Resolution and *in Vitro* and Initial *in Vivo* Evaluation of Isomers of Iodine-125-Labeled 1-Azabicyclo[2.2.2]oct-3-yl α-Hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate: A High-Affinity Ligand for the Muscarinic Receptor

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1-Azabicyclo[2.2.2]oct-3-yl α-hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate (IQNP, **1**), is a highly selective ligand for the muscarinic acetylcholinergic receptor (mAChR). There are eight stereoisomers in the racemic mixture. The optical isomers of α-hydroxy-α-phenyl-α-(1-propyn-3-yl)acetic acid were resolved as the α-methylbenzylamine salts, and the optical isomers of 3-quinuclidinol were resolved as the tartrate salts. The *E* and *Z* isomers were prepared by varying the reaction conditions for the stannylation of the triple bond followed by purification utilizing flash column chromatography. *In vitro* binding assay of the four stereoisomers containing the (*R*)-(−)-3-quinuclidinyl ester demonstrated that each isomer of **1** bound to mAChR with high affinity. In addition, (*E*)-(−)-(−)-IQNP demonstrated the highest receptor subtype specificity between the m1 molecular subtype (K_D, nM, 0.383 ± 0.102) and the m2 molecular subtype (29.6 ± 9.70). *In vivo* biodistribution studies demonstrated that iodine-125-labeled (*E*)-(−)-(+)-**1** cleared rapidly from the brain and heart. In contrast, iodine-125-labeled (*E*)-(−)-(−)-, (*Z*)-(−)-(−)-, and (*Z*)-(−)-(+)-**1** have high uptake and retention in mAChR rich areas of the brain. It was also observed that (*E*)-(−)-(−)-IQNP demonstrated an apparent subtype selectivity *in vivo* with retention in M_1 (m1, m4) mAChR areas of the rain. In addition, (*Z*)-(−)-(−)-IQNP also demonstrated significant uptake in tissues containing the M_2 (m2) mAChR subtype. These results demonstrate that the iodine-123-labeled analogues of the (*E*)-(−)-(−)- and (*Z*)-(−)-(−)-IQNP isomers are attractive candidates for single-photon emission-computed tomographic imaging of cerebral and cardiac mAChR receptor densities.

Introduction

The muscarinic acetylcholinergic receptor (mAChR) has been well studied, and four subtypes have been identified as M_1–M_4 by classical pharmacological methods.[1,2] More recently, mAChR has been cloned, and five distinct molecular subtypes were identified as m1–m5.[3–8] The M_1 subtype has been reported to correspond to m1, m4, and m5, the M_2 to m2, and the M_3 to the m3 subtype. The distribution of these muscarinic subtypes vary throughout the body.[9] For example, the lung contains both m2 and m4 subtypes, while the cortex and hippocampus contain significant quantities of the m1 subtype in addition to lesser concentrations of the m2–m4 subtypes. The m2 subtype is found in high concentrations in the thalamus, cerebellum, and heart. The mAChR subtypes have been postulated to play an important role in many physiological and behavioral responses in addition to dementias such as Parkinson's and Alzheimer's diseases.[10–17] These observations have stimulated interest in the ability of imaging the density or distribution of cerebral and cardiac mAChR noninvasively *in vivo* utilizing external imaging techniques.

We recently reported the synthesis and evaluation of a new ligand, 1-azabicyclo[2.2.2]oct-3-yl α-hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate (IQNP, **1**), which demonstrates high cerebral uptake and specificity *in vivo* for mAChR.[18] IQNP is an analogue of 1-azabicyclo-[2.2.2]oct-3-yl α-hydroxy-α-(4-iodophenyl)-α-phenylacetate (4-IQNB, **2**), a high-affinity muscarinic antagonist. 4-IQNB has been utilized in single-photon emission-computed tomography (SPECT) studies of healthy individuals and patients with dementias.[19–25] However, there have been several problems identified which would be expected to limit the routine use of **2** in patient studies. These include a low reported radiolabeling yield (~20%)[26] using the triazine decomposition method and the prolonged time period required between administration and scanning (~24 h) due to the slow clearance of nonspecific binding. IQNP is readily radioiodinated with high specific activity and demonstrates significant cerebral uptake and rapid vascular clearance. Therefore iodine-123-labeled IQNP (**1**) may be an important candidate for further study and potential clinical studies for the evaluation of mAChR density by SPECT.

IQNP (**1**) contains two chiral centers analogous to the asymmetric centers in 4-IQNB, the 3-carbon of the 3-quinuclidinyl ring (a) and the 2-carbon of the acetate

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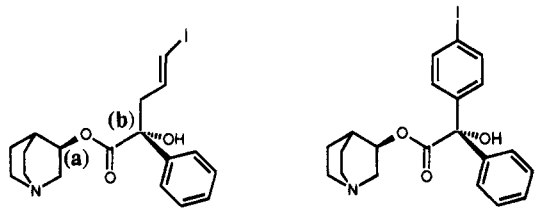


1 2

Figure 1.

moiety (b) (Figure 1). Unlike 4-IQNB, however, **1** may have either *Z* (*cis*) or *E* (*trans*) orientation of the iodide on the double bond. The presence of two chiral centers and the stereochemistry of the vinyl iodide result in eight possible stereoisomers in the racemic mixture. The various isomers of 4-IQNB (**2**) have been separated and evaluated both *in vitro* and *in vivo*. (*S*,*S*)-4-IQNB has a low affinity for mAChR and has been used in rat studies to estimate the degree of nonspecific ligand binding.[27,28] *In vivo* studies with (*R*,*R*)-4-IQNB demonstrate mAChR receptor-mediated localization,[28-30] and iodine-123-labeled (*R*,*R*)-4-IQNB has been used in SPECT studies in healthy individuals and patients with dementias.[19-24] Although (*R*,*S*)- and (*R*,*R*)-4-IQNB have similar affinity for mAChR and metabolic clearance, (*R*,*S*)-4-IQNB has a 13-fold faster dissociation rate from the receptor and an association rate which is 2–3-fold faster as compared to that of (*R*,*R*)-4-IQNB.[31,32] However, the various stereoisomers of 4-IQNB have not been reported to be mAChR subtype-selective *in vivo*.

In this paper we report the resolution of the various stereoisomers of **1** and describe the results of the *in vitro* binding assays and an *in vivo* biodistribution study in female rates of the four isomers which contain the (*R*)-(−)-3-quinuclidinyl ester to evaluate the optimum stereochemistry for the maximum affinity and subtype selectivity for mAChR.

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. Ketanserine tartrate was purchased from Research Biochemicals Inc. (Natick, MA), and (±)-QNB oxalate was prepared from a literature method.[33] All other chemicals and solvents were analytical grade and used without further purification. Sodium [^{125}I]iodide was purchased from New England Nuclear (specific activity ca. 2200 Ci/mmol). Sep-Pak (C_{18}) columns were purchased from Waters, Inc. Thin layer chromatographic analyses (TLC) were performed using 250 μm layers of silica gel coated on glass (Alltech). Analytical high-performance liquid chromatographic (HPLC) analysis was performed using a Whatmann Partisil 5 (3.9 mm × 30 cm) column with an on-line Waters Model 454 variable wavelength detector at 254 nm. Purification of the radioiodinated ligands was performed by HPLC using a Waters Nova Pak (7.8 mm × 30 cm) column with an on-line Beckman Model 170 radioisotope detector and monitoring at 254 nm. 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). Elemental analyses were determined at Galbraith Laboratories (Knoxville, TN). Optical rotations were recorded using a Model MDL SR6 half-circle polarimeter from Polyscience (Niles, IL). Concentration (*c*) of the specific rotation is reported as g/mL. The iodine-125-labeled samples were counted in a Packard Minaxi 5000 sodium iodide auto-γ-counter. Melting points were determined using a Hoover melting point Model 245 apparatus and are uncorrected.

Resolution of 3-Quinuclidinol. (*R*)-(−)- and (*S*)-(+)-3-Quinuclidinol were resolved according to the published procedures.[34]

Ethyl α-Hydroxy-α-phenyl-α-(1-propyn-3-yl)acetate (3). Compound **3** was prepared according to a modified procedure published previously.[18] Ethyl benzoylformate (32.99 g, 185.1 mmol), propargyl bromide (44.06 g, 370.3 mmol), zinc dust (30.26 g, 462.8 mmol), and anhydrous tetrahydrofuran (100 mL) were added to a flask under argon. A water-cooled condenser was attached, and the solution was stirred in an oil bath at ~80 °C until the reaction proceeded on its own. This reaction, which may be vigorous, was moderated with an ice bath when necessary. When the reaction had subsided, the solution was refluxed for 4 h. The mixture was filtered and diluted with ethyl acetate (200 mL). The resulting solution was washed with a 20% ammonium acetate solution (2 × 150 mL) and then dried over sodium sulfate. The organic solution was evaporated to dryness in vacuo to afford an orange oil. The product was first purified by flash column chromatography using silica gel and eluted with hexane:ethyl acetate (9:1) followed by another column of silica gel eluted with hexane:ethyl acetate (92:8) to afford **3** as a pale orange oil (32.05 g, 79.3%): ^{1}H NMR ($CDCl_3$) δ 7.61 (m, 2H), 7.32 (m, 3H), 4.26 (m, 2H), 4.02 (s, 1H), 3.21–3.12 (d of d, 1H, J = 2.6 Hz), 2.86–2.78 (d of d, 1H, J = 2.6 Hz), 2.03 (t, 1H, J = 2.6 Hz), 1.27 (t, 3H); ^{13}C 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 gel, hexane:ethyl acetate (9:1)] R_f = 0.25.

