], scopolamine (**3**)[24] and dexetimide (DEX, **4**)[25] (Figure 1). Compound **2** was studied in the brain of primates but due to the short half-life of carbon-11, the pharmacokinetics were not able to be studied accurately. Scopolamine (**3**), meanwhile, did not demonstrate accurately the known differences in receptors density at late time periods in an initial human study.

There have been only a few examples of fluorine-18 labeled muscarinic ligands reported in the literature. Dexetimide, a high affinity muscarinic antagonist, has been reported to be labeled with fluorine-18 in either the 2 (**5**) or 4 (**6**) position of the phenyl ring (Figure 2)[26]. However the preparation of these analogues was long and the radiochemical yield was low making the routine production for patient studies unfeasible. Preliminary studies in rats demonstrated the 4-fluoro analogue to be more stable to *in vivo* defluorination than the 2-fluoro analogue. More recently a bromine-76 analogue of dexetimide (**7**) (Figure 2) has been reported for the potential use as a PET imaging agent[27].

There are also only a couple examples of muscarinic ligands labeled with iodine-123 for use in SPECT studies. The first example of an iodinated ligand developed for the *in vivo* detection of m-AChR was 3-quinuclidinyl 4-iodobenzilate (4-IQNB, **8**)[28]. It was shown in studies with rats that the uptake of **8** in m-AChR rich areas of the brain parallel the concentration of m-AChR[29,30]. Compound **8** has also been used in studies of patients with dementia and healthy individuals[15-17,31,32]. The major disadvantages for the routine use of **8** is the low radiochemical yield (~20 %) and the long time required between the injection of **8** and the time required for the accumulation of the ligand in m-AChR rich tissues in a concentration to allow imaging (~24 hours). The increased lipophilicity of the molecule by the replacement of a hydrogen by an iodide may account for the slow accumulation. Iododexetimide (**9**) (Figure 3), has also been prepared and evaluated in animals and healthy individuals[33,34]. These studies show that **9** has high initial uptake in cerebral m-AChR rich areas and has potential for routine SPECT studies.

These examples shown above, although they demonstrate high affinity for m-AChR, they do not demonstrate selectivity over receptor subtypes. A ligand which would display receptor subtype is of importance since the receptor subtypes play an important role in various dementias. It has been shown in *in vitro* binding assays that QNB (**10**) (Figure 4), a high affinity muscarinic antagonist, is able to be modified by replacement of one of the phenyl rings with a variety of groups and still retain affinity for the receptor[35]. This type of modification also allows for the ligand to demonstrate a higher affinity for certain subtypes of the muscarinic receptor. An example is 3-quinuclidinyl atrolactate (**11**) (Figure 4) in which a phenyl group was replaced with a methyl group. The (S,R) isomer of QNA is more selective for the M_1 m-AChR subtype over the M_2 receptor subtype[36]. We therefore explored the preparation of an analogue of QNB in which one of the phenyl rings was replaced with a group that would allow for iodine to be readily incorporated in high yield under mild reaction conditions.

There are a variety of methods which allow for the facile incorporation of radioiodine into molecules of biological interest[37,38] and are shown in Scheme 1. These methods include the electrophilic iododemetallation of trialkylstannyl, boronic acid, mercuric acetates, or thallium difluoroacetate intermediates. The iododestannylation reaction has also been shown to be stereospecific, therefore if the E trialkylstannyl intermediate is utilized, the iodinated product has the E configuration. Other methods involve the copper(I) assisted iodo for bromo or iodo exchange, however this method can result in a lower specific activity and may not be a suitable method for radiolabeling in studies where high specific activity is required.

Preparation of IQNP (12).

The preparation of 4-IQNB (**8**) utilizing the copper(I) assisted iodo for iodo exchange[31] or the use of a boronic acid intermediate[39] has been reported in an attempt to overcome the low radiochemical yield of **8**. However, in both these cases, the specific activity was low due to the addition of carrier **8** or iodine to achieve the final radioiodinated product. We therefore investigated the development of a new ligand which would overcome these problems of low radiochemical yield and specific activity. Since it has been shown that it was possible to replace one of the phenyl rings of **10** with a variety of different groups and still retain affinity for m-AChR the replacement of a phenyl group with an iodo allyl group was investigated[40]. This modification would allow for the readily incorporation of iodine in a metabolically stable position. This new ligand, 1-azabicyclo[2.2.2]oct-3-yl α-hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate (IQNP, **12**), was prepared as shown in Scheme 2. Ethyl α-hydroxy-α-phenyl-α-(1-propyn-3-yl)acetate (**13**) was prepared by treatment of ethyl benzoylformate with propargyl bromide in the presence of zinc dust.. Compound **13** was then transesterified with 3-quinuclidinol to afford 1-azabicyclo[2.2.2]oct-3-yl α-hydroxy-α-phenyl-α-(1-propyn-3-yl)acetate (**14**). Treatment of **14** with excess tributyltin hydride in the presence of a slight molar excess of the 2,2-azobis-(2-methylpropionitrile) (AIBN), a radical initiator, afforded a mixture of (E)- and (Z)-1-azabicyclo[2.2.2]oct-3-yl α-hydroxy-α-phenyl-α-(1-tributylstannyl-1-propen-3-yl)acetate (**15**). Since complete separation of the two isomers by flash column chromatography could not be achieved, the isomeric mixture was utilized in the next step of the reaction. Compound **15** was then treated with iodine in chloroform to afford a mixture of (E)- and (Z)-1-azabicyclo[2.2.2]octyl-3-yl α-hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate (**12**). As above, the (E)- and (Z)-isomers of **3** could not be effectively separated and therefore **12** was initially evaluated as the racemic mixture.

