Preparation and Properties of (*R*)-(−)-1-Azabicyclo[2.2.2]oct-3-yl-(*R*)-(+)-α-hydroxy-α-(4-[^{125}I]iodophenyl)-α-phenyl Acetate and (*R*)-(−)-1-Azabicyclo[2.2.2]oct-3-yl-(*S*)-(−)-α-hydroxy-α-(4-[^{125}I]iodophenyl)-α-phenyl Acetate as Potential Radiopharmaceuticals

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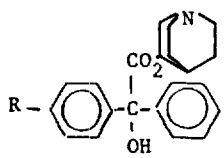
Abstract □ rac-4-Nitrobenzilic acid was synthesized and resolved with quinidine and quinine to give the corresponding (*R*)- and (*S*)-salts. The resolved diastereomeric salts were converted to (*R*)- and (*S*)-4-nitrobenzilic acids and subsequent esterification gave their corresponding ethyl esters. Transesterification with (*R*)-(−)-3-quinuclidinol afforded (*R*)-(−)-1-azabicyclo[2.2.2]oct-3-yl-(*R*)-(+)-α-hydroxy-α-(4-nitrophenyl)-α-phenyl acetate and (*R*)-(−)-1-azabicyclo[2.2.2]oct-3-yl-(*S*)-(−)-α-hydroxy-α-(4-nitrophenyl)-α-phenyl acetate. After hydrogenation, the (*R,R*)- and (*R,S*)-amines were converted to the respective triazene derivatives. The triazene derivatives reacted with sodium [^{125}I]iodide to give (*R*)-(−)-1-azabicyclo[2.2.2]oct-3-yl-(*R*)-(+)-α-hydroxy-α-(4-[^{125}I]iodophenyl)-α-phenyl acetate and (*R*)-(−)-1-azabicyclo[2.2.2]oct-3-yl-(*S*)-(−)-α-hydroxy-α-(4-[^{125}I]iodophenyl)-α-phenyl acetate. The evaluation of their affinities to muscarinic acetylcholine receptors (MAcChR) shows that (*R*)-(−)-1-azabicyclo[2.2.2]oct-3-yl-(*S*)-(−)-α-hydroxy-α-(4-[^{125}I]iodophenyl)-α-phenyl acetate exhibits an affinity for the MAcChR from corpus striatum that is approximately threefold lower than that of (*R*)-(−)-1-azabicyclo[2.2.2]oct-3-yl-(*R*)-(+)-α-hydroxy-α-(4-[^{125}I]iodophenyl)-α-phenyl acetate.