Resolution of α-Hydroxy-α-phenyl-α-(1-propyn-3-yl)-acetic Acid (4). Compound **3** (11.77 g, 53.9 mmol) was added to a 1 M ethanolic sodium hydroxide solution (80 mL), and the mixture was stirred at 60 °C for 3 h. The solution was then cooled to room temperature and diluted with 200 mL of water followed by washing with ethyl acetate (100 mL). The aqueous solution was made acidic by the slow addition of a 6 M HCl solution. The solution was washed with diethyl ether (2 × 100 mL), and the organic solution was dried over magnesium sulfate and evaporated to dryness to afford racemic **4** as an orange oil. Racemic **4** was added to diethyl ether (100 mL) and the solution heated to boiling in a water bath. (−)-α-Methylbenzylamine (6.61 g, 54.5 mmol) was added to the boiling solution, and the solution was then allowed to set at room temperature for 18 h. The resulting precipitate was filtered and the solid washed with ether. The (−)-α-methylbenzylamine salt was recrystallized twice from water to afford the product as a pale yellow solid (4.41 g, 52.7%): mp 154–6 °C; $[\alpha]^{20}_D$ = −10.5° (c = 0.10, ethanol).

The salt was then dissolved in a 3 M HCl solution (200 mL) and the acidic solution washed with diethyl ether (2 × 200 mL). The ether solution was dried over magnesium sulfate and evaporated to dryness to afford (−)-**4** as an orange oil (2.70 g, 100%): $[\alpha]^{20}_D$ = −10.7° (c = 0.27, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.62–7.57 (m, 2H), 7.40–7.30 (m, 3H), 3.24 (d of d, 1H, J = 2.6 Hz), 3.16 (d of d, 1H, J = 2.6 Hz), 2.03 (t, 1H, J = 2.6 Hz); ^{13}C NMR ($CDCl_3$) δ 177.69 (CO), 138.82 (C), 128.42 (CH), 127.97 (CH), 125.31 (CH), 78.47 (C), 77.29 (C), 71.89 (CH), 30.71 (CH_2).

The mother liquors were dissolved in 3 M HCl (200 mL), and the solution was washed with diethyl ether (2 × 150 mL). The ether solution was dried over magnesium sulfate and evaporated to dryness to afford an orange oil (7.39 g). The recovered acid was added to diethyl ether (100 mL), and the solution was heated to boiling in a water bath. (+)-α-Methylbenzylamine (4.83 g, 39.8 mmol) was added to the boiling solution, and the mixture was then allowed to set at room temperature for 18 h. The resulting precipitate was filtered and the solid washed with ether. The (+)-α-methylbenzylamine salt was recrystallized twice from water to afford the product as a pale yellow solid (3.85 g, 46.0%): mp 143–4 °C; $[\alpha]^{20}_D$ = +11.8° (c = 0.08, ethanol).

The salt was then dissolved in a 3 M HCl solution (200 mL) and the acidic solution washed with diethyl ether (2 × 200 mL). The ether solution was dried over magnesium sulfate

and evaporated to dryness to afford (+)-**4** as an orange oil (2.36 g, 100%): $[\alpha]^{20}_D$ = +12.7° (*c* = 0.24, chloroform); ^{1}H NMR ($CDCl_3$) δ 7.62–7.57 (m, 2H), 7.36–7.29 (m, 3H), 3.23–3.14 (d, 1H, *J* = 2.6 Hz), 2.92–2.82 (d of d, 1H, *J* = 2.6 Hz), 2.03 (t, 1H, *J* = 2.6 Hz); ^{13}C NMR ($CDCl_3$) δ 177.29 (CO), 138.97 (C), 128.57 (CH), 127.92 (CH), 125.48 (CH), 79.62 (C), 77.24 (C), 72.07 (CH), 30.87 (CH_2).

(−)-Ethyl α-Hydroxy-α-phenyl-α-(1-propyn-3-yl)acetate ((−)-3). (−)-**4** (2.70 g, 14.2 mmol) was dissolved in ethanol (75 mL), and sulfuric acid (1 mL) was slowly added to the solution with stirring. The resulting mixture was refluxed for 5 h and allowed to cool to room temperature. A saturated solution of sodium bicarbonate (100 mL) was slowly added and the solution washed with diethyl ether (2 × 150 mL). The ether solution was dried over magnesium sulfate and evaporated to dryness to afford an orange oil. The product was purified by Kugelruhr distillation under vacuum (0.1 mmHg), and (−)-**3** distilled over at 95 °C as a pale oil (2.80 g, 90.2%): ^{1}H NMR ($CDCl_3$) δ 7.62–7.57 (m, 2H), 7.40–7.29 (m, 3H), 4.37–4.18 (m, 2H), 4.01 (s, 1H), 3.21–3.13 (d of d, 1H), 2.88–2.78 (d of d, 1H), 2.04 (t, 1H), 1.28 (t, 3H); ^{13}C NMR ($CDCl_3$) δ 173.19 (CO), 139.87 (C), 128.19 (CH), 128.04 (CH), 125.23 (CH), 79.02 (C), 77.20 (C), 70.98 (CH), 62.73 (CH_2), 30.85 (CH_2), 14.02 (CH_3); $[\alpha]^{20}_D$ = −13.6° (*c* = 0.28, chloroform); TLC [silica gel, hexane:ethyl acetate (9:1)] R_f = 0.25.

(+)-Ethyl α-Hydroxy-α-phenyl-α-(1-propyn-3-yl)acetate ((+)-3). (+)-**3** was prepared from (+)-**4** in an analogous manner as above to afford the product as a pale oil (2.40 g, 88.5%): ^{1}H NMR ($CDCl_3$) δ 7.62–7.57 (m, 2H), 7.40–7.29 (m, 3H), 4.37–4.18 (m, 2H), 4.01 (s, 1H), 3.21–3.13 (d of d, 1H), 2.88–2.78 (d of d, 1H), 2.04 (t, 1H), 1.28 (t, 3H); ^{13}C NMR ($CDCl_3$) δ 173.15 (CO), 139.87 (C), 128.14 (CH), 127.99 (CH), 125.20 (CH), 78.99 (C), 77.18 (C), 70.96 (CH), 62.68 (CH_2), 30.83 (CH_2), 13.98 (CH_3); $[\alpha]^{20}_D$ = +18.4° (*c* = 0.24, chloroform); TLC [silica gel, hexane:ethyl acetate (9:1)] 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 (2.28 g, 17.9 mmol) and benzene (125 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 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 hot solution was transferred, via double-tipped needle technique to remove unreacted sodium metal, into a flask which contained a solution of (−)-**3** (1.72 g, 7.9 mmol) in benzene (100 mL). This solution had also been previously refluxed for 1 h under argon utilizing a Dean–Stark trap to remove moisture. The resulting solution was then refluxed using a Dean–Stark trap for 18 h. The solution was then cooled to room temperature and evaporated to dryness under vacuum. The residue was diluted with water (200 mL) and washed with chloroform (2 × 150 mL). The chloroform layer was then washed with water (2 × 100 mL), dried over magnesium sulfate, and evaporated to dryness to afford a dark brown oil. The product was purified by flash column chromatography [silica gel, methylene chloride:methanol:ammonium hydroxide (95:5:1)] to afford (−)-(−)-**5** as an orange oil (1.11 g, 47.2%): ^{1}H NMR ($CDCl_3$) δ 7.62–7.55 (m, 2H), 7.41–7.27 (m, 3H), 4.89 (m, 1H), 4.5 (bs, 1H), 3.24–3.06 (m, 2H), 2.99–2.47 (m, 6H), 2.16–1.37 (m, 5H), 2.03 (t, 1H); ^{13}C NMR ($CDCl_3$) δ 172.99 (CO), 140.05 (C), 128.24 (CH), 128.11 (CH), 125.24 (CH), 79.09 (C), 77.19 (C), 73.78 (CH), 71.08 (CH), 54.70 (CH_2), 47.14 (CH_2), 46.18 (CH_2), 30.78 (CH_2), 25.07 (CH), 24.28 (CH_2), 19.49 (CH_2); $[\alpha]^{20}_D$ = −4.5° (*c* = 0.11, chloroform); TLC [silica gel, chloroform:methanol (8:2)] R_f = 0.49.

