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Synthesis and Muscarinic Receptor Activity of Ester Derivatives of 2-Substituted 2-Azabicyclo[2.2.1]heptan-5-ol and -6-ol

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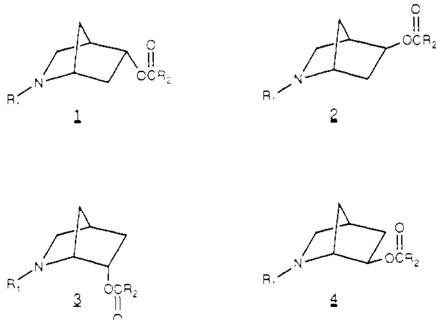
Radioligand binding affinities of four new muscarinic antagonists and six potential muscarinic agonists which possess the 2-alkyl-2-azabicyclo[2.2.1]heptane ring system have been determined in rat heart, rat brain, and m_1- or m_3-transfected CHO cell membrane preparations to examine the selectivity for subtypes of muscarinic receptor. The efficacies of the potential muscarinic agonists were determined by the ratio of binding affinities against [^{3}H]QNB and [^{3}H]Oxo-M. Four muscarinic antagonists which have the 2,2-diphenylpropionate side chain at either the C_5 (5-endo or 5-exo) or the C_6 (6-endo or 6-exo) positions did not discriminate between the subtypes of muscarinic receptors. The 2,2-diphenylpropionate 5-endo substituted compound was the most potent, showing affinities between 4.23×10^{-10} and 1.18×10^{-9} M in rat heart, rat brain, and m_1- or m_3-transfected CHO cell membrane preparations. The rank order of ester potency was 5-endo > 5-exo > 6-endo > 6-exo. A molecular modeling study based on the pharmacophore developed for azaprophen was used to account for the relative potency of these antagonists. Six potential muscarinic agonists which have acetoxy groups in the C_5 or C_6 position with an *N*-methyl or *N*-benzyl substituent did not discriminate subtypes of muscarinic receptors and had affinities between 6.63×10^{-6} and 4.76×10^{-5} M in rat heart, rat brain, and m_1- or m_3-transfected CHO cell membrane preparations. *exo*-2-Methyl-5-acetoxy-2-azabicyclo[2.2.1]heptane was the most efficacious partial agonist.

Introduction

The cholinergic hypothesis[1,2] is a major neurochemical base for current Alzheimer's research. This has resulted in a renewed interest in the muscarinic receptor(s) field. As an extension of our study on the structure–activity relationship of muscarinic ligands,[3-5] we prepared the acetate and 2,2-diphenylpropionate esters of *endo*- and *exo*-2-alkyl-2-azabicyclo[2.2.1]heptan-5-ol (1 and 2) and



endo- and *exo*-2-alkyl-2-azabicyclo[2.2.1]heptan-6-ol (3 and 4). Radioligand binding affinities of these esters were determined in rat heart, rat brain, and m_1- or m_3-transfected CHO cell membrane preparations to examine the selectivity for subtypes of muscarinic receptor. A molecular modeling study was conducted to account for the relative potencies of the isomers. Muscarinic agonist efficacies of the acetate analogues were determined by the ratio of binding affinities against [^{3}H]QNB and [^{3}H]Oxo-M.[6,7]

The ratio of affinities of muscarinic ligands in displacing agonist and antagonist radioligands was introduced by Freedman et al.[6,7] as an index of efficacy at cerebral cortical receptors. A relationship between the index ([^{3}H]-NMS:[^{3}H]Oxo-M) and functional efficacy was indicated since high and low ratios corresponded to high and low phosphatidylinositol turnover, respectively.[1,2,8] In a previous publication,[4] we found the ratios [^{3}H]NMS:[^{3}H]-Oxo-M and [^{3}H]QNB:[^{3}H]Oxo-M for a ligand series to be not significantly different and to approximate literature data.[4]

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Results

Synthesis. The synthesis of the azabicyclic alcohols 6, 14, and 15 and the azabicyclo alcohol acetate 11 have been reported by Portoghese and co-workers.[9a,b] We investigated these methods for the synthesis of our target com-