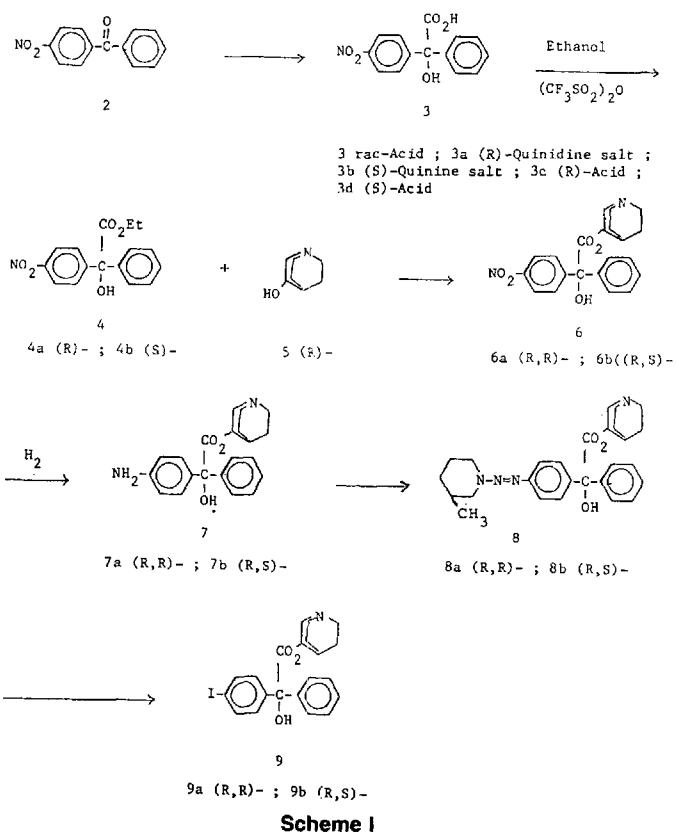
In continuation of our efforts to develop potent and effective radiotracers that bind to the muscarinic acetylcholine receptors (MAcChR), we have synthesized a number of derivatives of 3-quinuclidinyl benzilate (QNB) substituted with stable isotopes of the routinely used gamma-emitting radionuclides iodine-123 and iodine-125, bromine-75 and bromine-77, and fluorine-18.[1] Introduction of iodine-127[1] or iodine-125[2] at the 4′ position (see structure) does not significantly reduce the affinity to the MAcChR from ventricular muscle of rat and dog or from caudate/putamen of rat and rabbit. In an effort to obtain more information on the structure–activity relationships, the partially resolved (*R*)-(−)- or (*S*)-(+)-3-quinuclidinyl-(*R,S*)-α-hydroxy-α-(4-[^{125}I]iodophenyl)-α-phenyl acetates were synthesized.[3] Evaluation of their affinities to MAcChR shows that the (*R*)-(−)-3-quinuclidinyl derivative has a much higher affinity than the (*S*)-(+)-3-quinuclidinyl derivative. Additionally, Inch et al.[4] showed that the (*R*)-isomer of stereoisomeric β-diethylaminoethyl benzilate (benactyzine) derivatives (i.e., only chiral carbon in the ester moiety), exhibits 10-fold greater activity than the (*S*)-stereoisomer. We therefore focused our interests on the synthesis of [(*R,R*)[^{125}I]4-IQNB] and [(*R,S*)[^{125}I]4-IQNB]. The (*R,R*)[^{125}I]4-IQNB exhibits, as expected, a threefold higher affinity than the (*R,S*)[^{125}I]4-IQNB. The kinetics of dissociation of the two diastereomers, however, exhibit greater differences.



R = I

Results

Chemistry—The rac-4-nitrobenzilic acid (**3**) reacted with quinidine, and the resulting pure salt (**3a**) was treated with HCl to obtain the (*R*)-acid (**3c**; see Scheme I). The mother



Scheme I

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liquor which was left after filtration of the quinidine salt was converted back to the acid and reacted with quinine, and the resulting salt (**3b**) was converted to the (*S*)-acid (**3d**) by HCl. The ethyl esters of the resolved forms of acid (**4a** and **4b**) were prepared by adding a few milliliters of trifluoromethanesulfonic anhydride to a solution of the desired acid in anhydrous ethanol and heating under reflux for 12 h. Transesterification of the resulting ethyl esters with (*R*)-(−)-3-quinuclidinol (**5**) in the presence of sodium metal provided the (*R*)-(−)-3-quinuclidinyl-(*R*)-(+)- or (*R*)-(−)-3-quinuclidinyl-(*S*)-(−)-α-hydroxy-α-(4-nitrophenyl)-α-phenyl acetates (**6a** and **6b**, respectively). Compounds **6a** and **6b** were hydrogenated at room temperature in methanol in the presence of palladium-polyethylenimine to give the respective amines (**7a, 7b**). The triazenes (**8a, 8b**) were synthesized from reaction of the diazonium intermediates with 3-methylpiperidine. Treatment of **8a** and **8b** with sodium [^{125}I]iodide in the presence of trifluoromethanesulfonic acid in trifluoroethanol (TFE) afforded **9a** and **9b**. The assignment of *R*- and *S*-configurations to the acid moiety is by analogy to the compounds reported by Inch and co-workers,[4,5] in which the *R*-isomers exhibit the higher affinities.