IQNP (**12**) was radioiodinated by the procedure shown in Scheme 3, which involves iododestannylation of **15** by treatment with sodium [^{125}I]iodide utilizing a 3.0 % solution of hydrogen peroxide as the oxidizing agent followed by purification. [^{125}I]-**12** was obtained in an overall radiochemical yield of ~60 % with a specific activity of ~1500 mCi/ μmol.

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. We therefore investigated the use of hydrogen peroxide as the oxidizing agent. The use of

hydrogen peroxide avoids the formation of potential chlorinated by-products therefore making the final purification of the ligand less complicated. We observed that the use of a 30% hydrogen peroxide solution resulted in an unacceptable low yield of [^{125}I]-**12** (< 5.0%) with the formation of several unknown radioiodinated by-products. The use of a 3.0% hydrogen peroxide solution resulted in greater than 80% crude radiochemical yield and greater than 60% radiochemical yield after high performance liquid chromatography (HPLC) purification.

Therefore the replacement of a iodophenyl ring with an iodoallyl group allows the rapid incorporation of radioiodine in high yield with high specific activity and overcomes the problems observed in the radiolabeling of 4-IQNB.

In Vitro and In Vivo Evaluation of IQNP (12)

Radioligand binding data for IQNP (**12**) and reference muscarinic agents are shown in Table 1. 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 **12** binds with high affinity to the M_1, M_2, and M_3 subtype muscarinic receptor, however, **12** did not demonstrate selectivity between the subtypes of m-AChR.

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]-**12** 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]-**12** (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. 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.

The results of a biodistribution study of [^{125}I]-**12** in female rats over a six hour time period is shown in Table 2. These data demonstrate that those cerebral regions which are rich in m-AChR (striatum and cortex)[41] have the highest accumulation of radioactivity over the time period studied. The cerebellum, which contains a low concentration of m-AChR[42], demonstrated a low initial uptake of radioactivity but this level of activity decreased over the time 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 six hours to values of 6.8:1 and 7.8:1, respectively. This increase in the ratios resulted from the washout of non-specifically bound activity which occurred more rapidly than the washout of activity in the striatum and cortex. This study demonstrates that IQNP (**12**) has high uptake in tissues that are rich in m-AChR. When these results are compared to a similar study utilizing 4-IQNB (**8**), the uptake of **12** is significantly higher therefore the replacement of the iodophenyl ring with an iodoallyl group enhances the cerebral uptake of activity.

A series of blocking studies were then performed to determine the specificity and selectivity of **12** for m-AChR. For these studies the various challenge ligands (5 mg/kg) were injected in female rats one hour before injection of [^{125}I]-**12** and another set of rats were injected with [^{125}I]-**12** as a control group. Three hours after the injection of [^{125}I]-**12** the rats were killed and the various tissues were treated as described above. The receptor specific ligands that were employed in these studies were: (+)-butaclamol (D_2/D_1 dopamine antagonist), (-)-butaclamol (inactive enantiomer), spiperone (D_2 dopamine antagonist), S-(+)-dexetimide (muscarinic antagonist), (±)-QNB (muscarinic antagonist), and ketanserin (5-HT_2 antagonist). The dose chosen for this blocking study was sufficient to appreciably block the various receptors over the time course of the experiment[33,43]. The results of this study, shown in Figure 5, clearly illustrate that only dexetimide and QNB, both muscarinic antagonists, block the uptake of [^{125}I]-**12** in m-AChR rich areas. This study also demonstrated that the binding of [^{125}I]-**12** was displaced by a post-injection of (±)-QNB (Figure 6) therefore uptake of the ligand was due to a receptor mediated process and not simply a measure of blood flow to these areas in the brain and heart.

These data demonstrate that the replacement of an iodophenyl group with an iodoallyl group allows the ligand to retain affinity and selectivity for m-AChR.

Resolution of the Stereoisomers of IQNP (11)

Since the 3-carbon of the quinuclidinyl ring and the 2-carbon of the acetate moiety are

asymmetric centers and the configuration around the double bond can be either Z (cis) or E (trans) racemic **12** contains 8 stereoisomers. It has been reported that the R configuration of the 3-quinuclidinyl moiety is 100 times more active than the S configuration[44]. It has also been shown that the various stereoisomers of 4-IQNB have different biological properties as demonstrated by *in vivo* studies in rats[45]. Our initial studies in the evaluation of the stereoisomers of **12** was therefore, to prepare **12** utilizing R-3-quinuclidinol. 3-Quinuclidinol was resolved as its tartrate salt[46] and (R)-IQNP was then prepared in an analogous manner as the racemic compound (Scheme 2). The results of a biodistribution study in female rats is shown in Figure 6 and it was observed the uptake of activity increases to about double the level in the cortex (m-AChR rich) as compared to racemic **12**. The same increase in radioactivity was also observed in the striatum, another m-AChR rich tissue. This result is in agreement with 4-IQNB (**8**) and QNB (**10**) in which the R isomer of the 3-quinuclidinyl moiety imparts greater biological activity to the ligand.