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(6) Saunders, J.; Cassidy, M.; Freedman, S. B.; Harley, E. A.; Iversen, H. L.; Kneen, C.; Macleod, A. M.; Merchant, K. J.; Snow, R. J.; Baker, R. Novel Quiniclidine-Based Ligands for the Muscarinic Cholinergic Receptor. *J. Med. Chem.* 1990, 33, 1128–1138.
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(9) (a) Portoghese, P. S.; Sepp, D. T. 2-Azatricyclo[2.2.1.0.1.6]-heptane: Synthesis and Conversion to 2-Azabicyclo[2.2.1]-heptanes. *Tetrahedron* 1973, 29, 2253–2256. (b) Portoghese, P. S.; Lattin, D. L. Bicyclic Bases (1). Stereoselective Synthesis of exo and endo-N-Benzyl-6-hydroxy-2-azabicyclo-[2.2.1]heptane. *J. Heterocycl. Chem.* 1972, 9, 395–397.

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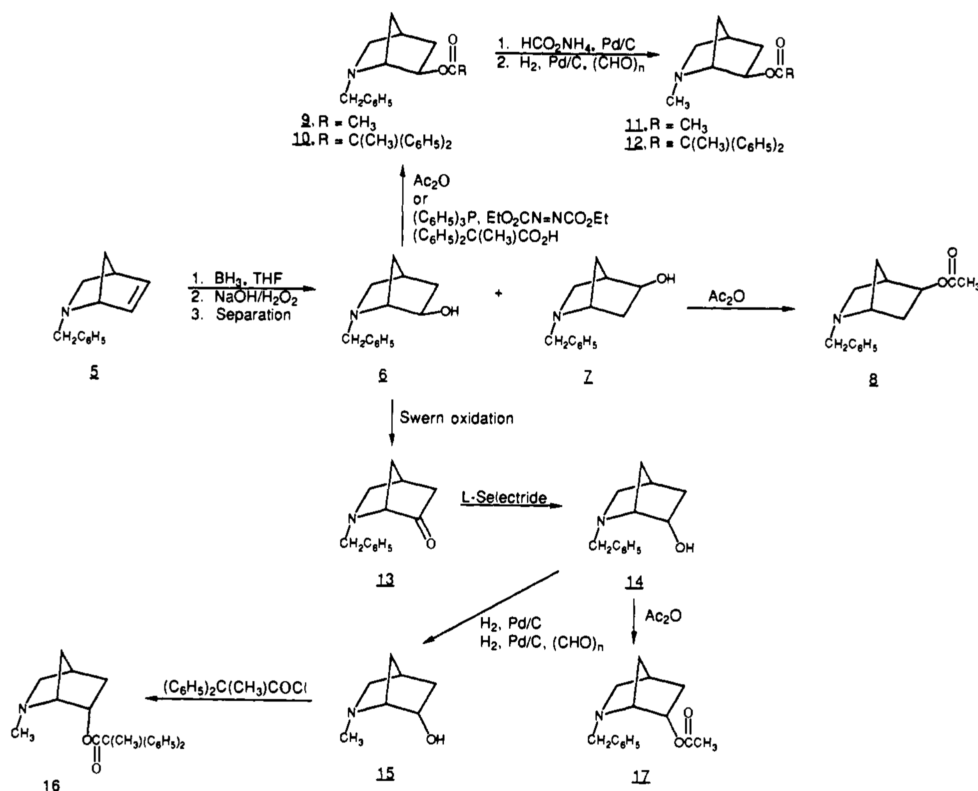
Scheme I