(−)-1-Azabicyclo[2.2.2]oct-3-yl (+)-α-Hydroxy-α-phenyl-α-(1-propyn-3-yl)acetate ((−)-(+)-5). (−)-(+)-**5** was prepared in an analogus manner as above using (−)-3-quinuclidinol (3.98 g, 31.3 mmol) and (+)-**3** (2.73 g, 12.5 mmol) to afford the product as an orange oil (1.78 g, 47.5%): ^{1}H NMR ($CDCl_3$) δ 7.61–7.55 (m, 2H), 7.41–7.27 (m, 3H), 4.87–4.81 (m, 1H), 4.52 (bs, 1H), 3.29–3.16 (m, 2H), 2.89–2.70 (m, 6H), 2.09 (m, 1H), 1.99–1.96 (m, 1H), 1.72–1.32 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 173.08 (CO), 140.09 (C), 128.24 (CH), 128.12 (CH), 125.24 (CH), 79.23 (C), 77.08 (C), 74.05 (CH), 71.20 (CH), 54.88 (CH_2), 47.12 (CH_2), 46.18 (CH_2), 30.85 (CH_2), 24.98 (CH), 24.25 (CH_2), 19.48 (CH_2); $[\alpha]^{20}_D$ = +41.8° (*c* = 0.11, chloroform); TLC [silica gel, chloroform:methanol (9:1)] R_f = 0.27.

(*E*)-(−)-1-Azabicyclo[2.2.2]oct-3-yl (−)-α-Hydroxy-α-phenyl-α-[1-(tributylstannyl)-1-propen-3-yl]acetate ((*E*)-(−)-(−)-6). To a clean dry flask under argon was added (−)-(−)-**5** (1.04 g, 3.5 mmol), anhydrous toluene (25 mL), 2,2-azobis(2-methylpropionitrile) (AIBN) (692.7 mg, 4.2 mmol), and tributyltin hydride (1.62 g, 5.6 mmol). The solution was stirred in an oil bath at 70 °C for 3 h under argon, cooled to room temperature, diluted with methylene chloride, and washed with water (2 × 100 mL). The organic layer was dried over magnesium sulfate and evaporated to dryness to afford the product as a semisolid. The product was purified by repeated flash column chromatography [silica gel, chloroform:methanol:ammonium hydroxide (98:2:1)] to afford (*E*)-(−)-(−)-**6** as a pale yellow oil free of the minor amount of *Z* isomer (987.7 mg, 47.2%): ^{1}H NMR ($CDCl_3$) δ 7.62–7.56 (m, 2H), 7.37–7.25 (m, 3H), 6.13–6.04 (d, *J* = 19.01 Hz, 1H), 6.01–5.95 (t, *J* = 5.09 Hz, 1H), 4.83–4.77 (m, 1H), 4.11 (bs, 1H), 3.15–3.01 (m, 2H), 2.89–2.39 (m, 6H), 2.02–1.18 (17H), 0.96–0.74 (15H); ^{13}C NMR ($CDCl_3$) δ 174.19 (CO), 142.22 (C-H), 141.38 (C), 133.69 (Sn-C), 127.89 (CH), 127.51 (CH), 125.39 (CH), 77.84 (C), 73.21 (CH), 54.87 (CH_2), 47.94 (CH_2), 47.12 (CH_2), 46.21 (CH_2), 29.01 (CH_2), 27.24 (CH_2), 25.26 (CH), 24.33 (CH_2), 19.64 (CH_2), 13.71 (CH_3), 9.45 (CH_2); $[\alpha]^{20}_D$ = −12.5° (*c* = 0.01, chloroform); TLC [silica gel, chloroform:methanol (9:1)] R_f = 0.48.

(*E*)-(−)-1-Azabicyclo[2.2.2]oct-3-yl (+)-α-Hydroxy-α-phenyl-α-[1-(tributylstannyl)-1-propen-3-yl]acetate ((*E*)-(−)-(+)-6). (*E*)-(−)-(+)-**6** was prepared in an analogous manner as above using (−)-(+)-**5** (730.4 mg, 2.4 mmol), AIBN (487.8 mg, 3.0 mmol), and tributyltin hydride (1.08 g, 3.7 mmol) to afford the product as a pale yellow oil (673.0 mg, 47.2%): ^{1}H NMR ($CDCl_3$) δ 7.63–7.58 (m, 2H), 7.38–7.26 (m, 3H), 6.17–6.08 (d, *J* = 19.01 Hz, 1H), 6.04–5.98 (t, *J* = 5.09 Hz, 1H), 4.80–4.76 (m, 1H), 4.08 (bs, 1H), 3.21–3.03 (m, 2H), 2.91–2.67 (m, 6H), 1.92–1.90 (1H), 1.64–1.19 (1H), 1.64–1.19 (16H), 0.98–0.72 (15H); ^{13}C NMR ($CDCl_3$) δ 174.26 (CO), 142.17 (C-H), 141.51 (C), 134.01 (Sn-C), 127.96 (CH), 127.60 (CH), 125.45 (CH), 77.71 (C), 73.55 (CH), 55.04 (CH2), 48.01 (CH_2), 47.28 (CH_2), 46.32 (CH_2), 29.03 (CH_2), 27.25 (CH_2), 24.99 (CH), 24.29 (CH_2), 19.64 (CH_2), 13.72 (CH_3), 9.48 (CH_2); $[\alpha]^{20}_D$ = +29.0° (*c* = 0.07, chloroform); TLC [silica gel, chloroform:methanol (9:1)] R_f = 0.47.

(*Z*)-(−)-1-Azabicyclo[2.2.2]oct-3-yl (−)-α-Hydroxy-α-phenyl-α-[1-(tributylstannyl)-1-propen-3-yl]acetate ((*Z*)-(−)-(−)-6). (−)-(−)-**5** (226.6 mg, 0.8 mmol) was added to a dry flask with hexamethylphosphoramide (HMPA) (1.0 mL) and tributyltin hydride (0.6 g, 2.2 mmol). The solution was stirred in an oil bath at 50 °C for 24 h. The solution was then cooled to room temperature, diluted with water (100 mL), and washed with ethyl acetate (100 mL). The organic fraction was then washed with water (2 × 100 mL), dried over magnesium sulfate, and evaporated to dryness to afford a pale oil. The product was purified by repeated flash column chromatography [silica gel, methylene chloride:methanol:ammonium hydroxide (97:3:1)] to afford (*Z*)-(−)-(−)-**6** as a pale yellow oil free of the *E* isomer (110.4 mg, 23.2%): ^{1}H NMR ($CDCl_3$) δ 7.62–7.58 (m, 2H), 7.40–7.26 (m, 3H), 6.49–6.36 (pent, *J* = 6.1 Hz, 1H), 6.07–6.00 (d, *J* = 12.7 Hz, 1H), 4.87–4.81 (m, 1H), 3.85 (bs, 1H), 3.18–2.42 (m, 8H), 2.06–2.00 (m, 1H), 1.77–1.15 (m, 16H), 0.96–0.65 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 174.34 (CO), 142.17 (C-H), 141.60 (C), 133.68 (Sn-H), 128.13 (CH), 127.68 (CH), 125.46 (CH), 77.46 (C), 73.67 (CH), 55.00 (CH_2), 47.23 (CH_2), 46.33 (CH_2), 29.18 (CH_2), 27.36 (CH_2), 25.21 (CH), 24.43 (CH_2), 19.64 (CH_2), 13.73 (CH_3), 10.29 (CH_2); TLC [silica gel, chloroform:methanol (9:1)] R_f = 0.49.

(*Z*)-(−)-1-Azabicyclo[2.2.2]oct-3-yl (+)-α-Hydroxy-α-phenyl-α-[1-(tributylstannyl)-1-propen-3-yl]acetate ((*Z*)-(−)-(+)-6). (*Z*)-(−)-(+)-**6** was prepared in an analogous manner as above using (−)-(+)-**5** (235.4 mg, 0.8 mmol), HMPA (1.4 mL), and tributyltin hydride (757.4 mg, 2.6 mmol) to afford the product as a pale yellow oil (158.8 mg, 17.8%): ^{1}H NMR ($CDCl_3$) δ 7.62–7.56 (m, 2H), 7.39–7.27 (m, 3H), 6.51–6.38 (pent, *J* = 6.9 Hz, 1H), 6.09–6.02 (d, *J* = 12.7 Hz, 1H), 4.85–4.80 (m, 1H), 3.85 (bs, 1H), 3.30–2.62 (m, 8H), 1.97–1.93 (m,

1H), 1.68–1.16 (m, 16H), 0.97–0.71 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 174.35 (CO), 141.51 (C-H), 141.23 (C), 133.83 (Sn-H), 128.09 (CH), 127.64 (CH), 125.49 (CH), 77.36 (C), 73.72 (CH), 55.18 (CH_2), 47.32 (CH_2), 46.33 (CH_2), 29.18 (CH_2), 27.36 (CH_2), 24.98 (CH), 24.34 (CH_2), 19.43 (CH_2), 13.73 (CH_3), 10.31 (CH_2); $[\alpha]^{20}_D = -31.5°$ ($c = 0.02$, chloroform); TLC [silica gel, chloroform:methanol (9:1)] $R_f = 0.49$.