The stereoisomers of the acetate moiety were then separated by the use of quinine and quinidine. The E and Z configuration of the iodide on the terminal end of the double bond could be controlled by varying the reaction conditions of the stannylation reaction. The use of toluene as a solvent and a radical initiator produces the E configuration while the use of an aprotic solvent and mild reaction conditions affords the Z isomer. The four isomers of **12** with the R configuration at the 3-quinuclidinyl center were then prepared in a similar manner to that used for racemic **12** as shown in Scheme 2. The results of an initial *in vivo* biodistribution study in female rats are shown in Table 3. It was observed that while E-(R,S)-**12** washes out quickly from the brain and heart (m-AChR rich tissues), the other three isomers demonstrated significant uptake and retention of radioactivity in m-AChR rich tissues of the brain (cortex, striatum) up to 6 hours post-injection. The major difference observed in these three stereoisomers was Z-(R,R)-**12** also demonstrated significant uptake in cerebellum and heart, regions high in the M_2 m-AChR subtype[30]. These results suggest that muscarinic receptor subtype selectivity is influenced by the configuration of the iodide on the double bond of IQNP. Work is continuing on *in vitro* binding assays and the determination of the selectivity and specificity of these isomers of **12** for m-AChR.

Preparation and Initial Biological Evaluation of a Brominated IQNP Analogue (16)

Recent interest has focussed on the use of radiopharmaceuticals labeled with bromine-76 for use in the study of neurotransmitter systems by PET. Bromine-75 has a favorable half-life ($t_{1/2}$=1.6 h) for receptor binding studies and is cyclotron-produced from $^{75}As(^{3}He,3n)^{75}Br$. Since

bromine chemistry is similar to iodine, the preparation of a brominated analogue "BrQNP" of IQNP was investigated. BrQNP (**16**) was prepared as shown in Scheme 4 and is analogous to take employed in the preparation of IQNP. An initial *in vivo* blocking study was then preformed in which the ability of unlabeled BrQNP to block the uptake of [^{125}I]-Z-(R,R)-IQNP (**12**) was studied. The results of this study is shown in Figure 7 and it was observed that BrQNP (2 mg/kg) blocks the uptake of **12** by approximately 60% in both the heart and brain while there was no observable difference in the level of activity in the blood. Work is in progress to radiolabel BrQNP (**16**) in order to evaluate the cerebral uptake, receptor specificity and selectivity of this potential ligand for the study of m-AChR by PET.

Conclusion

1-Azabicyclo[2.2.2]oct-3-yl α-hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate (IQNP, **12**) was developed as a new ligand to overcome the low radioiodination yield and the long time required for imaging studies utilizing 4-IQNB (**8**), a ligand used in the study of m-AChR by SPECT. The radiolabeling of **12** 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-([^{125}I]-1-iodo-1-propen-3-yl)-α-phenylacetate ([^{125}I]-**12**) 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 six hours. 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 hours, which decreased to about 1:1 over the time frame of the experiment. 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 [^{125}I]-**12** in m-AChR rich tissues. These results demonstrate that IQNP (**12**) is specific and selective for m-AChR and demonstrates higher cerebral uptake as compared to 4-IQNB (**8**). Preliminary studies on the separation of the various stereoisomers of IQNP demonstrated that the asymmetric centers and configuration of the iodine play an important role on the binding to the different muscarinic receptor subtypes. E-(R,R)-IQNP was observed to have high uptake in regions of high M_1 m-AChR subtype while Z-(R,R)-IQNP demonstrated high uptake in both M_1 and M_2 subtype rich tissues. A brominated analogue "BrQNP" (**16**) was also prepared and initial *in vivo* studies demonstrated the ability of this new ligand to block the uptake

of radioiodinated IQNP in m-AChR rich tissues. Work is continuing on the development and characterization this new class of QNB analogues in an attempt to prepare a m-AChR ligand which displays high m-AChR subtype specificity for use in the study of various dementias by nuclear medicine techniques.

Acknowledgement

The authors thank C. R. Lambert for assistance with the biodistribution studies and Dr. D. L. DeHaven-Hudkins for the in vitro bind assay data. Research was supported by the Office of Health and Environmental Research, U.S. Department of Energy, under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc.

References

1. J.-P. Changeux, *Scientific American* November, 58 (1993).
2. K. J. Reinikainen, P. J. Riekkinen, T. Halonen, and M. Laakso, *Life Sci.* **41**, 453 (1987).
3. K. W. Lange, F. R. Wells, M. N. Rossor, P. Jenner, and C. D. Marsden, *Lancet* **2**, 1279 (1989).
4. K. Mizukawa, N. Ogawa, Y. H. Sora, Y. H.and I Sora, *Nerosci. Lett.* **81**, 105 (1987).
5. E. Perry, Br. J. Psychiat. 152, 737 (1988).
6. P. K. Narang, and N. R. Cutler, *Prog. Neuro-Psychopharmacol. Biol. Psychiat.* **10**, 519 (1986).
7. N. R. Sims, D. M. Bowen, S. J. Allen, C. C. T. Smith, D. Neary, D.J. Thomas, and A. N. Davison, *J. Neurochem.* **40**, 503 (1983).
8. W. S. Messer Jr., M. M. Bohnett, and J. Stibbe, *Neurosci Lett.* **116**, 184 (1990).
9. A. Norberg, I. Alafuzoff, and B. Winblad, *J. Neurosci. Research* **31**, 103 (1992).
10. E. Mutschler, U. Moser, J. Wess, and G. Lambrecht, "Progress in Pharmacology and Clinical Pharmacology; Stuttgart: New York, Gustav Fischer Verlag, Vol. 7/1, 13 (1989).
11. E. G. Peralta, A. Ashkenazi, J. W. Winslow, D. H. Smith, J. Ramachandran, and D. J. Capon, *EMBO J.* **6**, 3923 (1987).
12. T. I. Bonner, N. J. Buckley, A. C. Young, and M. R. Brann, *Science*, **237**, 527 (1987).
13. T. I. Bonner, A. C. Young, M. R. Brann, and N. J. Buckley, *Neuron.* **7**, 403 (1988).
14. N. J. Buckley, T. I. Bonner, C. M. Buckley, and M. R. Brann, *Mol. Pharmacol.* **35**, 469 (1989).
15. B. L. Holman, R. E. Gibson, T. C. Hill, W. C. Eckelman, M. Albert, and R. C. Reba,

JAMA **254**, 3063 (1985).