Pharmacology—The equilibrium association constants (K_A) for (*R,R*)- and (*R,S*)[^{125}I]4-IQNB (**9a** and **9b**) were determined by saturation analysis as previously described,[6] using at least eight concentrations of the radioligands and using rat corpus striatum as the source of the MAcChR. The K_a values were determined by the method of Scatchard[7] and confirmed using SCAFIT from the LIGAND system of programs.[8] Each K_A is the result of 27 to 46 determinations. The dissociation rate constant (K_{-1}) for (*R,S*)[^{125}I]4-IQNB was also determined as previously described,[6] using MAcChR from the rat corpus striatum. We have found that the dissociation kinetics of [^{125}I]4-IQNB from the receptor isolated from the corpus striatum is well described by a monoexponential; that is, no more than 10% of the MAcChR concentration in the corpus striatum exhibits rapid dissociation kinetics for (*R,R*)[^{125}I]4-IQNB.[6] The dissociation rate profiles for both (*R,S*)- and (*R,R*)[^{125}I]4-IQNB were monoexponential and K_{-1} values were obtained by linear regression of the ln percent bound versus time. The number of determinations for each parameter is provided in Table I.

Discussion

In Table I, data are presented which show a comparison of the relative affinities of (*R*)-(−)-3-quinuclidinyl-(*R*)-(+)-α-hydroxy-α-(4-[^{125}I]iodophenyl)-α-phenyl acetate (**9a**), (*R*)-(−)-3-quinuclidinyl-(*S*)-(−)-α-hydroxy-α-(4-[^{125}I]iodophenyl)-α-phenyl acetate (**9b**), and [^{3}H](*R*)-(−)-3-quinuclidinyl benzilate for the MAcChR from rat corpus striatum. The in vitro properties of the radioligands (**9a** and **9b**) indicate that neither **9b**, which has an affinity slightly less than that of (*R*)-QNB, nor **9a**, which has an affinity slightly greater than (*R*)-QNB, differ significantly from QNB, but the affinity of the (*R,S*)-diastereomer is significantly lower than that of the (*R,R*)-isomer. The small differences in affinity, however, mask a large difference in kinetic properties: the dissociation rate constant of (*R,S*)[^{125}I]4-IQNB is 13-fold greater than that of (*R,R*)[^{125}I]4-IQNB. These results imply that the association rate constant of the (*R,S*)-diastereomer must be fourfold larger than that of the (*R,R*)-isomer. The radiochemical yields for the two isomers are essentially the same as is the average specific activity. The range for specific activities obtained is 410 to 2300 Ci/mmol (overestimating the theoretical maximum by 5% results from inaccuracies in the receptor–radioligand method for determining the specific activity). Specific activities <2200 Ci/mmol, the specific activity of the [^{125}I]NaI provided by the manufacturer, are the result of tailing of an unlabeled side product which elutes before [^{125}I]4-IQNB from the HPLC column. In two syntheses, the specific activities for (*R,R*)[^{125}I]4-IQNB and (*R,S*)[^{125}I]4-IQNB were determined to be 410 and 405 Ci/mmole, respectively, while the specific activity of the products increased to 1600 and 1870 Ci/mmole, respectively, by a second chromatographic pass on HPLC.

Experimental Section

Chemistry—Melting points were determined on a Fisher-Johns apparatus. Optical rotations were determined by polarimetry on a Zeiss polarimeter. The IR spectra of the compounds, neat or in KBr pellet, were obtained in a Beckman model IR 20A spectrophotometer. Elemental analyses were performed by Galbraith Laboratories (Knoxville, TN). The results obtained are within ±0.4% of the theoretical values. 4-Nitrobenzophenone, 3-quinuclidinol, quinidine, and quinine were obtained from Aldrich Chemical, Milwaukee, WI. [^{3}H]3-Quinuclidinyl benzilate (QNB) was purchased from New England Nuclear (37.2 Ci/mmol). Sodium [^{125}I]iodide was purchased from Amersham Corporation, Arlington Heights, IL.

(*R,S*)-α-Hydroxy-α-(4-nitrophenyl)-α-phenylacetic acid (**3**) was prepared from 4-nitrobenzophenone (**2**) and trimethylsilyl cyanide by a reported method.[3] The (*R*)-enantiomer of 3-quinuclidinol (**5**) was prepared by methods reported by Grob et al.[9] and Ringldahl et al.[10]

