allowed to come to room temperature. After a water wash, the chloroform solution was dried with magnesium sulfate, filtered, and evaporated to dryness to give 2.3 g of the product as an oil. The material was purified by dry column chromatography (10% methanol/chloroform) and 1.3 g of the product was obtained as a light amber oil which crystallized at room temperature. After trituration with ethyl ether, 0.8 g (44%) of the product was obtained as a cream-colored solid: mp 104–5 °C.

***N*-[2-(4-Morpholinyl)ethyl]-2-chloro-*cis*-5a,6,7,8,9,9a-hexahydrodibenzofuran-4-carboxamide (28).** To a stirred mixture of acid **5** (2.0 g, 8 mmol) and triethylamine (1.22 g, 12 mmol) in chloroform (12.0 mL) at 0 °C was first added ethyl chloroformate (0.95 g, 9 mmol), and then after 2 h of stirring, a solution of 4-(2-aminoethyl)morpholine (1.14 g, 9 mmol) in chloroform (3.0 mL) was added and stirring was continued overnight. It was then diluted with chloroform (100 mL), washed with water, dried over anhydrous magnesium sulfate, and evaporated to dryness to give 3.30 g of an oily product which was purified on a silica gel column (60% ethyl acetate/hexanes) to give 2.2 g (80%) of a white solid (**28**): mp 99 °C; MS m/z = 364 (M^+).

***N*-[2-(4-Imidazolyl)ethyl]-2-chloro-*cis*-5a,6,7,8,9,9a-hexahydrodibenzofuran-4-carboxamide (27).** By the same procedure as above, 2.0 g (8 mmol) of acid **5** and 0.99 g (9 mmol) of 4-(2-aminoethyl)imidazole gave 3.12 g of an oily mixture which was purified on a silica gel column (8% methanol/chloroform) to give 1.19 g (43%) of **27** as a white solid: mp 209–10 °C; MS m/z = 345 (M^+).

***N*-[2-(*N*',*N*'-Diethylamino)ethyl]-2-chloro-*cis*-5a,6,7,8,9,9a-hexahydrodibenzofuran-4-carboxamide (24).** By the same procedure as above, 1.0 g (0.004 mol) of racemic acid **5** and 0.6 g (0.005 mol) of *N*',*N*'-diethylenediamine gave an oily product which was taken up in ether, washed with 1 N sodium hydroxide and then water, dried over magnesium sulfate, filtered, and evaporated to dryness to give an oily product which was chromatographed over silica gel column (1:1 ethyl acetate/hexanes) to give a solid product (**24**) (0.84 g): mp 96–8 °C; MS m/z = 350 (M^+); ^{1}H NMR ($CDCl_3$) δ 8.15 (s, 1 H), 7.88 (m, 1 H), 7.17 (m, 1 H), 4.81 (m, 1 H), 3.52 (m, 2 H), 3.22 (m, 1 H), 2.57 (m, 6 H), 1.68 (m, 8 H), 1.03 (m, 6 H).

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Synthesis and in Vitro Biological Profile of All Four Isomers of the Potent Muscarinic Agonist 3-(3-Methyl-1,2,4-oxadiazol-5-yl)-1-azabicyclo[2.2.1]heptane

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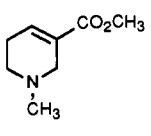
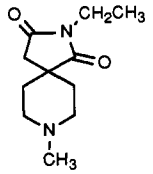
The four stereoisomers of the muscarinic agonist **7** have been synthesized from enantiomerically pure *exo*-azanorbornane esters (**13a,b**). The esters were obtained in optically active form by separation of the carboxamide diastereomers **12a,b**, formed from the borane complex of *exo*-azanorbornane-3-carboxylate **10** and a chiral amine auxiliary. Using the known chirality of (*R*)-α-methylbenzylamine, an X-ray analysis was accomplished on **12a** in order to determine the absolute configuration of the azanorbornane C4 chiral center. Each of the chiral esters **13a,b** was separately transformed into the oxadiazoles with concomitant epimerization at C3 of the azanorbornane ring to afford the thermodynamic equilibrium mixture of isomers. Chromatographic separation followed by analysis of each isomer by NMR and GC allowed the absolute stereochemistry of all four isomers of **7** to be confirmed. Full biological evaluation in biochemical and pharmacological assays revealed that the 3*R*,4*R* isomer was the most active on receptor binding studies and the most potent on the pharmacological preparations, showing a 50-fold increase in potency at the M_2 and M_3 sites compared to M_1.

The finding that specific cholinergic deficits present in brain tissues of patients diagnosed as having Alzheimer's disease[1] has led to the hypothesis that an enhancement of cholinergic neurotransmission may alleviate the deficits in memory and cognition. The clinical trials of a number of directly acting muscarinic agonists such as arecoline (**1**),[2] RS86 (**2**),[3] and pilocarpine (**3**)[4] have proved disappointing.