16. D. R. Weinberger, R. Gibson, R. Coppola, D. W. Jones, S. Molchan, T. Sunderland, K. F. Berman, and R. C. Reba, *Arch. Neurol.* **48**, 169 (1991).
17. J. Wyper, D. Brown, J. Patterson, J. Owens, R. Hunter, E. Teasdale, and J. McCulloch, *Eur. J. Nucl. Med.* **20**, 379-386 (1993).
18. I. Aubert, D. M. Araujo, D. Cecyre, Y. Robitaille, S. Gauthier, and R. Quirion, *J. Neurochem.* **58**, 529 (1992).
19. A.-L. Svensson, I. Alafuzoff, and A. Nordberg, *Brain Res.* **596**, 142 (1992).
20. D. D. Flynn, D. A. Weinstein, and D. C. Mash, *Ann. Neurol.* **29**, 256 (1991).
21. A. Nordberg, I. Alaguzoff, and B. Winblad, *J. Neurosci. Research* **31**, 103 (1992).
22. C. Prenant, L. Barre, and C. Crouzel, *J. of Label. Comp. Radiopharm.* **27**, 1257 (1989).
23. G. K. Mulholland, C. A. Otto, D. M. Jewett, M. R. Kilbourn, R. A. Koeppe, P. S. Sherman, N. A. Pietry, J. E. Carey, E. R. Atkinson, S. Archer, K. R. Freys, and D. E. Kuhl, *J. Nucl. Med.* **33**, 423 (1992).
24. K. A. Frey, R. A. Koeppe, G. K. Mulholland, D. Jewett, R. Hichwa, R. L. E. Ehrenkaufer, J. E. Carey, D. M. Wieland, D. E. Kuhl, and B. W. Agranoff, *J. Cereb. Blood Flow Metab.* **12**, 147 (1992).
25. R. F. Dannals, B. Langstrom, H. T. Ravert,. A. A. Wilson, and H. N. Wagner, Jr., *Appl. Radiat. Isot.* **39**, 291 (1988).
26. D.-R. Hwang, C. S. Dence, Z. A. McKinnon, C. J. Mathias, and M. J. Welch, *Nucl. Med. Biol.* **18**, 247 (1991).
27. M. Kassiou, C. Loc'h, M. Bottlander, R. M. Lambrecht, A. Katsifis, L. Schmid, M. Ottaviani, M. Maziere, and B. Maziere, In, Proceedings of the 10th International Symposium on Radiopharmaceutical Chemistry, Kyoto, Japan, October 25-28, 229, 1993.
28. W. J. Rzeszotarski, W. C. Eckelman, B. E. Francis, D. A. Simms, R. E. Gibson, E. M. Jagoda, M. P. Grissom, R. R. Eng, J. J. Conklin, and R. C. Reba, *J. Med. Chem.* **27**, 156 (1984).
29. R. E. Gibson, T. A. Schneidau, V. I. Cohen, V. Sood, J. Ruch, J. Melograna, W. C. Eckelman, and R. C. Reba, *J. Nucl. Med.* **30**, 1079 (1989).
30. R. E. Gibson, Muscarinic Acetylcholine Receptors, in Quantitative Imaging. Neuroreceptors, Neurotransmitters, and Enzymes: Frost, J. J., Wagner, Jr., H. N. Eds.: Raven Press, New York, NY, 129 (1990).
31. J. Owens, T. Murray, J. McCulloch, and D. Wyper, *J. Labelled Cmpd. Pharm.* **31**, 45 (1992).
32. W. C. Eckelman, R. C. Reba, W. J. Rzeszotarski, R. E. Gibson, T. Hill, B. Holman, T. Budinger, J. J. Conklin, R. Eng,and M. P. Grissom, *Science* **223**, 291 (1984).

33. A. A. Wilson, R. F. Dannals, H. T. Ravert, J. J. Frost, and H. N. Wagner, Jr., *J. Med. Chem.* **32**, 1057-1062 (1989).

34. H. W. Mueller-Gaertner, A. A. Wilson, R. F. Dannals, H. N. Wagner, Jr., and J. J. Frost, *J. Nucl. Med.* **32**, 981 (1991).

35. W. J. Rzeszotarski, R. E. Gibson, W. C. Eckelman, D. A. Simms, E. M. Jagoda, N. L. Ferreira, and R. C. Reba, *J. Med. Chem.* **25**, 1103 (1982).

36. W. J. Rzeszotarski, D. W. McPherson, J. W. Ferkany, W. J. Kinnier, L. Noroha-Blob, and A. Kirken-Rzeszotarski, *J. Med. Chem.* **31**, 1463 (1988).

37. R. H. Seevers, and R. E. Counsell, R. E., *Chem. Rev.* **82**, 575 (1982).

38. P. C. Srivastava, F. F. Knapp, Jr., G. W. Kabalka, and S. A. Kunda, *Synthetic Comm.* **15**, 355 (1985).

39. G. W. Kabalka, Y.-Z. Gai, and S. Mathur, *Nucl. Med. Biol.* **16**, 359 (1989).

40. D. W. McPherson , D. L. DeHaven-Hudkins, A. P. Callahan and F. F. Knapp, Jr., *J. Med. Chem.* **36**, 848 (1993).

41. R. M. Kobayashi, M. Palkovits, R. E. Hruska, R. Rothschild, and H. I. Yamamura, *Brain Res.* **154**, 13 (1978).

42. K. A. Frey, R. L. E. Ehrenkaufer, S. Beaucage, and B. W. Agranoff, *J. Neurosci.* **5**, 421 (1985).

43. R. E. Gibson, D. J. Weckstein, E. M. Jagoda, W. J. Rzeszotarski, R. C. Reba, and W. C. Eckelman, *J. Nucl. Med.* **25**, 214 (1984).