Syntheses—*(R)-(+)-α-Hydroxy-α-(4-nitrophenyl)-α-phenylacetic Acid (3c)*—The racemic acid (**3**; 27.3 g, 0.1 mol) was added to quinidine (32.44 g, 0.1 mol), dissolved in boiling ethyl acetate, and maintained at room temperature for 1 d. The salt which crystallized was filtered and, after 3–4 recrystallizations from the same solvent, had a constant mp of 122–126 °C and rotation $[\alpha]_D^{24}$ of +121.2° (c 0.32, pyridine): yield 72%; TLC [silica gel, toluene:HOAc (9:1)] R_f 0.24. The quinidine salt (**3a**) was treated with excess 6M HCl and extracted with ethyl acetate. The extract was dried over $MgSO_4$ and the solvent was removed under reduced pressure to give an oily yellow product (**3c**): yield 81%; rotation $[\alpha]_D^{24}$ +49.4° (c 1.34, acetone); TLC [silica gel, toluene: HOAc (9:1)] R_f 0.3; IR (KBr:1715 (C=O) cm^{-1}.

Anal.—Calc. for $C_{14}H_{11}NO_5$: C,H,N.

(S)-(−)-α-Hydroxy-α-(4-nitrophenyl)-α-phenylacetic Acid (3d)—The mother liquor remaining after the first filtration of the quinidine salt was concentrated, converted back to the acid (10.92 g, 0.04 mol) by treating with excess 6M HCl, and then added to quinine (12.97 g, 0.04 mol) dissolved in boiling ethanol. The salt which crystallized was filtered and, after five recrystallizations from methanol:acetonitrile (50:50), had a constant mp of 172–174 °C and a rotation $[\alpha]_D^{24}$ of −76.2° (c 0.26, pyridine): yield 64%; TLC [silica gel, toluene:HOAc (9:1)] R_f 0.26. The resulting pure quinine salt of the (*S*)-acid (**3b**) was treated with excess 6M HCl and extracted with ethyl acetate. The extract was washed with water and dried over $MgSO_4$. The solvent evaporated under reduced pressure gave (**3d**): yield 74%; rotation $[\alpha]_D^{24}$ −49.1° (c 1.34, acetone); TLC [silica gel, toluene:HOAc (9:1)] R_f 0.30; IR (KBr):

Table I—Characteristics of [^{125}I]-Labeled (*R,R*)-4-IQNB and (*R,S*)-4-IQNB

Radioligand	K_a, M^{-1} (N)	K_{-1}, min^{-1} (N)	SA, Ci/mmol (N)	% Yield (N)
(*R,R*)-[^{125}I]4-IQNB(**9a**)	8.93 × 109 (46) (±1.36 × 109)	0.0049 (19) (±0.0004)	1172 (14) (±213)	15.5 (14) (±1.1)
(*R,S*)-[^{125}I]4-IQNB (**9b**)	2.90 × 109 (27) (±0.45 × 109)	0.0654 (9) (±0.011)	1087 (9) (±211)	13.4 (8) (±1.9)
(*R*)-[^{3}H]QNB	6.30 × 109 (73) (±0.92 × 109)	0.012[a]	—	—

[a] Data from ref 5.

1715 (C=O) cm^{-1}.

Anal.—Calc. for $C_{14}H_{11}NO_5$: C,H,N.

*(R)-(+)-Ethyl-α-hydroxy-α-(4-nitrophenyl)-α-phenyl Acetate (**4a**)*—To a solution of **3c** (40.9 g, 0.15 mol) in anhydrous ethanol (250 mL) was added 5 mL of trifluoromethanesulfonic anhydride. The mixture was heated at reflux for 12 h. The solvent was evaporated under reduced pressure. The residue was partitioned between ethyl acetate and saturated sodium bicarbonate. The organic layer was separated, washed with water, and dried over anhydrous $MgSO_4$. Removal of the solvent afforded **4a**: rotation $[\alpha]_D^{24}$ +43.6° (c 0.64, acetone); yield 38 g (84%); TLC [silica gel, toluene:HOAc (9:1)] R_f 0.62; IR: 3482, 2985, 1720, 1595, and 690 cm^{-1}.

Anal.—Calc. for $C_{16}H_{15}NO_5$: C,H,N.