(*E*)-(−)-1-Azabicyclo[2.2.2]oct-3-yl (−)-α-Hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate ((*E*)-(−)-(−)-1). (*E*)-(−)-(−)-**6** (508.6 mg, 0.9 mmol) was dissolved in chloroform (10 mL) and the solution stirred. A 0.5 M solution of iodine in chloroform was slowly added until the color of iodine persisted. The solution was then stirred for 18 h at room temperature followed by dilution with chloroform (100 mL). The solution was washed with a 10% sodium bisulfite solution (50 mL), a saturated sodium bicarbonate solution (50 mL), and water (50 mL). The chloroform solution was dried over magnesium sulfate and evaporated to dryness to afford a yellow oil. The product was purified by flash column chromatography [silica gel, chloroform:methanol:ammonium hydroxide (95:5:1)] to afford (*E*)-(−)-(−)-**1** as a clear oil (296.3 mg, 80.6%): mp 174–6 °C (oxalate, from ether/ethanol); ^{1}H NMR ($CDCl_3$) δ 7.57–7.52 (m, 2H), 7.39–7.27 (m, 3H), 6.59–6.44 (pent, 1H), 6.19–6.12 (d, $J = 14.5$ Hz, 1H), 4.85–4.70 (m, 1H), 4.6 (bs, 1H), 3.15–2.61 (m, 7H), 2.46–2.38 (m, 1H), 2.02–1.99 (m, 1H), 1.76–1.40 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 173.41 (CO), 140.83 (C), 139.96 (CH), 128.11 (CH), 127.76 (CH), 125.05 (CH), 78.64 (CI), 77.26 (C), 73.40 (CH), 54.60 (CH_2), 46.88 (CH_2), 46.00 (CH_2), 45.71 (CH_2), 25.08 (CH_2), 24.18 (CH_2), 19.36 (CH_2); $[\alpha]^{20}_D = -20.2°$ ($c = 0.02$, chloroform); TLC [silica gel, chloroform:methanol (9:1)] $R_f = 0.36$; HPLC [mobile phase, methylene chloride:ethanol:triethylamine (97:3:0.0003); flow rate = 1.0 mL/min] $t_R = 13$ min. Anal. ($C_{20}H_{24}INO_7 \cdot 0.5H_2O$) Calcd: C, 45.64; H, 4.79; N, 2.66. Found: C, 45.75; H, 4.58; N, 2.66.

(*E*)-(−)-1-Azabicyclo[2.2.2]oct-3-yl (+)-α-Hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate ((*E*)-(−)-(+)-1). (*E*)-(−)-(+)-**1** was prepared in an analogous manner as above using (*E*)-(−)-(+)-**6** (645.6 mg, 1.1 mmol) to afford the product as a clear oil (334.5 mg, 71.2%): mp 184–8 °C (oxalate, from ether/ethanol); ^{1}H NMR ($CDCl_3$) δ 7.58–7.52 (m, 2H), 7.40–7.26 (m, 3H), 6.64–6.50 (m, 1H), 6.26–6.19 (d, $J = 14.6$ Hz, 1H), 4.84–4.80 (m, 1H), 4.1 (bs, 1H), 3.30–3.19 (m, 1H), 3.02–2.62 (m, 7H), 1.96–1.95 (m, 1H), 1.69–1.29 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 173.70 (CO), 140.88 (C), 140.00 (CH), 128.24 (CH), 127.92 (CH), 125.21 (CH), 78.97 (CI), 77.24 (C), 73.94 (CH), 55.00 (CH_2), 47.22 (CH_2), 46.27 (CH_2), 45.88 (CH_2), 25.00 (CH_2), 24.27 (CH_2), 19.41 (CH_2); $[\alpha]^{20}_D = +39.5°$ ($c = 0.03$, chloroform); TLC [silica gel, chloroform:methanol (9:1)] $R_f = 0.38$; HPLC t_R = 11 min. Anal. ($C_{20}H_{24}INO_7$) Calcd: C, 46.43; H, 4.68; N, 2.71. Found: C, 46.93; H, 4.87; N, 2.71.

(*Z*)-(−)-1-Azabicyclo[2.2.2]oct-3-yl (−)-α-(1-iodo-1-propen-3-yl)-α-phenylacetate ((*Z*)-(−)-(−)-1). (*Z*)-(−)-(−)-**1** was prepared in an analogous manner as above using (*Z*)-(−)-(−)-**6** (154.9 mg, 0.26 mmol) to afford the product as a clear oil (33.6 mg, 30.3%): mp 135–65 °C (oxalate, from ether/ethanol); ^{1}H NMR ($CDCl_3$) δ 7.64–7.54 (m, 2H), 7.43–7.26 (m, 3H), 6.39–6.22 (m, $J = 7.8$ Hz, 1H), 4.87–4.79 (m, 1H), 4.1 (bs, 1H), 3.18–2.35 (m, 8H), 2.14–1.25 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 174.00 (CO), 140.88 (C), 139.91 (CH), 128.32 (CH), 128.04 (CH), 125.43 (CH), 85.48 (CI), 77.27 (C), 74.16 (CH), 55.00 (CH_2), 47.24 (CH_2), 46.31 (CH_2), 44.66 (CH_2), 25.17 (CH_2), 24.43 (CH_2), 19.68 (CH_2); TLC [silica gel, chloroform:methanol (9:1)] $R_f = 0.34$; HPLC $t_R = 14$ min. Anal. ($C_{20}H_{24}INO_7 \cdot 0.5H_2O$) Calcd: C, 45.64; H, 4.79; N, 2.66. Found: C, 45.35; H, 4.90; N, 2.62.

(*Z*)-(−)-1-Azabicyclo[2.2.2]oct-3-yl (+)-α-Hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate ((*Z*)-(−)-(+)-1). (*Z*)-(−)-(+)-1 was prepared in an analogous manner as above using (*Z*)-(−)-(+)-**6** (136.7 mg, 0.23 mmol) to afford the product as a clear oil (31.4 mg, 31.9%): mp 138–45 °C (oxalate, from ether/ethanol); ^{1}H NMR ($CDCl_3$) δ 7.63–7.54 (m, 2H), 7.42–7.26 (m, 3H), 6.41–6.37 (m, $J = 7.8$ Hz, 1H), 6.36–6.26 (m, 1H), 4.85–4.81 (m, 1H), 4.1 (bs, 1H), 3.23–2.62 (m, 8H), 1.95–1.92 (m, 1H), 1.66–1.23 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 174.28 (CO), 140.87 (C), 135.58 (CH), 128.27 (CH), 128.00 (CH), 125.49 (CH), 85.51 (CI), 77.09 (C), 74.04 (CH), 55.05 (CH_2), 47.29 (CH_2), 46.34 (CH_2), 36.86 (CH_2), 24.94 (CH_2), 24.19 (CH_2), 19.25 (CH_2); TLC [silica gel, chloroform:methanol (9:1)] $R_f = 0.31$; HPLC $t_R = 12$ min. Anal. ($C_{20}H_{24}INO_7 \cdot 0.5H_2O$) Calcd: C, 45.64; H, 4.79; N, 2.66. Found: C, 45.38; H, 4.90; N, 2.62.

(*E*)-(−)-1-Azabicyclo[2.2.2]oct-3-yl (−)-α-Hydroxy-α-(1-[^{125}I]iodo-1-propen-3-yl)-α-phenylacetate ([^{125}I]-(*E*)-(−)-(−)-1). (*E*)-(−)-(−)-**6** (100 μL, 1.2 mg/mL in ethanol), 0.1 M HCl (150 μL), and sodium iodide-125 (5.0 mCi in 0.1 M NaOH) in ethanol (100 μL) were added to a 2.0 mL reaction vial. The solution was stirred briefly, and a 3% H_2O_2 solution (50 μL, 30% solution diluted with distilled water before use) was added. The vial was sealed, and the mixture was stirred at room temperature for 1 h. To this solution was then added a 10% sodium bisulfite solution (250 μL). After stirring a few minutes, a saturated sodium bicarbonate solution (1.0 mL) was slowly added. The resulting solution was transferred to a syringe containing a C_{18} Sep Pak column. The reaction vial was rinsed with acetonitrile (100 μL) followed by water (1.0 mL). These washes were transferred to the syringe containing the product and diluted to 10 mL with water. The solution was passed through the Sep-Pak column, and the Sep-Pak column was washed with water (2 × 5 mL) followed by acetonitrile (250 μL). The product was removed from the Sep-Pak column with ~5 mL of a chloroform:methanol solution (8:2) and evaported to dryness under a stream of argon. The product was then taken up in a minimum amount of HPLC solvent and injected into the HPLC instrument [mobile phase, methylene chloride:ethanol:triethylamine (98:2:0.0002); flow rate, 2.0 mL/min; retention time ~ 10 min]. The fractions containing [^{125}I]-(*E*)-(−)-(−)-**1** were pooled and evaporated to dryness under argon to afford 3.1 mCi of [^{125}I]-(*E*)-(−)-(−)-**1** (65.0%) with a specific activity of >1000 mCi/μmol. [^{125}I]-(*E*)-(−)-(−)-**1** was analyzed by comparing the TLC [silica gel, methylene chloride:methanol (8:2), R_f = 0.4] and HPLC mobility to that of the cold compound. In both cases [^{125}I]-(*E*)-(−)-(−)-**1** cochromatographed with the cold standard. 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.