pounds but found the routes shown in Scheme I to be more convenient. Thus, hydroboration of 2-benzyl-2-azabicyclo[2.2.1]heptan-5-ene (**5**)[10] followed by a hydrogen peroxide oxidative workup gave the 6-*exo*-alcohol **6** with only traces of the 5-*exo*-alcohol **7**. Acylation of **6** and **7** with acetic anhydride gave **9** and **8**, respectively. Treatment of **6** with triphenylphosphine and diethyl azodicarboxylate and 2,2-diphenylpropionic acid in tetrahydrofuran gave *exo*-diphenylpropionate **10** as the sole product. The retention of configuration on Mitsunobu esterification of **6** was not expected; however, other Mitsunobu reactions have been reported to proceed with retention of configuration.[11] Catalytic transfer hydrogenation with ammonium formate[12] was used to remove the *N*-benzyl group from **9** and **10**. Catalytic reductive amination of the debenzylated intermediates using paraformaldehyde provided **11** and **12**.

endo-2-Methyl-2-azabicyclo[2.2.1]heptan-6-ol (**15**) was prepared by Swern[13] oxidation of **6** to ketone **13**, which on reduction with L-Selectride gave *endo*-alcohol **14**. Acetylation of **14** gave **17**. Catalytic debenzylation/reductive amination of **14** provided the desired *N*-methyl *endo*-alcohol **15**. Acylation of **15** with 2,2-diphenylpropionyl chloride gave **16**. Acetylation of **15** gave *endo*-2-methyl-2-azabicyclo[2.2.1]heptan-6-ol acetate. However, all attempts to prepare a crystalline salt were unsuccessful.

Compound **11** was identical to a sample prepared by the stereoselective route reported by Portoghese.[9a] This combined with a comparison of the NMR spectral properties of **11**, **6**, **14**, and **15**, given in the Experimental Section, to the NMR data reported by Portoghese[9a,b] established the correctness of the structures shown in Scheme I.

The synthesis used to prepare the 2-methyl-2-azabicyclo[2.2.1]heptan-5-ol esters is shown in Scheme II. *N*-

Scheme II

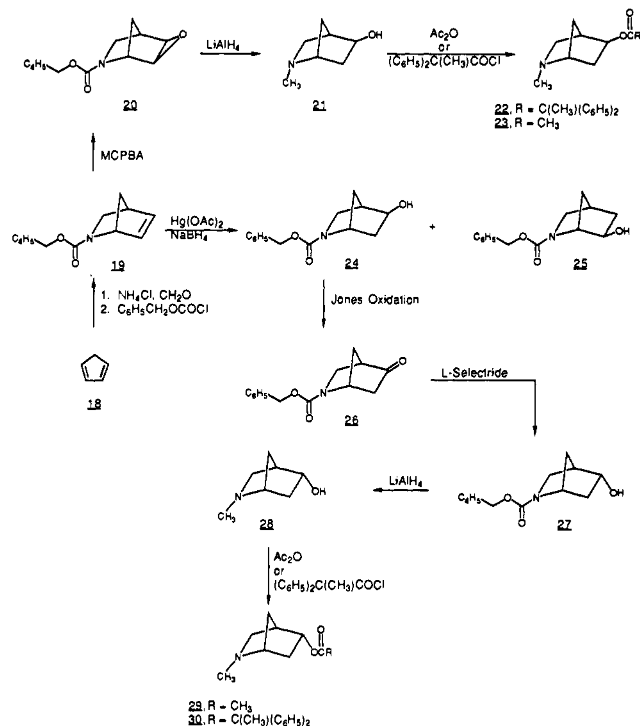


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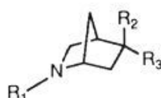
Table I. Selected ^{1}H-NMR Data[a]



compd	R_1	R_2	R_3	C5-hydrogen δ	C5-hydrogen $J_{5,6}$ Hz ($W_{1/2}$)
21	CH_3	OH	H	3.81 (dm)	7.04 (13)
28	CH_3	H	OH	4.28 (brm)	(24)
24	$C_6H_5CH_2OC(O)$	OH	H	3.91 (dm)	9.00 (12)
27	$C_6H_5CH_2OC(O)$	H	OH	4.21 (brm)	(23)
exo-**31**[b]	$pCH_3C_6H_4SO_2$	OH	H	3.87 (dm)	7.00 (13)
endo-**31**[b]	$pCH_3C_6H_4SO_2$	H	OH	4.18 (brm)	(21)

[a] All experimental chemical shifts were obtained in $CDCl_3$ solution and are expressed in parts per million downfield from TMS. Multiplicities are denoted by dm (doublet of multiplet) or brm (broad multiplet). [b] Taken from ref 14.