44. J. R. Lever, U. A. Scheffel, M Stathis, J. L. Musachio, and H. N. Wagner, Jr., *Life Sci.* **46**, 1967 (1990).

45. A. Meyerhoffer, *J. Med. Chem.* **15**, 994 (1972).

46. V. I. Cohen, W. J. Rzeszotarski, R. E. Gibson, L. H. Fan, and R. C. Reba, *J. Pharm. Sci.* **78**, 833 (1989).

47. B. Ringdahl, B. Resul, and R. Dahlbom, *Acta. Pharm. Suec.* **16**, 281 (1979).

Figure Legends.

Figure 1. Examples of carbon-11 labeled muscarinic ligands.

Figure 2. Examples of fluorine-18 and bromine-76 labeled muscarinic ligands.

Figure 3. Examples of radioiodinated muscarinic ligands.

Figure 4. QNB (**10**) and QNA (**11**).

Figure 5. Challenge studies of [^{125}I]-**12** with various receptor specific ligands[40].

Figure 6. Challenge study with [^{125}I]-**12** and (±)-QNB (**10**)[40].

Figure 7. Comparison of the uptake of cortex activity of racemic [^{125}I]-**12** and R-[^{125}I]-**12**

Figure 8. Challenge study with [^{125}I]-**12** and "BrQNP" (**16**).

Scheme legends.

Scheme 1. Methods for radioiodination.

Scheme 2. Preparation of IQNP (**12**).

Scheme 3. Preparation of [^{125}I]-**12**.

Scheme 4. Preparation of "BrQNP" (**16**).

Table 1. Muscarinic Binding Affinity of **12** and Various Muscarinic Antagonists.

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	0.76	0.12
Pirenzepine	5.21	267	28
R-QNB	0.15	0.03	0.16
IQNP	**0.78**	**1.06**	**0.27**

Data are the mean ±SEM (n=3)

From reference 40.

Table 2. Biodistribution of [^{125}I]-**12** in Female Fisher Rats·

mean percent injected dose/gram ± SD at time (min)

Organ	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
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
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

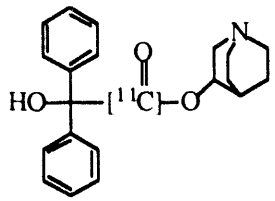
Group of 5 rats/time point.

Table 3. Evaluation of the Various Isomers of [^{125}I]-**12** in Female Rats At 6 Hours.

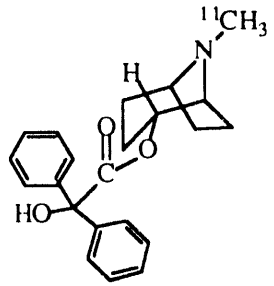
mean percent injected dose/gram ± SD at time (min)

Tissue	E-(R,R)	E-(R,S)	Z-(R,R)	Z-(R,S)
cortex	1.38±0.31	0.04±0.00	1.52±0.25	1.15±0.17
striatum	1.22±0.20	0.02±0.02	1.32±0.22	0.97±0.16
cerebellum	0.04±0.01	0.01±0.01	0.28±0.05	0.10±0.02
heart	0.10±0.02	0.06±0.01	0.70±0.15	0.34±0.05
blood	0.09±0.02	0.11±0.01	0.13±0.02	0.12±0.02

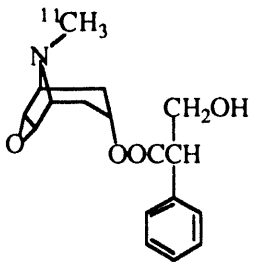
Group of 5 rats/time point.



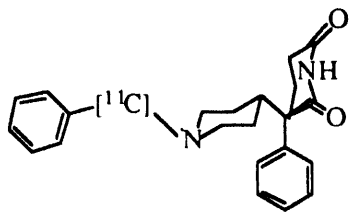
1



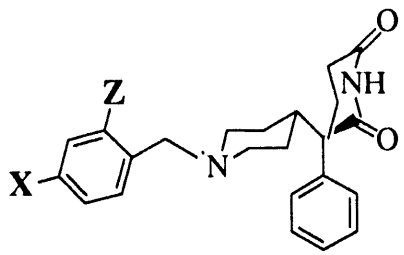
2



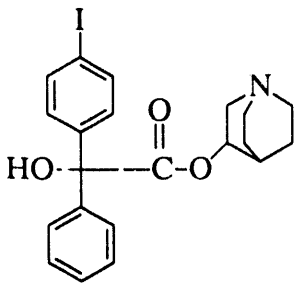
3



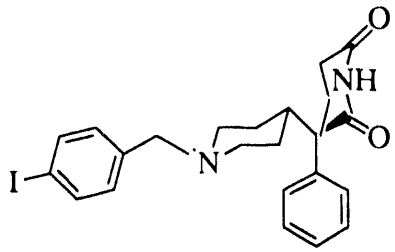
4



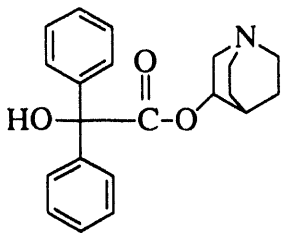
5) X=H Y=^{18}F
6) X=^{18}F Y=H
7) X=^{76}Br Y=H