1



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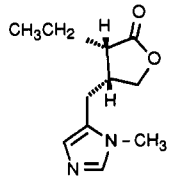

3

We have previously suggested[5] that these results may be understood in terms of the low cortical efficacy of these ligands. Within the cerebral cortex the postsynaptic pirenzepine-sensitive muscarinic receptors (coupled to phosphatidylinositol turnover) lack an effective receptor reserve, and therefore partial agonists such as **1**, **2**, and **3** produce a small maximal response relative to a full agonist such as carbachol (**4**).[6,7]

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(1) Perry, E. K. The Cholinergic Hypothesis - ten years on. *Br. Med. Bull.* **1986**, *42*, 63–69.
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(3) Mouradian, M. M.; Mohr, E.; Williams, J. A.; Chase, T. N. No response to high dose Muscarinic Agonist Therapy in Alzheimer's disease. *Neurology* **1988**, *38*, 606–608.
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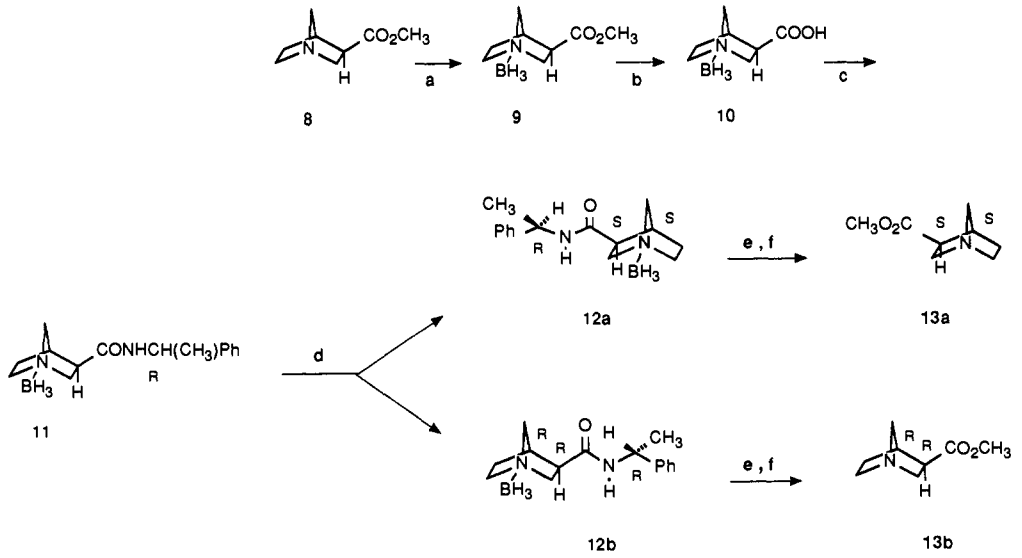
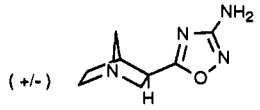
Scheme I[a]



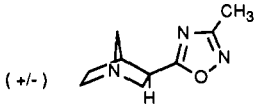
[a] Reagents: (a) BH_3·THF, THF, −78 °C; (b) NaOH, H_2O, MeOH; (c) NEt_3, iBuOCOCl, (*R*)-α-methylbenzylamine, CH_2Cl_2; (d) chromatography on silica; (e) concentrated HCl; (f) $SOCl_2$, MeOH.

Recent studies from this laboratory have described[8,9] (*exo*-azanorbornyl)oxadiazole analogues (**5** and **6**) which were shown to be among the most potent and efficacious muscarinic agonists known. Both **5** and **6** are tertiary amines and are capable of CNS penetration; therefore, they are suitable ligands for the study of muscarinic receptors within the central nervous system. Cortical muscarinic receptors may bear similarity to those present in sympathetic ganglia (M_1), and agonists have been evaluated in the isolated superior cervical ganglion relative to their ability to stimulate muscarinic receptors in the isolated heart (M_2) and small intestine (M_3) preparations.[9]



5

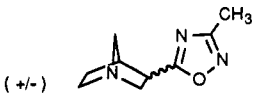
exo diastereomer



6

exo diastereomer

This study reports on the synthesis and isolation of all four isomers of 3-(3-methyl-1,2,4-oxadiazol-5-yl)-1-azabicyclo[2.2.1]heptane (**7**) and the determination of the absolute stereochemistry of each isomer by X-ray crystallography on the borane complex of one of the chiral azanorbornane intermediates. Full in vitro biological characterization of all four isomers in muscarinic biochemical and pharmacological assays is discussed.



7

mixture of all four isomers

Synthetic Chemistry and X-ray Crystallography

In order to obtain all four isomers of **7** we required a method of obtaining sufficient quantities of the enantiomers of the ester **8**[8,10] in optically pure form (>95% ee). Initially the crystallization of chiral acid salts using known optically pure acids from the chiral pool (e.g. tartaric acid and its derivatives, camphorsulfonic acid) was investigated. However, although some degree of resolution was achieved, we were unable to obtain satisfactory optical purity. We therefore decided to use the carboxylate functionality of **8** to introduce a chiral auxiliary in order to obtain diastereomers which could be separated by physical means. An amide derivative was chosen since carboxylic amides retain a higher degree of rigidity when compared to carboxylic esters, allowing a greater chance of diastereomeric separation. The carboxamide **15** was synthesized from the corresponding carboxylic acid chloride, and although the two diastereomers could be separated by careful column chromatography on neutral alumina, the use of **15** had two major problems. Firstly, the synthesis of **15** on scale-up produced highly variable yields, and secondly it was observed that **15**, when in solution (and during column chromatography) as its free base, was unstable, giving rise to polar degradation products. Both of these problems, we believed, were due to the presence of a basic nitrogen moiety in the molecule. Reduction of this basicity was therefore required, and this was accomplished by formation of the stable borane complex.