(Carbobenzyloxy)-2-azabicyclo[2.2.1]heptan-5-ene (**19**) was prepared by a hetero-Diels–Alder reaction of cyclopentadiene (**18**) and the iminium ion prepared from ammonium chloride and formaldehyde to give *N*-norazabicyclo[2.2.1]heptan-5-ene.[10] Without isolation this intermediate was converted to the desired **19** by acylation with benzyl chloroformate. Oxidation of **19** with *m*-chloroperoxybenzoic acid gave the epoxide **20**, which yielded 5-*exo*-alcohol **21** on reduction with lithium aluminum hydride. Acylation of **21** with 2,2-diphenylpropionyl chloride or acetic anhydride gave **22** and **23**, respectively.

If **19** were subjected to oxymercuration followed by sodium borohydride reduction, a 2:1 mixture of 5-*exo*- and 6-*exo*-alcohols **24** and **25** was obtained which could be separated by flash chromatography. Jones oxidation of **24** gave **26**, which on reduction with L-Selectride provided **27**. Lithium aluminum hydride reduction of **27** gave the *N*-methyl 5-*endo*-alcohol **28**. Esterification of **28** with acetic anhydride or diphenylpropionyl chloride gave **29** and **30**, respectively.

The structural assignment of the esters shown in Scheme II was based on their elemental analysis and NMR spectral properties. Since they were shown to be isomeric with the esters prepared in Scheme I, the ester function must be connected to the azabicyclo[2.2.1]heptane ring at the 5-position. The 5-endo and 5-exo assignments were based on a comparison of the chemical shift and coupling constant or $W_{1/2}$ of the 5-proton of compounds **21**, **24**, **27**, and **28** to those of *exo*- and *endo*-*N*-tosyl-5-hydroxy-2-azabicyclo[2.2.1]heptane (**31**), which had been previously characterized by Portoghese and co-workers.[14] The 5-endo and 5-exo spectral data are summarized in Table I. The chemical shifts of 3.81 and 3.91 ppm for **21** and **24**, respectively, are very close to the 3.87 ppm reported for *exo*-**31**.[14] The 4.18 ppm value reported[14] for *endo*-**31** is almost identical to the 4.28 and 4.21 ppm observed for **28** and **27**, respectively. In addition, the coupling constants or $W_{1/2}$ values for **21** and **24** were similar to those of *exo*-**31** whereas the values for **28** and **27** were like those of *endo*-**31**.[14]

Biological. Competition against [^{3}H]QNB. Table II presents the potency of four muscarinic antagonists for the inhibition of binding of [^{3}H]QNB in rat heart and m_1- or m_3-transfected CHO cell line membrane preparations.

(14) Portoghese, P. S.; Lattin, D. L.; Telang, V. G. Bicyclic Bases (1, 2). Synthesis and Stereochemical Studies of Chiral 5-Substituted N-Tosyl-2-azabicyclo[2.2.1]heptane. *J. Heterocycl. Chem.* **1971**, *8*, 993–998.