(*E*)-(−)-1-Azabicyclo[2.2.2]oct-3-yl (+)-α-Hydroxy-α-(1-[^{125}I]iodo-1-propen-3-yl)-α-phenylacetate ([^{125}I]-(*E*)-(−)-(+)-1). [^{125}I]-(*E*)-(−)-(+)-**1** was prepared in the same manner as above to afford the product in 63.8% yield.

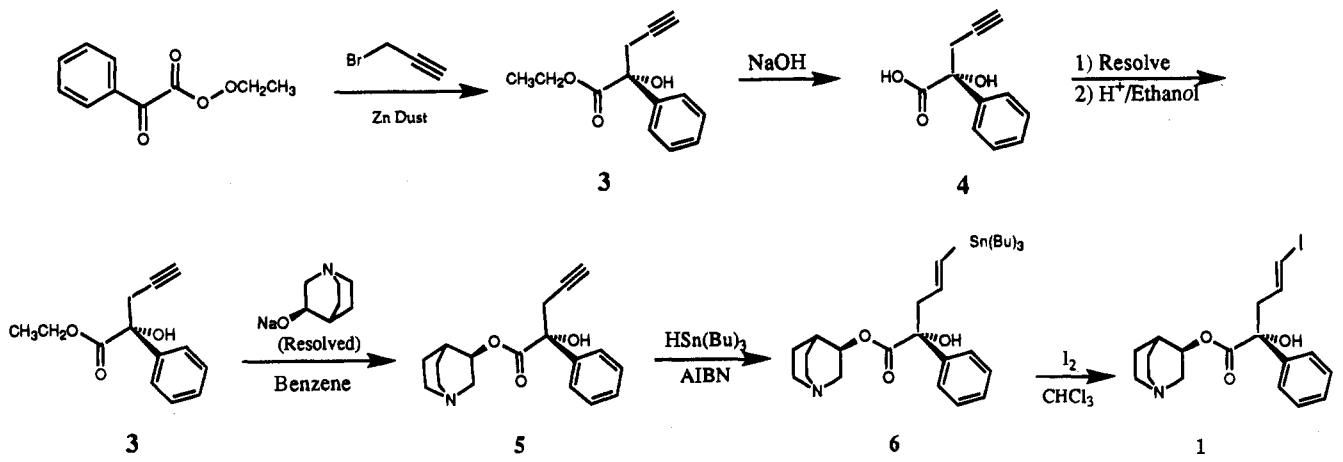
(*Z*)-(−)-1-Azabicyclo[2.2.2]oct-3-yl (−)-α-Hydroxy-α-(1-[^{125}I]iodo-1-propen-3-yl)-α-phenylacetate ([^{125}I]-(*Z*)-(−)-(−)-1). [^{125}I]-(*Z*)-(−)-(−)-**1** was prepared in the same manner as above to afford the product in 50.8% yield.

(*Z*)-(−)-1-Azabicyclo[2.2.2]oct-3-yl (+)-α-Hydroxy-α-(1-[^{125}I]iodo-1-propen-3-yl)-α-phenylacetate ([^{125}I]-(*Z*)-(−)-(+)-1). [^{125}I]-(*Z*)-(−)-(+)-**1** was prepared in the same manner as above to afford the product in 16.2% yield.

***In Vitro* Binding Assays. Tissue preparation**: Membranes containing m1, m2, and m4 receptors were isolated from CHO cells that had been transfected with these genes. These transfected cell lines were grown as previously described.[35] Cell membranes were prepared by lysing cells in 10 mM TRIS-HCl, pH 7.2, and 2 mM EDTA. Cell membranes were resuspended in the TRIS/EDTA buffer at a protein concentration of 3 mg/mL and stored at −70 °C until use.

Determination of K_D values: The K_D values for the muscarinic ligands were determined by competitive ligand-binding assay with [^{3}H]QNB as the radiotracer. Competition curves were generated with 12 concentrations of unlabeled compounds: 10^{-12}–10^{-6} M. The compounds were dissolved in 100% ethanol and added to 4 mL of tris(hydroxymethyl)-aminomethane-buffered (10 mM, pH 7.4) 0.9% saline containing 2.5×10^{-10} M [^{3}H]QNB at a final concentration of 0.5% ethanol. Aliquots (0.1 mL) of cell membranes were added; the mixture was vortexed followed by incubation at 30 °C for 2 h. The incubation mixture was rapidly filtered on a GF/C filter paper. The filter was washed with 15 mL of ice cold saline, air-dried, placed in Cytoscint (ICN Biomedicals, Inc.) scintillation cocktail, and counted for 2 min each. Data were analyzed with Ligand program.[36] The K_D values were obtained

Scheme 1



from pooled data of at least two determinations in duplicate on separate days.

Biodistribution Studies. 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 ORNL Animal Care and Use Committee. For these studies the radioiodinated ligand was dissolved in 100 μL of ethanol followed by the addition of 50 μL of 0.1 M HCl solution and diluted to 10 mL with normal saline. Following intravenous injection (1−2 μCi) into a lateral tail vein of the metophane-anesthetized rat, the animals 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 metophane 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.[37] Blood samples were obtained from the heart cavity after removal of the heart.

For the blocking experiment, either (±)-QNB or ketanserine was injected (5 mg/kg) in a group of animals 1 h prior to injection of [^{125}I]-(*R*)-IQNP (1−2 μCi). One group of animals was injected with [^{125}I]-(*R*)-IQNP as a control. The animals were then killed 3 h postinjection of the radiolabeled ligand. The various tissues were removed, treated, and analyzed as described above.

Results and Discussion

3-Quinuclidinyl esters having the *R*-(−) configuration at the 3-carbon have been shown *in vitro* to exhibit a higher affinity for mAChR when compared to the *S*-(+) configuration. For example, the *R* isomer of stereoisomeric β-(diethylamino)ethyl benzilate derivatives exhibits a 10-fold greater activity than the *S* isomer,[38] and both (*R*)-(−)-3-quinuclidinyl 4-iodobenzilate and (*R*)-(−)-3-quinuclidinyl benzilate demonstrate higher affinities for mAChR than the corresponding (*S*)-(+)-3-quinuclidinyl compounds.[30,32,39] An increase in the affinity for mAChR was also observed for other (*R*)-3-quinuclidinyl ester derivatives of QNB.[33] Therefore, we initially prepared iodine-125-labeled (*E,Z*)-(*R*)-1-azabicyclo[2.2.2]oct-3-yl (*R,S*)-α-hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate ((*R*)-**1**) using (*R*)-(−)-3-quinuclidinol transesterification with the racemic ethyl ester **3** as shown in Scheme 1. This synthetic scheme is analogous to that used in the preparation of racemic IQNP.

The results of the *in vivo* evaluation of iodine-125-labeled (*R*)-**1** ([^{125}I]-(*R*)-**1**) are shown in Table 1, and the activity observed in the cortical and striatal regions (high mAChR concentration[9]) increases by a factor of 2 as compared to the level of activity observed for racemic

Table 1. Biodistribution of Iodine-125-(*E,Z*)-(*R*)-(*R,S*)-IQNP ([^{125}I]-(*R*)-**1**) at 3 h ($n = 5$)

	mean injected dose/g (±SD)			
organ	racemic IQNP	(*R*)-**1**	preblocking (±)-QNB	preblocking ketanserine
blood	0.25 ± 0.04	0.18 ± 0.01	0.10 ± 0.01	0.14 ± 0.01
heart	0.34 ± 0.06	0.47 ± 0.05	0.08 ± 0.02	0.59 ± 0.06
cortex	0.36 ± 0.08	0.80 ± 0.15	0.03 ± 0.04	0.64 ± 0.08
striatum	0.39 ± 0.06	0.87 ± 0.10	0.04 ± 0.01	0.73 ± 0.07
cerebellum	0.07 ± 0.00	0.13 ± 0.01	0.01 ± 0.00	0.16 ± 0.03

IQNP at 3 h. It was also observed that the uptake of the ligand was blocked by the preinjection of (±)-QNB (5 mg/kg) 1 h prior to the injection of [^{125}I]-(*R*)-**1**, and the animals were killed 3 h later. The same protocol using ketanserine (serotonin 5HT$_2$ antagonist) demonstrated no decrease in the cerebral uptake of [^{125}I]-(*R*)-**1** (Table 1). These results demonstrate that the *R* configuration of the quinuclidinyl ring of IQNP is partly responsible for the affinity of the ligand to the muscarinic receptor and are in agreement with data reported for other ligands containing the 3-quinuclidinyl ester. In addition, the results of this study are in agreement with those data reported previously for racemic **1**[18] and indicate that the increased [^{125}]-(*R*)-**1** uptake is occurring at the muscarinic receptor site as evidenced by the ability of (±)-QNB to block the uptake of radioactivity, as opposed to the ligand binding at another receptor site as shown by the failure of ketanserine to block the uptake of [^{125}I]-(*R*)-**1**.