The ester **8** was reacted with BH_3·THF at −78 °C to afford the crystalline borane complex **9** in 73% yield

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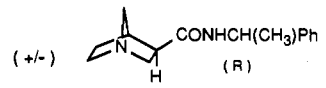


15

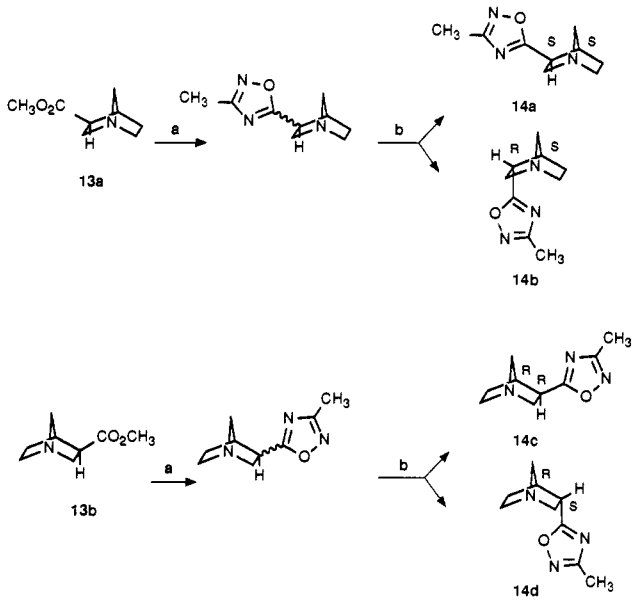
(Scheme I). Saponification of the ester using NaOH followed by amide formation under mixed anhydride conditions gave **11**. It was important to use a chiral auxiliary of good optical purity, and only batches of (*R*)-(+)-α-methylbenzylamine which were ≥96% ee (chiral GC) were employed. Compound **11** was subjected to column chromatography on silica to obtain the pure diastereomers **12a** (26%) and **12b** (17%). It was important to assess the diastereomeric purity of the isomers at this stage since the absolute stereochemistry at C4 was now fixed and accurate determination of the optical purity of the esters (**13a, 13b**) was difficult by HPLC due to lack of a UV chromophore. The purities of **12a** and **12b** were shown by HPLC to be 98.8% and 96.0% de, respectively. Racemization at C4 was not possible in the subsequent steps leading to optically pure carboxylic esters **13a** and **13b** which were confirmed exo at C3 by NMR[8] and were >99% chemically pure by GC. We now had in hand samples of optically pure azanorbornyl esters which could be used to synthesize 1,2,4-oxadiazoles. Each ester (**13a, 13b**) was taken in turn and converted to the 3-methyl-1,2,4-oxadiazole derivatives (**14a–d**) using chemistry previously described[8] (Scheme II). The purity of each isomer was assessed by both GC and HPLC (>99% in all four isomers), and the optical purity was the same as the diastereomeric purity of the amides **12a** and **12b**, since the absolute stereochemistry at C4 is fixed and the relative stereochemistry at C3 confirmed by NMR.[8]

In order to identify the stereochemistry of the diastereomeric amides **12a** and **12b**, crystals of **12a** were grown from EtOAc, and the absolute configuration was assigned by X-ray analysis. From the *R* configuration of the chiral auxiliary the absolute configuration of **12a** was shown to be 3*S*,4*S* in the azanorbornane ring (Figure 1), thus allowing the absolute configuration of all four isomers of the oxadiazoles (**14a–d**) to be determined.

Results

Receptor Binding Studies. The isomers **14a–d** were evaluated in the previously described paradigm[9] which can be used to measure the affinity of ligands for the muscarinic receptors present in the rat cerebral cortex and also to predict relative efficacy. This assay measures the ability of compounds to displace low concentrations of the muscarinic agonist [^{3}H]oxotremorine-M from the high-affinity state of the receptor and also to displace the muscarinic antagonist [^{3}H]-*N*-methylscopolamine from the low affinity state. The NMS/OXO-M ratio obtained has been shown to be predictive of the ability of the ligand to stimulate PI hydrolysis.[9] In this assay full agonists have a high NMS/OXO-M ratio (>1000) whereas antagonists show a ratio close to unity.[9] The results (Table I) reveal that the exo enantiomers (**14a, 14c**) have identical predicted cortical efficacy for the muscarinic receptor (NMS/OXO-M ratio = 1200); however, the 3*R*,4*R* enantiomer (**14c**) has 10-fold greater affinity at both the high affinity (OXO-M, K_{app} = 0.027 nM) and low affinity (NMS, K_{app} = 32 nM) states of the cortical muscarinic receptor. The racemate **6** was shown to have intermediate affinity (K_{app} = 100 nM) at the low affinity state.[9] The endo enantiomers (**14b, 14d**) have identical affinities (2200 nM) for the low affinity (NMS) state of the receptor (Table I), and both compounds possess a marginal increase in cortical efficacy over their exo isomers.

Scheme II[a]



[a] Reagents: (a) NaH, THF, $CH_3C(=NOH)NH_2$; (b) chromatography on alumina.

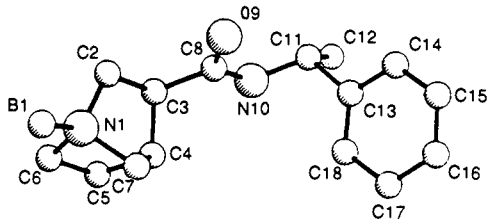


Figure 1. Computer-generated picture of **12a** derived from the X-ray coordinates.