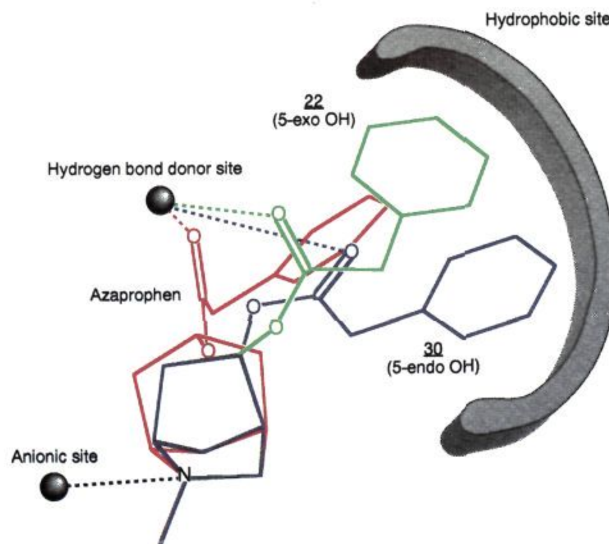


Figure 1. Schematic drawing of the alignment of azaprophen, compound **22**, and compound **30** to the proposed muscarinic pharmacophore. All protons, the methyl, and one phenyl group of the ester side chain are not shown for clarity.

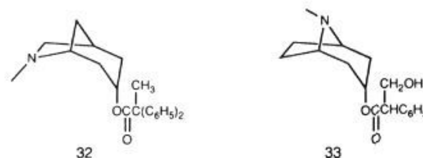
These antagonists did not discriminate between the subtypes of muscarinic receptors. The 2-methyl-2-azabicyclo[2.2.1]heptan-5-ol esters **22** and **30** were more potent than 6-ol esters **12** and **16**. In addition, 5-*endo*-ester **30** is about 2 times more potent than 5-*exo*-ester **22** in heart and m_1 cell lines but shows similar affinity to the m_3 receptor. 6-*endo*-Ester **16** is about 2–3 times more potent than the 6-*exo*-ester in all systems. The rank order of potency was 5-*endo*-ester > 5-*exo*-ester > 6-*endo*-ester > 6-*exo*-ester in all systems.

Table III presents the binding affinities of six putative muscarinic agonists in rat heart, rat brain, and m_1- or m_3-transfected CHO cell membrane preparations. 2-methyl- and 2-benzyl-2-azabicyclo[2.2.1]heptan-5-ol and 6-ol acetates **8**, **9**, **11**, **17**, **23**, and **29** did not discriminate between subtypes of muscarinic receptors, showing affinities between 6.63×10^{-6} and 4.76×10^{-5} M.

Competition against [^{3}H]QNB and [^{3}H]Oxo-M. Table IV presents the binding affinities of six potential agonists against [^{3}H]QNB and [^{3}H]Oxo-M in rat brain membrane preparations. Compound **23** shows the highest [^{3}H]QNB:[^{3}H]Oxo-M ratio of 42.78. The other acetates show ratios of below 10.

Discussion

The 6-methyl-6-azabicyclo[3.2.1]octane head group of azaprophen (**32**) is isomeric with the 8-methyl-8-azabicyclo[3.2.1]octane head group of atropine (**33**). We recently



described a pharmacophore model to show how both compounds could bind to the same receptor site.[3–5] In order to gain additional information on this pharmacophore model, we prepared the diphenylpropionate esters **12**, **16**, **22**, and **30**. Ester **30** which has an *endo*-2-methyl-2-azabicyclo[2.2.1]heptan-5-ol head group exhibits the greatest similarity to azaprophen. Esters **12** and **16** have the 2-methyl-2-azabicyclo[2.2.1]heptan-6-ol head

Table II. Inhibition of [^{3}H]QNB Binding by Muscarinic Antagonists in Rat Heart and m_1 CHO and m_3 CHO Cell Membrane Preparations

	heart			m_1 CHO			m_3 CHO		
compd	K_I	n_H	n	K_I	n_H	n	K_I	n_H	n
30	$1.18 \pm 0.06 \times 10^{-9}$	0.94	5	$4.23 \pm 1.30 \times 10^{-10}$	0.99	5	$5.22 \pm 0.90 \times 10^{-10}$	0.92	5
22	$4.07 \pm 0.45 \times 10^{-9}$	0.95	4	$8.76 \pm 1.90 \times 10^{-10}$	1.11	5	$5.33 \pm 0.86 \times 10^{-10}$	0.96	6
12	$2.01 \pm 0.28 \times 10^{-8}$	1.02	5	$5.31 \pm 1.77 \times 10^{-9}$	0.94	5	$8.33 \pm 1.60 \times 10^{-9}$	1.00	4
16	$6.15 \pm 2.99 \times 10^{-9}$	1.08	5	$2.20 \pm 0.58 \times 10^{-9}$	1.05	5	$4.03 \pm 0.90 \times 10^{-9}$	0.95	4