Resolution of the stereoisomers of the acetate moiety was investigated initially using quinine and quinidine as the resolving agents since these agents have been reported for the resolution of hydroxy monocarboxylic acids such as atrolactic acid[33] and 4-nitrobenzilic acid.[19,30] Ethyl α-hydroxy-α-phenyl-α-(1-propyn-3-yl)acetate (**3**) was saponified to the free acid followed by addition to a boiling solution of quinine in methanol. The crystals which precipitated from the solution were collected and recrystallized three times from methanol to afford the quinine salt of (+)-α-hydroxy-α-phenyl-α-(1-propyn-3-yl)acetic acid ((+)-**4**). The mother liquors were treated with a 2 M H_2SO_4 solution, and the recovered acid was added to a solution of quinidine in ethyl acetate. Upon cooling an orange oil was isolated. Since repeated attempts to crystallize this oil in a variety of organic solvents was unsuccessful, the use of other resolving agents was then investigated.

Since the optical isomers of α-methylbenzylamine have been successfully employed for resolution of α-hydroxy acids,[40,41] the use of these optically active amines was investigated for resolution of **3**. Following addition of (+)-α-methylbenzylamine to a boiling solution of racemic **4** in ether, the solid which precipitated from the solution was collected and recrystallized twice from water to afford the desired α-methylbenzylamine salt of (+)-**4**. Treatment of the salt in dilute acid afforded the desired (+)-**3**. The mother liquors were treated with dilute acid to recover enriched (−)-**4**. By the use of (−)-α-methylbenzylamine, (−)-**3** was isolated as described above. Transesterification of either (−)- or (+)-**3** with (*R*)-(−)-3-quinuclidinol afforded (−)-(−)- and (−)-(+)-**5**,[42] respectively.

Since iododestannylation reactions normally proceed with retention of stereochemistry,[43,44] the orientation of the tributylstannyl group on a double bond can be influenced by the choice of solvent used for the preparation of the vinyl tributylstannyl intermediate. The use of a slight excess of tributyltin hydride in toluene with a radical initiator affords primarily the *E* isomer,[45,46] while the use of an aprotic solvent such as hexamethylphosphoric triamide (HMPA) affords mainly the *Z* isomer.[47] As expected, reaction of either (−)-(−)- or (−)-(+)-**5** with a slight excess of tributyltin hydride in toluene with AIBN afforded primarily (*E*)-(−)-(−)- or (*E*)-(−)-(+)-**6**, respectively. The small amount of the *Z* isomer (<10%) which was produced was effectively removed by flash column chromatographic purification as confirmed by TLC and NMR analysis. Treatment of (*E*)-(−)-(−)- or (*E*)-(−)-(+)-**6** with iodine readily afforded *E*-(−)-(−)- or (*E*)-(−)-(+)-**1** with retention of the *E* configuration on the double bond as determined by NMR and HPLC analysis.

Since the *E* isomers were isolated as oils and a suitable crystal for X-ray analysis has not yet been prepared, the isomeric purity of (*E*)-(−)-(−)- and (*E*)-(−)-(+)-**1** was determined by HPLC analysis utilizing a normal phase column. The resolution of the stereoisomers of 3-quinuclidinol is well known[34] and has been utilized in the preparation of the various stereoisomers of 4-IQNB,[19,30] and therefore, this procedure was used for the resolution of 3-quinuclidinol. In addition, it was observed during the HPLC analysis of racemic IQNP that it was possible to effectively separate the stereoisomers of the acetate moiety utilizing a normal phase column. Thus, in this manner, the isomeric purity of (*E*)-(−)-(−)- and (*E*)-(−)-(+)-**1** was observed to be greater than 97%.

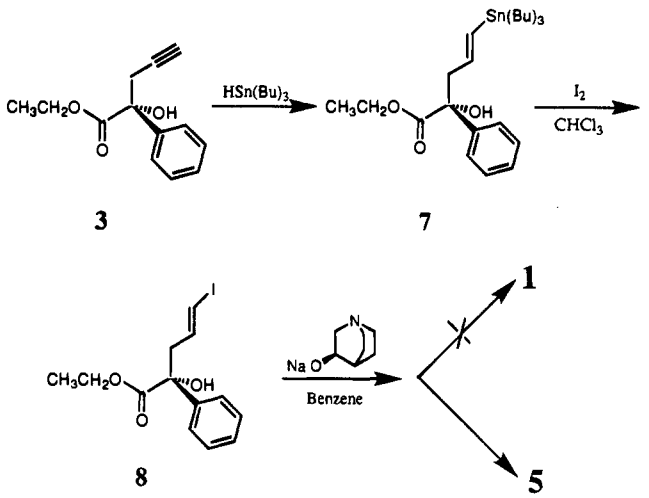
When the stannylation reaction was performed using HMPA as the solvent under a variety of reaction conditions (Table 2), only minor amounts of the (*Z*)-(−)-(−)- or (*Z*)-(−)-(+)-**6** isomer along with the *E* isomer was isolated from the reaction mixture. In addition, the yield and the ratio of E to Z isomers varied considerably even when the same reaction conditions were utilized (e.g., see entries 4 and 5 in Table 2). Therefore it appears that the *E* to *Z* ratio is not only dependent on the reaction conditions but also on the steric hindrance around the acetate center, which appears to favor formation of the *E* configuration. It was also observed that at greater than 30 °C, compound **5** decomposed and could not be recovered during the purification of the stannylated product. Surprisingly when (*Z*)-(−)-(−)- or

Table 2. Stannylation Products from the Preparation of *Z* Isomers

substrate (mmol)	tributyltin hydride (mmol)	HMPA (mL)	*T* (°C)	reaction time (h)	yield % *E*	yield % *Z*
(+)-**3** (3.2)	8.2	10	70	36	21.8	18.1
(−)-**3** (9.5)	23.8	15	80	65	36.2	18.0
(−)-**3** (13.6)	34.2	25	80	44	53.5	10.5
(+)-**3** (7.0)	20.4	10.5	50	72	3.1	11.8
(−)-**3** (8.6)	24.9	12.9	50	72	13.7	19.7
(+)-**3** (9.6)	27.9	15	50	96	35.6	3.9
(−)-**3** (6.6)	19.3	10	50	24	61.4	4.5
(−)-(+)-**6** (0.6)	2.5	0.9	40	22	18.7	31.0
(−)-(+)-**6** (2.1)	6.3	3.2	40	27	11.1	11.1
(−)-(−)-**6** (0.8)	2.2	1.0	55	45	18.8	23.2
(−)-(−)-**6** (1.3)	1.9	1.5	55	45	10.7	10.7
(−)-(−)-**6** (0.9)	2.6	1.4	55	45	20.0	8.2
(−)-(+)-**6** (1.5)	5.2	2.8	50	45	*a*	17.8
(−)-(−)-**6** (2.1)	5.6	10	70	50	7.8	3.4

[a] Not determined.

Scheme 2



(*Z*)-(−)-(+)-**6** was treated with iodine in chloroform, both (*E*)- and (*Z*)-(−)-(−)- or (*Z*)-(−)-(+)-**1**, respectively, were isolated in a ~20:80 ratio as determined by HPLC analysis as described above. As was observed with the racemic mixture, the *E* and *Z* isomers of IQNP could not be effectively separated by flash column chromatography. Due to the low yield and low observed optical rotation of (*Z*)-(−)-(−)-**6** and (*Z*)-(−)-(−)- and (*Z*)-(−)-(+)-**1**, the specific rotation of these compounds could not be accurately measured.

An alternate route was thus pursued for preparation of the *Z* isomers in an attempt to improve the yield and avoid the racemization of the vinyl iodide (Scheme 2). This route was chosen to simplify the chemistry by the addition of the quinuclidinyl moiety during the final step of the synthetic pathway. This reaction scheme would also decrease the steric hindrance around the acetate center by the absence of the 3-quinuclidinyl ring at the stannylation step, and therefore, the yield of the *Z* isomer would be expected to increase. To insure that this route would not cause racemization at any of the chiral centers, (*E*)-(−)-(+)-**1** was synthesized by this route and compared to the product isolated from Scheme 1. For this approach (+)-ethyl α-hydroxy-α-phenyl-α-(1-propyn-3-yl)acetate ((+)-**3**) was treated with tributyltin hydride in the presence of AIBN, and the resultant tributyltin intermediate was treated with iodine to afford (*E*)-(+)-**8**, as confirmed by NMR and TLC analysis. Transesterification with (−)-3-quinuclidinol af-

Table 3. Specific Rotations (c = g/mL, chloroform)

compd	specific rotation (deg)	compd (deg)	specific rotation (deg)
(−)-**4**	−10.7 (−10.5)[a]	(*E*)-(−)-(−)-**1**	−20.2
(+)-**4**	+12.7 (+11.8)[a]	(*E*)-(−)-(+)-**1**	+39.5 (+39.2)[b]
(−)-**3**	−13.6	(*E*)-(−)-**7**	−8.6
(+)-**3**	+18.4	(*E*)-(+)-**7**	+12.2
(−)-(−)-**5**	−4.5	(*Z*)-(−)-**7**	−10.4
(−)-(+)-**5**	+41.8	(*Z*)-(+)-**7**	+11.8
(*E*)-(−)-(−)-**6**	−12.5	(*E*)-(+)-**8**	+19.2
(*E*)-(−)-(+)-**6**	+29.0	(*Z*)-(−)-**8**	−10.1
(*Z*)-(−)-(+)-**6**	−31.5	(*Z*)-(+)-**8**	+14.0

[a] α-Methylbenzylamine salt in ethanol. [b] Prepared from Scheme 2.