Pharmacology. The four isomers were assessed for their relative potencies and efficacies in pharmacological preparations used to identify functional selectivity between muscarinic receptor subtypes. The tests used were the depolarizing response on the rat superior cervical ganglion[11] (mediated by M_1 receptors), the negative chronotropic effect on electrically driven guinea pig atria (mediated by M_2 receptors), and the contraction of the guinea pig myenteric plexus preparation (mediated by M_3 receptors). The results are shown in Table II. The exo enantiomers (**14a, 14c**) were full agonists in all three tissue preparations as shown by the relative maximum (RM > 0.9) compared to the standard muscarinic agonists muscarine and carbachol. The more potent 3*R*,4*R* enantiomer (**14c**) revealed some potency selectivity for the M_2/M_3 tissues compared to M_1 (M_1/M_2 = 62, M_1/M_3 = 53), whereas the 3*S*,4*S* enantiomer (**14a**) had virtually identical potencies. The endo enantiomers (**14b, 14d**) possessed similar potencies, both having a 5–10-fold increase on M_2/M_3 activity compared to M_1. In addition **14b** also showed a reduced efficacy (RM = 0.76) on the M_1 pharmacological assay.

Discussion

Recent studies from this laboratory[5,8] have reported that the protonated 1-azanorbornane and quinuclidine ring

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Table I. In Vitro Binding Data for the Isomers of 7 Compared to Standard Muscarinic Ligands

compound	absolute stereochem	relative stereochem	binding data: K_{app}, nM[a] [^{3}H]NMS[b]	[^{3}H]OXO-M[c]	ratio[d]
atropine	–	–	1.0	0.48	2.1
carbachol	–	–	22000	4.9	4500
14a	3*S*,4*S*	exo	370	0.32	1200
14b	3*R*,4*S*	endo	2200	1.6	1400
14c	3*R*,4*R*	exo	32	0.027	1200
14d	3*S*,4*R*	endo	2200	1.2	1800

[a] Displacement of tritiated radioligand from rat cortical membranes. Results are expressed as the geometric mean of the affinity constant (K_{app}) corrected for ligand occupancy. Each value is the geometric mean of at least three determinations performed on separate occasions. Each curve is typically four concentrations performed in triplicate. Variability in the determinations is ±12%. [b] Displacement of [^{3}H]-*N*-methylscopolamine. [c] Displacement of [^{3}H]oxotremorine-M. [d] The ratio of NMS/OXO-M K_{app}'s.

Table II. In Vitro Pharmacological Data for the Isomers of 7

compd	ganglion[a] (M_1) EC_{50}, nM[b]	RM[d]	atrium[a] (M_2) EC_{50}, nM	RM	ileum[a] (M_3) EC_{50}, nM	RM
14a	7.0	1.16	3.3	0.94	2.4	0.97
14b	90.0	0.76	19.0	0.96	16.0	0.93
14c	2.4	0.94	0.039	0.91	0.045	0.91
14d	110.0	0.98	14.0	0.92	13.0	0.90

[a] Assays described in full in the Experimental Section. [b] EC_{50} is the concentration required to produce half of the relative maximum. Each value is the geometric mean of at least two determinations (ganglion and atrium) or four determinations (ileum). Variability in the determinations is ±15%. [d] RM is the relative maximum, the amplitude of the response at which the dose–response curves exhibit a plateau relative to the amplitude of a response to muscarine (ganglion) or carbachol (atrium and ileum). Results are expressed as median.

systems could closely mimic the electrostatic charge distribution of the quaternary ammonium group present in classical muscarinic agonists such as muscarine. These studies led to the discovery of the potent muscarinic ligands (**5** and **6**) bearing a 1-azanorbornane ring system.[8,9] In order to further investigate the biological properties of **6** it was necessary to synthesize the individual enantiomers and their endo stereoisomers. Receptor binding studies revealed that the 3*R*,4*R* isomer (**14c**) displayed the highest affinity in cortical binding for the muscarinic receptor and was the most potent isomer on the three pharmacological preparations. Furthermore **14c** showed some selectivity (50–60-fold) toward the M_2 and M_3 receptor subtypes over the M_1 as revealed by its potency on the functional tissue preparations. The development of a simple binding model[12] for the muscarinic receptor (Figure 2) can be based on the absolute configuration of **14c**. All muscarinic ligands require the presence of a cationic head group which is involved in a primary electrostatic interaction with an Asp residue on the receptor protein (by analogy with the β-receptor[13]). The oxadiazole functionality in **14c** can form secondary interactions, allowing the overall binding mode to cause a conformational change of the receptor protein which is required for agonist activity. The muscarinic receptor contains three conserved aspartic acid residues[14] (Asp 71, 105, and 122). The Asp 105 could act as the primary binding site accessible to both agonists and

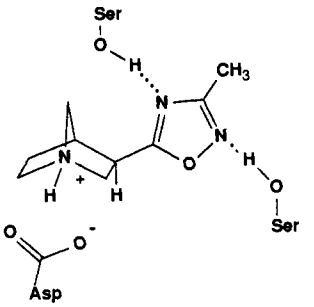

Figure 2. Simple binding model for **14c** at the muscarinic receptor.

antagonists (by analogy with Asp 113 of the β-adrenoceptor). However a small flexible agonist, such as **14c**, may penetrate further into the receptor to become highly bound to Asp 71 (corresponding to Asp 79 in the β-adrenoceptors) while allowing the oxadiazole nitrogens to achieve hydrogen-bond interactions. The reasons why the 3*R*,4*R* stereoisomer (**14c**) has M_2/M_3 potency selectivity over M_1, while its enantiomer (**14a**) is nonselective, is unclear based on current knowledge of the structural differences between the M_1, M_2, and M_3 receptor proteins.

In summary, the synthesis of all four stereoisomers of the 1,2,4-oxadiazole derivative **7** has been achieved from (3*S*,4*S*)- and (3*R*,4*R*)-methyl 1-azabicyclo[2.2.1]heptane-3-carboxylate (**13a** and **13b**), and the 3*R*,4*R* stereoisomer (**14c**) displayed the highest affinity for cortical muscarinic receptors in vitro. This stereoisomer was also the most active on muscarinic pharmacological preparations. In addition **14c** showed M_2/M_3 potency selectivity over M_1 (50-fold) whereas its enantiomer (**14a**) was nonselective.