*(S)-(−)-Ethyl-α-hydroxy-α-(4-nitrophenyl)-α-phenyl Acetate (**4b**)*—This compound was prepared from **3d** (27.3 g, 0.1 mol) and anhydrous ethanol (250 mL) by the procedure described above for the preparation of **4a**: yield 26 g (86%); rotation $[\alpha]_D^{24}$ −43.9° (c 0.64, acetone); TLC [silica gel, toluene:HOAc (9:1)] R_f 0.63; IR: 3480, 2980, 1720, 1595, and 685 cm^{-1}.

Anal.— Calc. for $C_{16}H_{15}NO_5$: C,H,N.

*(R)-(−)-1-Azabicyclo[2.2.2]oct-3-yl-(R)-(+)-α-hydroxy-α-(4-nitrophenyl)-α-phenyl Acetate (**6a**)*—A solution of 3.81 g (0.03 mol) of **5** in 250 mL of anhydrous benzene was refluxed for 1 h (Dean-Stark trap used to remove traces of water). Then, 0.4 g of sodium was added and the mixture was heated under reflux with stirring for 1 h. After removal of the remaining sodium, 6.02 g (0.02 mol) of **4a** was added and the reaction mixture was heated under reflux with stirring for 1 d. After the solvent was removed, the residue was partitioned between ethyl acetate and water. The organic layer was separated, washed with water, and dried over $MgSO_4$. After removal of the solvent, the residue was crystallized from acetonitrile to give 5.26 g of **6a**: yield 68%; rotation $[\alpha]_D^{24}$ + 84.6° (c 0.118, pyridine); mp 159–160 °C; TLC [silica gel, MeOH:NH_4OH (98:2)] R_f 0.68; HPLC [Bondapak C_{18}; 5 mM 1-octanesulfonic acid (pH 4, formic acid), MeOH:H_2O (60:40)]; IR (KBr): 2930, 1728, 1515, and 1240 cm^{-1}

Anal.—Calc. for $C_{21}H_{22}N_2O_5$: C,H,N.

*(R)-(−)-1-Azabicyclo[2.2.2]oct-3-yl-(S)-(−)-α-hydroxy-α-(4-nitrophenyl)-α-phenyl Acetate (**6b**)*—This compound was prepared from **5** and **4b** in the same manner as **6a**. A solid was obtained which was recrystallized from acetonitrile: yield 62%; mp 193–201 °C; rotation $[\alpha]_D^{24}$ −55° (c 0.181, pyridine); TLC [silica gel, MeOH:NH_4OH (98:2)] R_f 0.64; HPLC Bondapak C_{18}; 5 mM 1-octanesulfonic acid (pH 4, formic acid), MeOH:H_2O (60:40)]; IR (KBr): 2930, 1730, 1505, and 1225 cm^{-1}.

Anal.—Calc. for $C_{21}H_{22}N_2O_5$: C,H,N.

*(R)-(−)-1-Azabicyclo[2.2.2]oct-3-yl-(R)-(+)-α-hydroxy-α-(4-aminophenyl-α-phenyl Acetate (**7a**)*—To a solution of **6a** (0.382 g, 1 mmol) in 250 mL of methanol was added 150 mg of 1–2% palladium-polyethylenimine (PEI) powder and the mixture was shaken with hydrogen in a Parr pressure flask at 3 atm and room temperature for 72 h. The palladium-PEI was filtered off and the solvent was removed. The residue was dissolved in acetone, placed on a silica gel column, and eluted with acetonitrile. The fraction containing the product was evaporated to dryness under reduced pressure: yield 280 mg (79%); mp 65–68 °C; rotation $[\alpha]_D^{24}$ −43.8° (c 0.114, pyridine); TLC [MeOH:NH_4OH (98:2)] R_f 0.54; HPLC [Bondapak C_{18}; 5 mM 1-octanesulfonic acid (pH 4, formic acid), MeOH:H_2O (60:40)]; IR (KBr): 3445, 3360, 2930, 1715, 1610, and 1225 cm^{-1}.

Anal.—Calc. for $C_{21}H_{24}N_2O_3$: C,H,N.