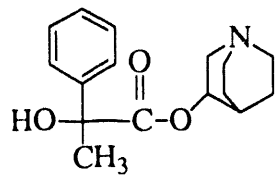
8



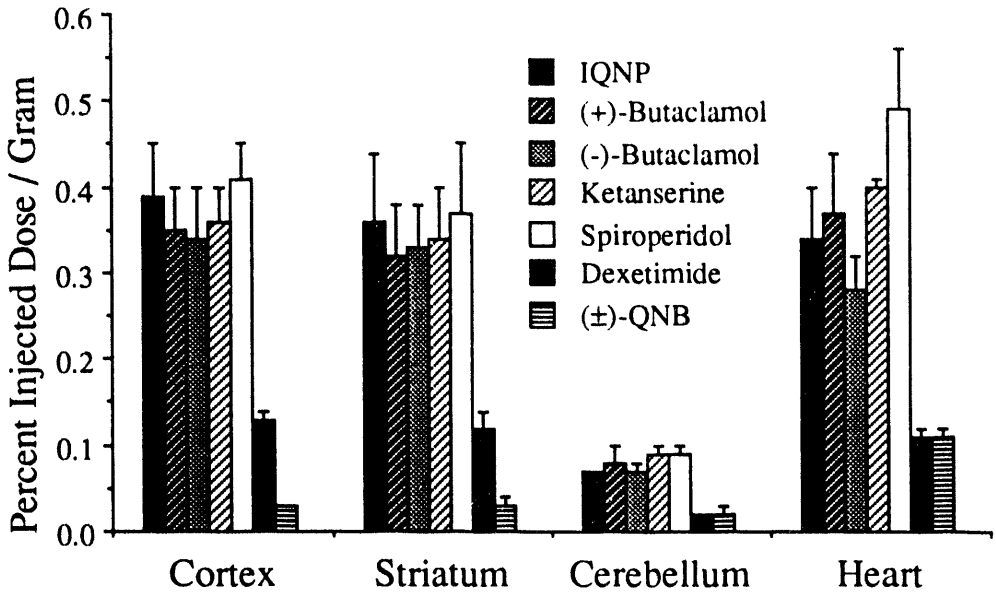
9

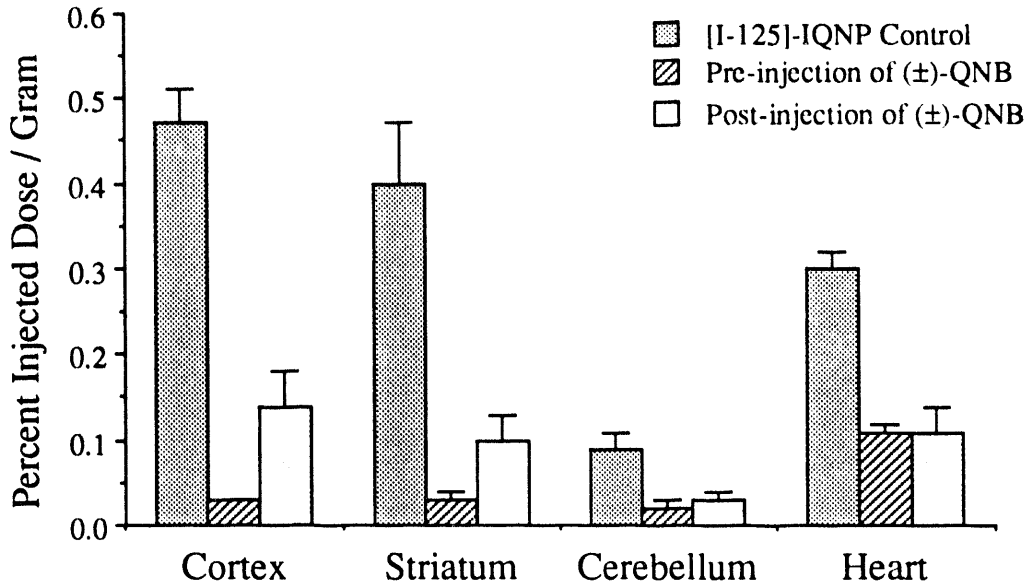


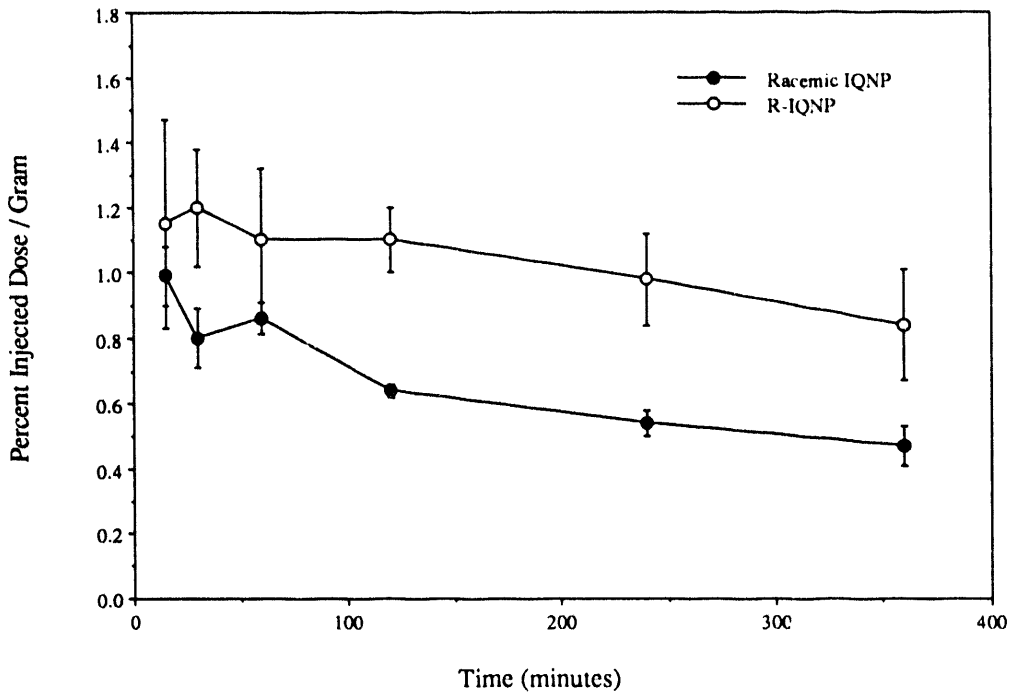
10

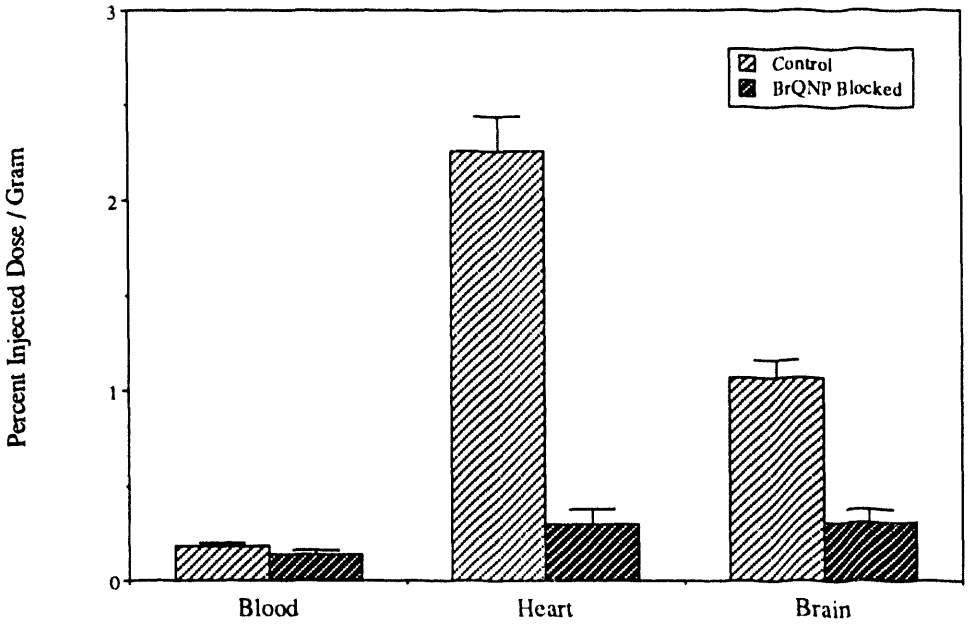


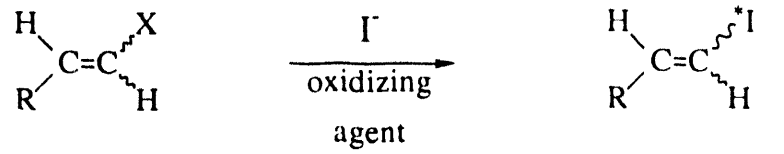
11


Percent Injected Dose / Gram
0.6
0.5
0.4
0.3
0.2
0.1
0.0
IQNP
(+)-Butaclamol
(-)-Butaclamol
Ketanserine
Spiroperidol
Dexetimide
(±)-QNB
Cortex
Striatum
Cerebellum
Heart

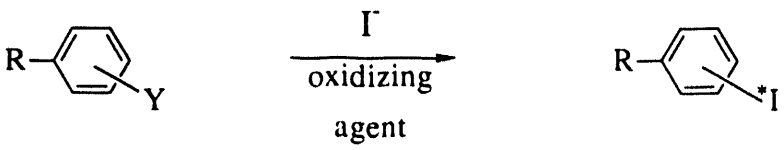

Percent Injected Dose / Gram
0.6
0.5
0.4
0.3
0.2
0.1
0.0
[I-125]-IQNP Control
Pre-injection of (±)-QNB
Post-injection of (±)-QNB
Cortex
Striatum
Cerebellum
Heart


Racemic IQNP
R-IQNP
Percent Injected Dose / Gram
Time (minutes)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
0
100
200
300
400