Experimental Section

Chemical Methods. General Directions. Except where otherwise stated, the following procedures were adopted: all ^{1}H NMR spectra were recorded at 360 MHz on a Bruker AM360 or at 250 MHz on a Bruker AC250 instrument, mass spectra with a VG 70-250 mass spectrometer, and infrared spectra on a Perkin-Elmer 782 IR spectrometer. GC was performed on a BP1 capillary column with a Perkin-Elmer gas chromatograph (8320). Optical rotations were measured by using a Perkin-Elmer 241 polarimeter. Organic solvents were purified when necessary by the methods described by D. D. Perrin, W. L. F. Armarego, and D. R. Perrin (*Purification of Laboratory Chemicals*; Pergamon: Oxford, 1966). Petroleum ether refers to that fraction having a boiling range of 60–80 °C. All solutions were dried over anhydrous sodium sulfate and evaporated on a Buchi rotary evaporator at reduced pressure. Thin-layer chromatography was carried out using silica (Merck Art 5719) or alumina plates (Merck Art 5550). Column chromatography was carried out using silica (Merck Art 7734) or neutral alumina (Merck Art 1077 or ICN, activity Brockmann Grade III). All novel compounds were fully characterized spectroscopically (^{1}H NMR and MS), and their chemical homogeneity was confirmed by GC or HPLC and elemental analysis of the appropriate salt form. Melting points are uncorrected.

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***exo*-Methyl 1-Azabicyclo[2.2.1]heptane-3-carboxylate Borane Complex 9.** BH_3·THF (242.5 mL of a 1 M THF solution, 0.24 mol) was added dropwise to a stirred solution of (±)-*exo*-methyl 1-azabicyclo[2.2.1]heptane-3-carboxylate[8,10] (15.0 g, 0.097 mol) in anhydrous THF (200 mL) at −78 °C under a nitrogen atmosphere. After 0.5 h at −78 °C, H_2O (20 mL) was added dropwise, and the mixture was stirred vigorously while warming to room temperature. The majority of the THF was removed by distillation, and further H_2O was added. The aqueous was extracted using CH_2Cl_2 (twice), and the combined organics were dried and then evaporated to give a pale yellow oil (16.5 g). This oil was purified by column chromatography on silica using EtOAc/*n*-hexane (1:2) to provide **9** as a colorless solid (12.0 g, 73%): mp 35–36 °C; R_f 0.35 in EtOAc/*n*-hexane (1:1) on silica plates; MS *m/z* 168 $(M - H)^+$; IR ν_{max} (film) 2450–2250 and 1735 cm^{-1}. Anal. ($C_8H_{16}BNO_2$) C, H, N.

***exo*-1-Azabicyclo[2.2.1]heptane-3-carboxylate Borane Complex 10.** A solution of NaOH (3.08 g, 77 mmol) in H_2O (40 mL) was added dropwise to a stirred, cooled (0 °C) solution of **9** (10.0 g, 59 mmol) in MeOH (80 mL). The mixture was allowed to warm to room temperature and then stirred for 4 h. The MeOH was evaporated, further H_2O (80 mL) was added, and then the aqueous was treated with citric acid (7.77 g, 40 mmol) and extracted with CH_2Cl_2 (three times). The combined organics were dried and then evaporated to dryness to give **10** as a colorless solid (7.05 g, 77%): mp 82–83 °C (Et_2O/petroleum ether (40–60)); R_f 0.25 in CH_2Cl_2/MeOH (19:1) on silica plates; MS *m/z* 154 $(M - H)^+$; IR ν_{max} (Nujol) 3300–2500, 2400–2250, and 1700 cm^{-1}. Anal. ($C_7H_{14}BNO_2$) C, H, N.

***N*-(1(*R*)-Phenylethyl)-1-azabicyclo[2.2.1]heptane-3-carboxamide Borane Complexes 12a and 12b.** NEt_3 (8.9 mL, 64 mmol) was added to a stirred, cooled (−20 °C) solution of **10** (8.0 g, 52 mmol) in anhydrous CH_2Cl_2 (100 mL) under a nitrogen atmosphere. Isobutyl chloroformate (7.5 mL, 57 mmol) was added dropwise, keeping the temperature below −20 °C. After 15 min (*R*)-(+)-α-methylbenzylamine (6.9 g, 57 mmol, >96% ee as assessed by chiral GC) was added, keeping the temperature below −20 °C. After 1 h at this temperature, H_2O (60 mL) was added and the mixture stirred while warming to room temperature. The organic layer was separated, and the aqueous layer was reextracted with CH_2Cl_2. The combined organics were dried and then evaporated to afford a solid which was purified by column chromatography on silica to afford **12a** (3.5 g, 26%) as a colorless crystalline solid followed by **12b** (2.2 g, 17%) as a colorless crystalline solid.