*(R)-(−)-1-Azabicyclo[2.2.2]oct-3-yl-(S)-(−)-α-hydroxy-α-(4-aminophenyl)-α-phenyl Acetate (**7b**)*—To a solution of **6b** (0.382 g, 1 mmol) in 250 mL of methanol was added 200 mg of 1–2% palladium-PEI and the mixture was shaken with hydrogen in a Parr pressure flask at 3 atm and room temperature for 48 h. The catalyst was filtered off and the solvent was removed under reduced pressure. The residue was dissolved in acetone, placed on a silica gel column, and eluted with acetonitrile. The fraction containing the product was evaporated to dryness under reduced pressure: yield 310 mg (88%); mp 79–82 °C; rotation $[\alpha]_D^{24}$ +85.32° (c 0.058, pyridine); TLC [MeOH:NH_4OH (98:2)] R_f 0.54; HPLC [Bondapak C_{18}; 5 mM 1-octanesulfonic acid (pH 4, formic acid), MeOH:H_2O (60:40)]; IR (KBr): 3445, 3360, 2930, 1715, 1610, and 1225 cm^{-1}.

Anal.—Calc. for $C_{21}H_{24}N_2O_3$: C,H,N.

*(R)-(−)-1-Azabicyclo[2.2.2]oct-3-yl-(R)-(+)-α-hydroxy-α-[4-[2-(3-methylpiperidin-1-yl)-1,2-diazaethylen-1-yl)]phenyl]-α-phenyl Acetate (**8a**)*—A solution of **7a** (105 mg, 0.3 mmol) in 3 mL of H_2SO_4 (10%) and 1 mL of acetone was cooled to 0 °C and treated with a solution of sodium nitrite (41.5 mg, 0.06 mmol) in 1 mL of water. A yellow suspension formed as the solution was stirred for 15 min at 0 °C, and then urea (35 mg) was added. A solution of 3-methylpiperidine (268 mg, 2.7 mmol) in water (3 mL) was added to the reaction and the mixture was stirred for 20 min at 0 °C. The reaction mixture was then made basic to pH 12 with 4M NaOH and was extracted with $CHCl_3$. The organic layer was washed with water and dried over $MgSO_4$. Rotary evaporation of the solvent afforded 114 mg (82%) of crude product. Column chromatography (silica gel, acetone as solvent) of this material afforded pure product: mp 164–167 °C; rotation $[\alpha]_D^{24}$ −46.6° (c 0.107, chloroform); TLC [silica gel, MeOH:NH_4OH (98:2)] R_f 0.62; HPLC [Bondapak C_{18}; 5 mM 1-octanesulfonic acid (pH 4, formic acid), MeOH:H_2O (60:40)]; IR(KBr): 2915, 1730, and 1428 cm^{-1}.

Anal.—Calc. for $C_{27}H_{34}N_4O_3$: C,H,N.

*(R)-(−)-1-Azabicyclo[2.2.2]oct-3-yl-(S)-(−)-α-hydroxy-α-[4-[2-(3-methylpiperidin-1-yl)-1,2-diazaethylen-1-yl)]phenyl]-α-phenyl Acetate (**8b**)*—This compound was prepared from **7b** and purified in the same manner as **8a**: yield 82%; mp 68–72 °C; rotation $[\alpha]_D^{24}$ +18.6° (c 0.269, chloroform); TLC [silica gel, MeOH:NH_4OH (98:2)] R_f 0.6; HPLC [Bondapak C_{18}; 5 mM 1-octanesulfonic acid (pH 4, formic acid), MeOH:H_2O (60:40)]; IR (KBr): 2920, 1735, and 1428 cm^{-1}.

Anal.—Calc. for $C_{27}H_{34}N_4O_3$: C,H,N.

*(R)-(−)-1-Azabicyclo[2.2.2]oct-3-yl-(R)-(+)-α-hydroxy-α-(4-[^{125}I]iodophenyl)-α-phenyl Acetate (**9a**)*—To a solution of sodium [^{125}I]iodide (20 mCi; in ~40 μL of 0.1 M NaOH in a conical vial), **8a** (0.5 mg, 0.00108 mol) in 75 μL of TFE was added. The pH was adjusted to 5.5–6 with methanesulfonic acid in TFE. The reaction mixture was heated at 78 °C for 1 h.