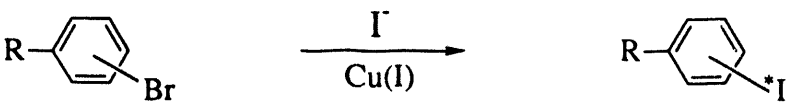

Percent Injected Dose / Gram
3
2
1
0
Control
BrQNP Blocked
Blood
Heart
Brain



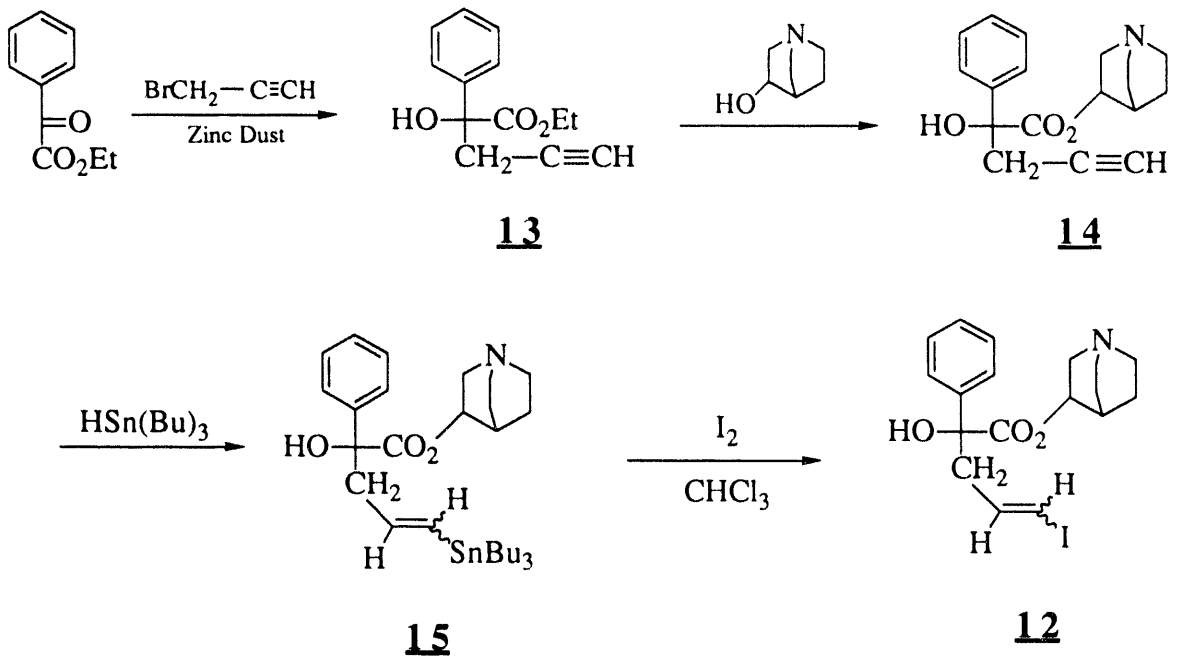
X = $B(OH)_2$, $SnMe_3$



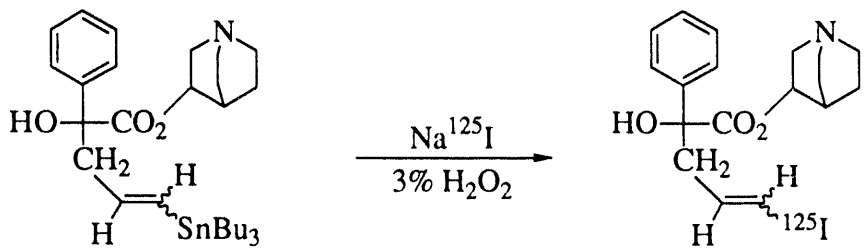
Y = $B(OH)_2$, $SnMe_3$, HgOAc, $Th(TFA)_2$



Scheme 1

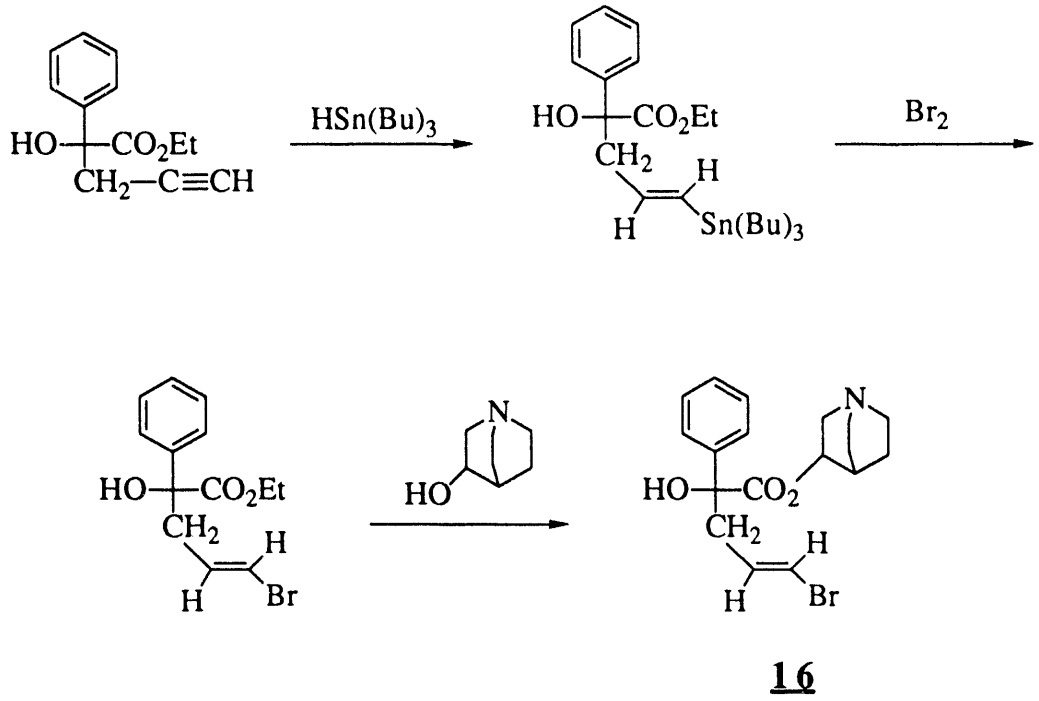

BrCH2—C≡CH
Zinc Dust
CO2Et
HO
CO2Et
CH2—C≡CH
13
HO
N
HO
CO2
CH2—C≡CH
14
HSn(Bu)3
HO
CO2
CH2
H
H
SnBu3
15
I2
CHCl3
HO
CO2
CH2
H
H
I
12

Scheme 2



15 **12**

Scheme 3


HO
CO2Et
CH2—C≡CH
HSn(Bu)3
HO
CO2Et
CH2
H
H
Sn(Bu)3
Br2
HO
CO2Et
CH2
H
H
Br
N
HO
HO
CO2
CH2
H
H
Br
N
16

Scheme 4

DATE
FILMED
8/25/94

END