12a: mp 151 °C; HPLC, 99.4% on an acetylated β-cyclodextrin column in 50% MeOH/50% H_2O; HRMS, found $(M - H)^+$ 257.1836, $C_{15}H_{22}BN_2O$ required (M − H) 257.1825; IR ν_{max} (Nujol) 3340, 2350, 2310, 2260, and 1645 cm^{-1}; $[\alpha]^{22}_D$ +64.0° (CH_2Cl_2, *c* = 0.5); ^1H NMR ($CDCl_3$) δ 1.48 (3 H, d, *J* = 7 Hz, $CHCH_3$), 1.52–1.70 (1 H, m, 5-CH), 1.91–2.02 (1 H, m, 5-CH) overlapped with 1.20–2.24 (3 H, broad resonance, BH_3), 2.40 (1 H, dd, *J* = 6.9 and 7 Hz, 3-CH), 2.64 (1 H, d, *J* = 10 Hz), 2.76 (1 H, d, *J* = 4 Hz, 4-CH), 2.79–2.90 (1 H, m), 2.98–3.08 (1 H, m), 3.08–3.20 (1 H, m), 3.24 (1 H, d, *J* = 9 Hz), 3.42–3.48 (1 H, m), 5.09 (1 H, quintet, *J* = 7 Hz, $CHCH_3$), 5.58–5.67 (1 H, m, NH), 7.19–7.42 (5 H, m, C_6H_5). Anal. ($C_{15}H_{23}BN_2O$) C, H, N.

12b: mp 176–178 °C; HPLC, 98% on an acetylated β-cyclodextrin column, 50% MeOH 50% H_2O; HRMS found $(M - H)^+$ 257.1833, $C_{15}H_{22}BN_2O$ requires (M − H) 257.1825; IR ν_{max} (Nujol) 3380–3200, 2340, 2310, 2260, and 1640 cm^{-1}; $[\alpha]^{22}_D$ +91.15° (CH_2Cl_2, *c* = 0.5); ^1H NMR ($CDCl_3$) δ 1.50 (3 H, d, *J* = 7 Hz, $CHCH_3$), 1.54–1.62 (1 H, m, 5-CH), 1.94–2.08 (1 H, m, 5-CH) overlapped with 1.36–2.18 (3 H, broad resonance, BH_3), 2.38 (1 H, dd, *J* = 6.6 and 7.3 Hz, 3-CH), 2.64 (1 H, d, *J* = 10 Hz), 2.78–2.89 (1 H, m) overlapped with 2.84 (1 H, d, *J* = 4 Hz, 4-CH), 2.93–3.00 (1 H, m), 3.07–3.18 (1 H, m), 3.26 (1 H, d, *J* = 9.6 Hz), 3.38–3.44 (1 H, m), 5.09 (1 H, quintet, *J* = 7 Hz, $CHCH_3$), 5.60–5.74 (1 H, m, NH), 7.24–7.45 (5 H, m, C_6H_5). Anal. ($C_{15}H_{23}BN_2O$) C, H, N.

(−)-*exo*-(3*S*,4*S*)-Methyl 1-Azabicyclo[2.2.1]heptane-3-carboxylate Hydrogen Oxalate (13a). **12a** (3.3 g, 12.8 mmol) was heated to reflux in concentrated HCl (30 mL) for 20 h. The solution was evaporated to dryness, and the residue was dissolved in aqueous NaOH solution (3.5 molar equiv), washed with Et_2O, reacidified with concentrated HCl, evaporated to dryness, and dried over P_2O_5 to afford a solid (7.3 g). $SOCl_2$ (2.79 mL, 38.4 mmol) was added to MeOH (30 mL) with stirring at −20 °C. The crude acid (7.3 g) was added, and the solution was allowed to warm to room temperature and then stirred for 20 h. The MeOH was evaporated, and the colorless residue was dissolved in H_2O. CH_2Cl_2 was added, and the aqueous layer was basified to pH = 10 using K_2CO_3. The organic layer was separated, and the aqueous layer was reextracted four times with CH_2Cl_2. The combined organics were dried then evaporated to dryness, and the residue was purified by column chromatography on silica using 6% MeOH/CH_2Cl_2 to afford **13a** free base as an oil (1.02 g, 51%). The hydrogen oxalate salt: mp 114–115 °C (MeOH); R_f 0.26 in CH_2Cl_2/MeOH (9:1) on silica plates; HRMS found M^+ 155.0984, $C_8H_{13}NO_2$ requires M 155.0946; $[\alpha]^{22}_D$ −3.2° (MeOH, *c* = 0.5). Anal. ($C_8H_{13}NO_2 \cdot C_2H_2O_4$) C, H, N.

(+)-*exo*-(3*R*,4*R*)-Methyl 1-Azabicyclo[2.2.1]heptane-3-carboxylate Hydrogen Oxalate (13b). **13b** was obtained from **12b** using the procedure described for **13a** above. The hydrogen oxalate salt: mp 115 °C (MeOH); R_f 0.26 in CH_2Cl_2/MeOH (9:1) on silica plates; HRMS found M^+ 155.0969, $C_8H_{13}NO_2$ requires M 155.0946; $[\alpha]^{22}_D$ +3.0° (MeOH, *c* = 0.5). Anal. ($C_8H_{13}NO_2 \cdot C_2H_2O_4$) C, H, N.

(3*S*,4*S*)- and (3*R*,4*S*)-3-(3-Methyl-1,2,4-oxadiazol-5-yl)-1-azabicyclo[2.2.1]heptane (14a and 14b). Acetamide oxime (281 mg, 3.8 mmol) was stirred with 4A molecular sieves (2 g) in anhydrous THF (20 mL) under a nitrogen atmosphere for 0.5 h. NaH (166 mg of a 55% oil dispersion) was added over 2 min. After addition the mixture was stirred at 50 °C for 1 h. A solution of **13a** free base (290 mg, 1.9 mmol) in anhydrous THF (5 mL) was added, and the mixture was heated at reflux with stirring for 2 h. The reaction mixture was cooled, H_2O (8 mL) was added, and the mixture was filtered and then evaporated. The residue was partitioned between H_2O (10 mL) and CH_2Cl_2 (20 mL). The organic layer was separated, and the aqueous layer was reextracted with CH_2Cl_2 (4 × 20 mL). The combined organics were dried and then evaporated to dryness to afford a pale yellow oil (320 mg). This oil was purified by column chromatography on neutral alumina using EtOAc/petroleum ether (3:1). Fractions were monitored by GC (isothermal, 160 °C), and those of >99% purity were combined. **14a** was obtained as a colorless oil (150 mg, 44%) and **14b** as a colorless oil (20 mg, 6%). In order to obtain a water-soluble solid form the *p*-toluenesulfonate salts were prepared.