Purification was carried out on an Altex 153 HPLC system. The mobile phase, composed of 5 mM 1-octanesulfonic acid in methanol: water (60:40), was acidified to pH 3.5 with formic acid. The fraction containing the iodinated derivative was collected and evaporated to dryness. The residue was diluted with ethyl acetate (4 mL), and sodium bicarbonate (3 mg) and water (2 mL) were added. The pH of the aqueous layer was 8. The aqueous layer was back extracted with portions of ethyl acetate. The ethyl acetate layer was combined and evaporated to dryness under reduced pressure. The final product had an average specific activity of 1200 Ci/mmol and an average radiochemical yield of 15.5% (n = 14). A solution of radioligand was prepared in 95% ethanol at a concentration of 1 mCi/mL and was stable under storage at −70 °C for at least 120 d.

*(R)-(−)-1-Azabicyclo[2.2.2]oct-3-yl-(S)-(−)-α-hydroxy-α-(4-[^{125}I]iodophenyl)-α-phenyl Acetate (**9b**)*—This compound was prepared from sodium [^{125}I]iodide (10 mCi; in ~20 μL of 0.1M NaOH in a conical vial), and **8b** (0.5 mg, 0.00108 mmol) in the same manner as **9a**. The average specific activity was 850 Ci/mmol, and the average radiochemical yield was 13.4% (n = 8).

Tissue Preparation—The M_1-acetylcholine receptor preparation was prepared as previously described.[6] The brains from freshly killed female Sprague-Dawley rats (200–250 g) were removed and placed on ice. The corpus striatum was removed, immediately frozen, and stored at −80 °C until used (we have stored tissue at this temperature for one year with no significant change in the receptor properties). Samples of 0.1 to 0.15 g of corpus striatum were homogenized in 20 mL of ice-cold 0.9% saline containing 10 mM Tris buffer (pH 7.4), 1.5 mM EDTA, and 10% sucrose, using a Polytron PC-U (medium speed, two bursts for 15 s each). The receptors were used without further purification. The 10% sucrose aids in maintaining a uniform suspension of the homogenate while sampling. The concentration of MAc-ChR was ~1 nM. Upon diluting in the assay system, the final concentration of receptor was ~20 pM.

Determination of Equilibrium Association Constants—Fractional tissue samples of 0.1 mL of the above homogenate were added to 5 mL of radioligand (eight concentrations) in Tris-buffered (10 mM, pH 7.4) 0.9% saline. After 2 h at room temperature, the incubates were filtered over GF/C filters (pretreated with 0.5% polyethylene imine to reduce radioligand interactions with the filters), washed with 9 mL of ice-cold saline, and air dried. The samples were counted in an auto gamma counter with a counting efficiency of 77%. Levels of nonreceptor binding to the tissue and filter paper were determined by incubation in the presence of 10^{-5} M atropine. Atropine blockade reduced ^{125}I -count rates to 0.5% of the total activity bound in the absence of atropine. Neither longer incubation times, nor continuous agitation of the incubation mix-

tures, nor increased temperatures (up to 30 °C) produced significant changes in the results obtained.

Although the specific activity of the [^{125}I]NaI is close to theoretical, we have found that small contamination from QNB generated in the reaction will effectively reduce the specific activity of the product. We therefore determined the specific activity by comparing the receptor concentration obtained using [^{3}H]3-quinuclidinyl benzilate with that obtained using the iodinated products. Assuming that the number of receptors occupied by the two radioligands is the same, the specific activity is obtained by the ratio of the count rates obtained for ^{3}H and ^{125}I and the specific activity of the tritiated standard, [^{3}H]QNB: specific activity of [^{125}I]4-IQNB = (dpm for ^{125}I/dpm for ^{3}H) × specific activity of ^{3}H.

Dissociation Rate Constants—Radioligand was added to the concentrated receptor preparation and equilibrated for 2 h at room temperature. Then, 0.1 mL of preparation was added to 5 mL of Tris-buffered 0.9% saline containing 10^{-5} M atropine (to prevent reassociation of radioligand) and incubated at 30 °C for times ranging from <10 s to 2 h. Nonreceptor binding was determined by conducting the initial equilibration with radioligand in the presence of 10^{-5} M atropine. The highest levels of nonreceptor binding were observed at the shortest times; but since they exhibited a log-linear dependence on time, values of nonreceptor binding which were not specifically determined could be interpolated.

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