Table 4. *In Vitro* Muscarinic Binding Affinity of the Stereoisomers of IQNP

	K_D (mean ± standard error, nM)				
subtype	(*E*)-(−)-(−)-**1**	(*E*)-(−)-(+)-**1**	(*Z*)-(−)-(−)-**1**	(*Z*)-(−)-(+)-**1**	(*R*)-QNB
m1	0.383 ± 0.102	6.83 ± 1.48	0.13 ± 0.04	0.23 ± 0.12	0.32 ± 0.05
m2	29.6 ± 9.70	41.2 ± 10.3	0.43 ± 0.06	0.62 ± 0.16	0.20 ± 0.03
m4	0.356 ± 0.020	2.39 ± 0.263	ND[a]	ND	ND

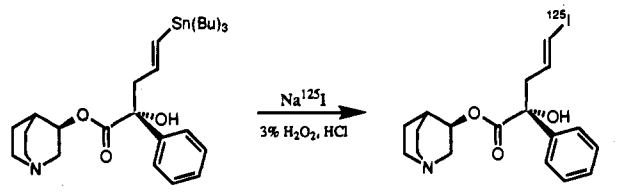
[a] ND = not determined.

forded (*E*)-(−)-(+)-**1**. The optical rotation and HPLC analysis of (*E*)-(−)-(+)-**1** prepared by both routes were in agreement (Table 3); therefore, this reaction sequence did not cause racemization at the chiral centers.

However, as observed before, reaction of either (+)- or (−)-**3** with tributyltin hydride in HMPA under a variety of conditions afforded both the *E* and *Z* isomers (Table 2). Repeated purification by flash column chromatography afforded the desired (*Z*)-(+)- or (*Z*)-(−)-**7** free of the *E* isomer, as determined by NMR and TLC analysis. In contrast to the approach described above, treatment of the *Z* isomers of **7** with iodide afforded the desired (*Z*)-(+)- or (*Z*)-(−)-**8** as determined by NMR and TLC analysis. It was observed that the specific rotations of the respective *E* and *Z* isomers of **7** along with the respective (*E*)- and (*Z*)-**8** isomers were similar (Table 2). However, transesterification of either (*Z*)-(+)- or (*Z*)-(−)-**8** with (−)-3-quinuclidinol under the usual conditions afforded only a minor amount of IQNP in which the configuration of the vinylic iodide had racemized and the major product isolated being the loss of the vinyl iodide to afford either (−)-(+)- or (−)-(−)-**5**. The conversion of (*Z*)-(+)-**8** to the free acid followed by the activation of the acid with carbodiimidazole and subsequent reaction with (−)-3-quinuclidinol[19] was also investigated; however, treatment of (*E*)-(+)-**8** with ethanolic sodium hydroxide caused, in addition to the saponification of the ethyl ester, rapid loss of the vinylic iodide to afford (+)-**4**.

The results of *in vitro* binding assays performed on the four IQNP stereoisomers are shown in Table 4. Due to the difficulty of preparing the *Z* isomers of **1** free of small amounts of the *E* isomers, the *Z* isomers tested in this assay contained ca. 20% of the corresponding *E* isomers. It was observed that all four of these isomers demonstrated a high binding affinity for the various mAChR subtypes. (*E*)-(−)-(−)-IQNP demonstrated a high mAChR subtype selectivity with nearly an 80-fold higher selectivity for m1 over the m2 mAChR subtype. In addition, (*E*)-(−)-(+)-IQNP demonstrated the lowest affinity for mAChR; however, the value was observed

Scheme 3



6 [^{125}I]-**1**

to be in the nanomolar range and is 10-fold selective for the m1 subtype over the m2 mAChR subtype. The *Z* isomers did not demonstrate mAChR subtype selectivity.

The radioiodination of the tributyltin isomers of **6** was accomplished in an analogous manner as that reported for the racemic mixture (Scheme 3). In the case of the *Z* stereoisomers, the *E* isomer formed during the iododestannylation, which was also observed in the cold chemistry, was effectively removed during HPLC purification of the final radioiodinated product and accounts for the lower yields observed for *Z* isomers.

The various isomers were labeled with iodine-125, and an initial *in vivo* biodistribution study in female Fischer rats was performed over 6 h, except for the (*E*)-(−)-(−)-IQNP, which was studied over 24 h. The results shown in Tables 5–8 demonstrate that (*E*)-(−)-(+)-IQNP (Table 5), although showing significant initial cerebral uptake, washed out rapidly from the brain. Although a kinetic analysis has not yet been performed with the various isomers, this rapid washout may be due in part to a more rapid dissociation rate from the receptor site as compared to the other isomers. This difference in the rate of dissociation of the ligand from the receptor site has been observed in a kinetic study of the isomers of 4-IQNB.[31] The biodistribution of (*E*)-(−)-(+)-IQNP in the other tissues evaluated is not markedly different from the other three isomers of **1**.

(*E*)-(−)-(−)-IQNP demonstrates high cerebral uptake in areas containing the M_1 (m1, m4) mAChR subtype by 15 min, and the level of activity decreases slowly over time (Table 6). By 6 h, the activity decreased significantly in all organs except for the cerebral M_1 regions (cortex, striatum, hippocampus) and the liver. This retention of activity in M_1 areas of the brain and the washout of activity in regions containing M_2 mAChR subtype (cerebellum, heart) demonstrate that (*E*)-(−)-(−)-IQNP has potential for the *in vivo* imaging of the M_1 mAChR subtype by SPECT.

(*Z*)-(−)-(−)-IQNP shows high uptake of activity in both M_1 and M_2 rich areas of brain and the heart (Table 7). As observed with (*E*)-(−)-(−)-IQNP, the activity is retained in regions containing the M_1 mAChR subtype over the time period studied. In addition, the uptake of activity in tissues containing M_2 subtype (heart, cerebellum) is significantly higher than was observed with (*E*)-(−)-(−)-IQNP. The heart to blood ratio after 4 h was observed to be 8:1. In addition, the activity level in the lung and liver was observed to be lower than that in the heart with the heart to lung and the heart to liver ratios of 2.1:1 and 2.2:1, respectively, after 4 h. These results indicate that (*Z*)-(−)-(−)-IQNP may have potential for imaging of M_2 myocardial receptor density.

(*Z*)-(−)-(+)-IQNP demonstrates similar biodistribution properties, with low liver and lung uptake, as the

Table 5. Biodistribution of Iodine-125-(*E*)-(−)-(+)-IQNP in Female Rats ($n = 5$)

	mean percent injected dose/g (±SD)					
organ	15 min	30 min	60 min	120 min	240 min	360 min
blood	0.29 ± 0.06	0.26 ± 0.04	0.20 ± 0.02	0.18 ± 0.05	0.15 ± 0.02	0.11 ± 0.01
liver	2.37 ± 0.42	2.14 ± 0.46	2.31 ± 0.30	1.91 ± 0.33	1.79 ± 0.18	1.48 ± 0.16
kidney	2.25 ± 0.37	1.88 ± 0.22	0.96 ± 0.17	0.56 ± 0.18	0.30 ± 0.03	0.21 ± 0.02
heart	0.75 ± 0.15	0.68 ± 0.11	0.38 ± 0.07	0.21 ± 0.05	0.12 ± 0.01	0.06 ± 0.01
lung	5.62 ± 1.05	3.78 ± 0.53	1.29 ± 0.28	0.54 ± 0.18	0.21 ± 0.02	0.10 ± 0.01
thyroid[a]	0.16 ± 0.03	0.24 ± 0.06	0.50 ± 0.18	0.85 ± 0.18	2.06 ± 0.53	1.64 ± 0.42
cortex	0.98 ± 0.09	0.81 ± 0.05	0.54 ± 0.02	0.20 ± 0.02	0.07 ± 0.00	0.04 ± 0.01
striatum	0.77 ± 0.14	0.72 ± 0.15	0.50 ± 0.09	0.19 ± 0.06	0.06 ± 0.03	0.02 ± 0.02
hippocampus	0.74 ± 0.18	0.74 ± 0.17	0.53 ± 0.13	0.25 ± 0.06	0.10 ± 0.03	0.02 ± 0.01
cerebellum	0.39 ± 0.09	0.22 ± 0.05	0.10 ± 0.02	0.04 ± 0.02	0.02 ± 0.00	0.01 ± 0.01
rest of brain	0.62 ± 0.14	0.43 ± 0.09	0.25 ± 0.06	0.11 ± 0.03	0.05 ± 0.00	0.03 ± 0.00

[a] Percent dose.