14a *p*-Toluenesulfonate: mp 143–144 °C (iPA/EtOAc); R_f 0.40 in EtOAc on alumina plates; GC (isothermal, 160 °C) >99.5% (t_R = 5.61 min); MS *m/z* 179 for M^+ of free base; $[\alpha]^{23}_D$ +14.4° (CH_2Cl_2, *c* = 0.5). Anal. ($C_9H_{13}N_3O \cdot C_7H_8O_3S$) C, H, N. **14b *p*-Toluenesulfonate**: mp 178–180 °C (iPA/EtOAc); R_f 0.28 in EtOAc on alumina plates; GC (isothermal, 160 °C) >99.5% (t_R = 5.39 min); MS *m/z* 179 for M^+ of free base; $[\alpha]^{23}_D$ −52.4° (CH_2Cl_2, *c* = 0.25). Anal. ($C_9H_{13}N_3O \cdot C_7H_8O_3S$) C, H, N.

(3*R*,4*R*)- and (3*S*,4*R*)-3-(3-Methyl-1,2,4-oxadiazol-5-yl)-1-azabicyclo[2.2.1]heptane (14c and 14d). **14c** and **14d** were obtained from **13b** as described above. **14c** was obtained as a colorless oil (352 mg, 52%) and **14d** as a colorless oil (55 mg, 8%).

14c *p*-Toluenesulfonate: mp 143 °C (iPA/EtOAc); R_f 0.40 in EtOAc on alumina plates; GC (isothermal, 160 °C) >99.5% (t_R = 5.57 min); MS *m/z* 179 for M^+ of free base; $[\alpha]^{23}_D$ −14.8° (CH_2Cl_2, *c* = 0.5). Anal. ($C_9H_{13}N_3O \cdot C_7H_8O_3S$) C, H, N.

14d *p*-Toluenesulfonate: mp 182–184 °C (iPA/EtOAc); R_f 0.28 in EtOAc on alumina plates; GC (isothermal, 160 °C) >99.5% (t_R = 5.37 min); MS *m/z* 179 for M^+ of free base; $[\alpha]^{23}_D$ +53.2° (CH_2Cl_2, *c* = 0.25). Anal. ($C_9H_{13}N_3O \cdot C_7H_8O_3S$) C, H, N.

Biochemical Methods. (a) Brain Membrane Preparation. Crude synaptosomal-mitochondrial membranes were prepared by homogenizing cerebral cortex from rat (250–300 g) in 0.32 M ice-cold sucrose (1/10, w/v) in a motor-driven Teflon/glass homogenizer at 500 rpm (10 strokes). The homogenate was centrifuged at 1000*g* for 15 min, and the resulting supernatant was centrifuged at 17000*g* for 20 min. This yielded the crude synaptosomal-mitochondrial pellet (P_2), which was used fresh or stored at −20 °C before use.

(b) Receptor Binding Studies. [^{3}H]-*N*-Methylscopolamine binding ([^{3}H]NMS) P_2 fractions were homogenized and resuspended at a final dilution of 1/600 (wet w/v) in ice-cold Krebs-HEPES buffer pH 7.4 (composition, mM: NaCl 118, KCl

4.7, $MgSO_4$ 1.2, $NaHCO_3$ 5, KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11, and HEPES 20). Binding of [^{3}H]NMS was determined with 0.01–1.0 nM ligand and nonspecific binding defined with 2 μM atropine. Displacing drugs were added in a volume of 10 μL to give a final assay volume of 1.0 mL. Incubations were initiated by adding 750 μL of membrane solution and were allowed to proceed for 60 min at 30 °C. Assays were terminated by filtration using a Brandell cell harvester and Whatman GF/B filters with 2 × 10-mL rinses in ice-cold saline (0.9% NaCl, w/v). Filters were then placed in 10 mL of scintillation fluid (Hydrofluor, National Diagnostics, NJ), and radioactivity was estimated by liquid scintillation spectrometry. Kinetic analysis has been previously reported[7] with a dissociation constant (K_D) of 0.14 ± 0.02 nM and a maximum binding capacity (B_{max}) of 1400 ± 340 fmol mg^{-1} protein (n = 5).

[^{3}H]Oxotremorine-M binding ([^{3}H]OXO-M) P_2 fractions were washed by resuspending in 10 mL of 20 mM HEPES buffer pH 7.4 and were centrifuged at 17000g for 20 min. The washed membranes were homogenized and resuspended at a final dilution of 1/100 (wet w/v) in ice-cold 20 mM HEPES buffer pH 7.4. Binding of [^{3}H]OXO-M was determined by use of 0.2–5 nM of ligand, and nonspecific binding was defined with 2 μM atropine. Displacing compounds were added in a volume of 10 μL to give a final assay volume of 1.0 mL. Incubations were initiated by adding 750 μL of membrane solution and were allowed to proceed for 40 min at 30 °C. Assays were terminated by filtration over Whatman GF/C filters presoaked in 0.05% polyethyleneimine, using a Brandell cell harvester. Samples were washed with 10 mL of ice-cold saline, and filters were placed in 10 mL of scintillation fluid (Hydrofluor, National Diagnostics, NJ), and radioactivity was estimated by liquid scintillation spectrometry. Scatchard analysis indicated a dissociation constant (K_D) of 0.68 ± 0.12 nM and a maximum binding capacity of 520 ± 160 fmol mg^{-1} protein.