Table 6. Biodistribution of Iodine-125-(*E*)-(−)-(−)-IQNP in Female Rats ($n = 5$)

	mean percent injected dose/g (±SD)						
organ	15 min	30 min	60 min	120 min	240 min	360 min	1440 min
blood	0.21 ± 0.01	0.18 ± 0.02	0.17 ± 0.01	0.20 ± 0.03	0.14 ± 0.03	0.09 ± 0.01	0.03 ± 0.00
liver	1.50 ± 0.13	1.38 ± 0.15	1.30 ± 0.09	1.58 ± 0.19	1.36 ± 0.27	1.22 ± 0.17	0.55 ± 0.08
kidney	3.21 ± 0.17	2.08 ± 0.25	1.28 ± 0.07	0.85 ± 0.12	0.35 ± 0.06	0.17 ± 0.03	0.05 ± 0.01
heart	1.65 ± 0.12	1.20 ± 0.16	0.71 ± 0.05	0.45 ± 0.03	0.23 ± 0.04	0.10 ± 0.02	0.04 ± 0.00
lung	6.60 ± 0.80	3.39 ± 0.44	1.83 ± 0.30	1.12 ± 0.16	0.40 ± 0.05	0.18 ± 0.04	0.03 ± 0.00
thyroid[a]	0.14 ± 0.01	0.19 ± 0.02	0.31 ± 0.06	0.73 ± 0.29	1.14 ± 0.20	2.13 ± 0.67	8.32 ± 1.71
cortex	1.34 ± 0.12	1.60 ± 0.20	1.50 ± 0.20	1.56 ± 0.18	1.48 ± 0.19	1.38 ± 0.31	0.72 ± 0.10
striatum	1.08 ± 0.10	1.27 ± 0.14	1.20 ± 0.22	1.21 ± 0.17	1.24 ± 0.18	1.22 ± 0.20	0.90 ± 0.11
hippocampus	1.04 ± 0.09	1.21 ± 0.16	1.13 ± 0.19	1.23 ± 0.15	1.23 ± 0.21	1.36 ± 0.54	0.73 ± 0.09
cerebellum	0.59 ± 0.06	0.47 ± 0.06	0.27 ± 0.02	0.16 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.05 ± 0.00
rest of brain	0.94 ± 0.08	1.02 ± 0.13	0.86 ± 0.11	0.85 ± 0.08	0.63 ± 0.09	0.58 ± 0.12	0.23 ± 0.03

[a] Percent dose.

Table 7. Biodistribution of Iodine-125-(*Z*)-(−)-(−)-IQNP in Female Rats ($n = 5$)

	mean percent injected dose/g (±SD)					
organ	15 min	30 min	60 min	120 min	240 min	360 min
blood	0.31 ± 0.03	0.24 ± 0.03	0.25 ± 0.04	0.23 ± 0.01	0.18 ± 0.05	0.13 ± 0.02
liver	1.44 ± 0.17	1.01 ± 0.12	0.86 ± 0.11	0.79 ± 0.07	0.66 ± 0.10	0.57 ± 0.08
kidney	2.58 ± 0.44	1.52 ± 0.26	1.15 ± 0.10	0.91 ± 0.30	0.52 ± 0.07	0.32 ± 0.05
heart	6.85 ± 0.90	5.92 ± 0.54	4.98 ± 0.80	2.36 ± 0.33	1.45 ± 0.23	0.70 ± 0.15
lung	8.05 ± 0.99	3.37 ± 0.57	1.99 ± 0.32	1.23 ± 0.12	0.69 ± 0.12	0.38 ± 0.06
thyroid[a]	0.21 ± 0.02	0.39 ± 0.04	0.72 ± 0.19	1.24 ± 0.27	2.32 ± 0.55	3.15 ± 0.50
cortex	1.31 ± 0.14	1.23 ± 0.20	1.48 ± 0.23	1.52 ± 0.28	1.56 ± 0.13	1.52 ± 0.25
striatum	1.04 ± 0.14	0.98 ± 0.11	1.25 ± 0.24	1.25 ± 0.24	1.36 ± 0.24	1.32 ± 0.22
hippocampus	0.93 ± 0.09	0.91 ± 0.13	1.17 ± 0.17	1.16 ± 0.21	1.17 ± 0.15	1.21 ± 0.24
pons/medulla	1.03 ± 0.12	0.96 ± 0.13	1.07 ± 0.18	0.98 ± 0.17	0.91 ± 0.12	0.76 ± 0.12
cerebellum	0.94 ± 0.12	0.80 ± 0.10	0.78 ± 0.13	0.57 ± 0.09	0.43 ± 0.05	0.28 ± 0.05
rest of brain	1.04 ± 0.13	1.00 ± 0.13	1.16 ± 0.17	1.17 ± 0.21	1.15 ± 0.12	1.06 ± 0.17

[a] Percent dose.

Table 8. Biodistribution of Iodine-125-(*Z*)-(−)-(+)-IQNP in Female Rats ($n = 5$)

	mean percent injected dose/g (±SD)					
organ	15 min	30 min	60 min	120 min	240 min	360 min
blood	0.22 ± 0.05	0.24 ± 0.03	0.21 ± 0.05	0.22 ± 0.03	0.16 ± 0.01	0.12 ± 0.02
liver	1.14 ± 0.15	1.32 ± 0.15	0.91 ± 0.23	1.05 ± 0.16	0.92 ± 0.10	0.83 ± 0.15
kidney	2.34 ± 0.38	1.70 ± 0.20	1.13 ± 0.30	0.65 ± 0.08	0.33 ± 0.02	0.22 ± 0.03
heart	3.35 ± 0.39	2.88 ± 0.36	1.94 ± 0.42	1.26 ± 0.12	0.60 ± 0.05	0.34 ± 0.05
lung	6.30 ± 1.26	3.43 ± 0.77	1.91 ± 0.50	0.99 ± 0.18	0.42 ± 0.03	0.24 ± 0.04
thyroid[a]	0.14 ± 0.03	0.33 ± 0.13	0.40 ± 0.10	1.20 ± 0.16	1.68 ± 0.12	1.65 ± 0.25
cortex	1.03 ± 0.15	1.25 ± 0.22	1.27 ± 0.25	1.45 ± 0.24	1.25 ± 0.14	1.15 ± 0.17
striatum	0.90 ± 0.18	0.97 ± 0.20	1.21 ± 0.21	1.24 ± 0.20	1.10 ± 0.17	0.97 ± 0.16
hippocampus	0.84 ± 0.09	0.81 ± 0.13	1.09 ± 0.17	1.10 ± 0.21	1.17 ± 0.15	1.21 ± 0.24
pons/medulla	0.78 ± 0.12	0.73 ± 0.11	0.73 ± 0.14	0.60 ± 0.08	0.42 ± 0.02	0.30 ± 0.06
cerebellum	0.64 ± 0.10	0.50 ± 0.06	0.41 ± 0.09	0.29 ± 0.05	0.16 ± 0.01	0.10 ± 0.02
rest of brain	0.84 ± 0.14	0.92 ± 0.15	0.94 ± 0.20	1.00 ± 0.18	0.76 ± 0.08	0.68 ± 0.15

[a] Percent dose.

corresponding (*Z*)-(−)-(−)-IQNP (Table 8). However,uptake in mAChR rich areas of the brain and heart is less than observed for (*Z*)-(−)-(−)-IQNP, and the levels of activity decrease at a faster rate. The rate of *in vivo* deiodination, as represented by the low uptake of radioactivity in the thyroid, was observed to be similar for all isomers evaluated. This result was surprising due to the ease in which the iodine was either racemized or lost from the vinylic position during the preparation of the *Z* isomers of IQNP.

Conclusions

The four stereoisomers of IQNP (**1**) which contain the (*R*)-(−)-3-quinuclidinyl ester have been resolved and radiolabeled with iodine-125. *In vitro* binding assays showed that these four isomers have a high affinity for mAChR with (*E*)-(−)-(−)-IQNP demonstrating the highest subtype selectivity with nearly an 80-fold higher selectivity for the m1 over the m2 molecular mAChR subtype. Biodistribution studies in female rats showed that while (*E*)-(−)-(+)-**1** demonstrated low uptake of radioactivity after 6 h, (*E*)-(−)-(−)-, (*Z*)-(−)-(−)-, and (*Z*)-(−)-(+)-**1** demonstrated significant uptake of activity in mAChR rich areas of the brain. In addition, these data suggest [^{123}I]-(*E*)-(−)-(−)-IQNP is a potential candidate for the *in vivo* imaging of cerebral M_1 (m1, m4) mAChR subtype density. In contrast, [^{123}I]-(*Z*)-(−)-(−)-IQNP demonstrates potential for imaging cardiac M_2 mAChR subtype due to a heart to blood ratio of 8:1 and heart to lung and heart to liver ratios of ca. 2:1 after 4 h.

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