Binding parameters were determined by nonlinear, least-squares regression analysis using RS1 (BBN Research Systems, Cambridge, MA) and a computerized iterative procedure written by Dr. A. Richardson, NRC Terlings Park.

Pharmacological Methods. (a) Rat Superior Cervical Ganglion. Superior cervical ganglia from male Sprague–Dawley rats were superfused in vitro as previous described.[11] The dc potential between the ganglion body and the internal carotid nerve was recorded across a greased gap with Ag/AgCl electrodes. Agonists were superfused with increasing concentrations for a 1-min period at 10-min intervals. Since the response did not return to base line during the 9-min wash period, calculations were made from the extrapolated base line. Before determining the dose–response relationship, a reproducible response to 1 μM (±)-muscarine chloride was obtained on each ganglion. Subsequent responses were all related to that depolarizing response, given an arbitrary value of 1.0. The maximum response and the concentration required to evoke half of that response (EC_{50}) were determined on a number of ganglia, each from a different rat. It should be noted that the dose–response relationship was determined by increasing the agonist concentration until the response levelled off.

(b) Guinea Pig Isolated Atria. Paired atria were removed from male guinea pigs (300–400 g weight) and suspended under 1 g of tension in Krebs bicarbonate solution containing 22 mM glucose. Preparations were paced by electrical field stimulation (3–4 Hz, 2–3 ms) with platinum electrodes. Following a 60-min equilibration period, noncumulative dose–response curves to muscarinic agonists were constructed, allowing exposure to any one application of test compound until a maximum negative chronotropic effect was obtained. A period of at least 45 min was allowed between each dose–response curve. Agonist potency (EC_{50}) and the maximum response relative to the maximum response to carbachol or muscarine were determined using RS1 (BBN Research Systems, Cambridge, MA) and the computerized procedure described above.

(c) Guinea Pig Ileum, Longitudinal Muscle–Myenteric Plexus Preparation. Preparations of longitudinal muscle with the myenteric plexus from the distal ileum of 300–400 g male guinea pigs were obtained as described by Rang (1964). Preparations were washed, suspended under 1 g of tension in glass organ baths containing 3 mL of Krebs bicarbonate solution at 37 °C, and allowed to equilibrate for at least 60 min. Isometric contractions to muscarinic agents were measured for at least 30 s until a clear peak of response was obtained. A period of at least 45 min was allowed between each test compound. Potency and maximum response relative to that for carbachol or muscarine were determined as for the atrium. Tissue responses were measured as changes in isometric tension in the ileum. The responses were then calculated as a percentage of the maximum response obtained relative to a dose of 1 μM carbachol. Agonist potency (EC_{50}) was determined by a nonlinear iterative curve-fitting procedure.

X-ray Crystallography. X-ray Crystal Structure Analysis of 12a. Crystals of **12a** ($C_{15}H_{23}BN_2O$, 2 formula units in unit cell) formed in space group $P2_12_12_1$ with a = 9.874 (3) Å, b = 17.559 (3) Å, c = 18.179 (6) Å for Z = 4 and a calculated density of 1.088 g/cm^3. An automatic four-circle diffractometer equipped with Cu Kα radiation (λ = 1.5418 Å) was used to measure 2677 potential diffraction peaks of which 963 were observed ($I \geq 3\sigma I$). Application of a multisolution tangent formula approach to the phase solution gave an initial model for the structure[15] which was subsequently refined with least-squares and Fourier methods. Anisotropic temperature parameters were refined for the non-hydrogen atoms, while isotropic temperature factors were applied to the hydrogens but not refined. The function $\sum\omega(|F_o| - |F_c|)^2$ with $\omega = 4F_o^2/\sigma^2(F_o^2)$ was minimized with full-matrix least-squares to give an unweighted residual of 0.065. Figure 1 is a computer-generated drawing of **12a** showing 3S,4S stereochemistry in the azanorbornane ring. All bond distances and angles are within chemically reasonable limits.

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Registry No. **8**, 115594-66-6; **9**, 133810-60-3; **10**, 133849-70-4; **12a**, 133963-76-5; **12b**, 133810-61-4; **13a**, 133963-80-1; **13a** free base, 133963-79-8; **13b**, 133963-78-7; **13b** free base, 133963-77-6; **14a**, 133943-40-5; **14a**·*p*-toluenesulfonate, 138602-62-7; **14b**, 138456-85-6; **14b**·*p*-toluenesulfonate, 138511-88-3; **14c**, 133989-15-8; **14c**·*p*-toluenesulfonate, 138602-63-8; **14d**, 138456-86-7; **14d**·*p*-toluenesulfonate, 138511-89-4; acetamide oxime, 22059-22-9.

Supplementary Material Available: Tables of the atomic positional and thermal parameters, bond distances, and bond angles for **12a** and microanalyses for all novel compounds (8 pages). Ordering information is given on any current masthead page.

(15) The following library of crystallographic programs was used. SHELXS-86: G. M. Sheldrick, University of Göttingen, West Germany (1986). PLUTO: W. D. S. Motherwell and W. Clegg, University of Cambridge, England (1978). A version of SDPV.3: Enraf-Nonius, Delft, The Netherlands (1985), locally modified for a Sun Microsystems computer.