Table III. Inhibition of [^{3}H]QNB Binding by Potential Muscarinic Agonists in Rat Heart, Rat Brain, and m_1 CHO and m_3 CHO Cell Membrane Preparations

	heart			brain			m_1 CHO			m_3 CHO		
compd	K_I	n_H	n	K_I	n_H	n	K_I	n_H	n	K_I	n_H	n
11	$2.49 \pm 0.11 \times 10^{-5}$	0.87	4	$1.75 \pm 0.13 \times 10^{-5}$	0.86	4	$2.16 \pm 0.23 \times 10^{-5}$	1.00	4	$2.80 \pm 0.73 \times 10^{-5}$	1.00	4
17	$1.51 \pm 0.20 \times 10^{-5}$	0.85	4	$6.63 \pm 0.64 \times 10^{-6}$	0.93	4	$7.01 \pm 1.96 \times 10^{-6}$	0.90	4	$1.88 \pm 0.11 \times 10^{-5}$	0.96	4
9	$1.53 \pm 0.05 \times 10^{-5}$	1.04	4	$2.00 \pm 0.04 \times 10^{-5}$	0.93	4	$2.26 \pm 0.37 \times 10^{-5}$	0.93	4	$4.76 \pm 0.92 \times 10^{-5}$	0.85	4
29	$1.74 \pm 0.34 \times 10^{-5}$	0.94	5	$1.15 \pm 0.27 \times 10^{-5}$	0.98	4	$2.19 \pm 0.77 \times 10^{-5}$	0.87	5	$2.77 \pm 0.52 \times 10^{-5}$	1.00	4
23	$1.01 \pm 0.27 \times 10^{-5}$	0.74	4	$9.84 \pm 1.50 \times 10^{-6}$	0.79	4	$1.75 \pm 0.13 \times 10^{-5}$	0.69	3	$1.20 \pm 0.20 \times 10^{-5}$	0.75	4
8	$1.03 \pm 0.09 \times 10^{-5}$	0.92	4	$1.48 \pm 0.25 \times 10^{-5}$	0.90	4	$1.89 \pm 0.21 \times 10^{-5}$	0.84	3	$2.69 \pm 0.14 \times 10^{-5}$	0.82	4

Table IV. Inhibition of [^{3}H]Oxo-M or [^{3}H]QNB Binding by Potential Muscarinic Agonists in Rat Brain Membrane Preparations

	[^{3}H]Oxo-M			[^{3}H]QNB			
compounds	K_I	n_H	n	K_I	n_H	n	QNB/Oxo-M
atropine	$4.59 \pm 1.65 \times 10^{-10}$	0.92	3	$3.37 \pm 0.48 \times 10^{-10}$	1.00	4	0.73
carbachol	$1.31 \pm 0.16 \times 10^{-8}$	1.11	4	$1.88 \pm 0.32 \times 10^{-5}$	0.56	4	1435.11
pilocarpine	$8.29 \pm 3.35 \times 10^{-8}$	0.95	4	$1.01 \pm 0.16 \times 10^{-6}$	0.95	5	12.18
11	$5.37 \pm 1.56 \times 10^{-6}$	1.05	4	$1.75 \pm 0.13 \times 10^{-5}$	0.86	4	3.26
17	$7.21 \pm 0.83 \times 10^{-6}$	0.90	4	$6.63 \pm 0.64 \times 10^{-6}$	0.93	4	0.92
9	$2.57 \pm 0.43 \times 10^{-6}$	1.03	5	$2.00 \pm 0.04 \times 10^{-5}$	0.93	4	7.78
29	$1.43 \pm 0.22 \times 10^{-6}$	1.04	5	$1.15 \pm 0.27 \times 10^{-5}$	0.98	4	8.04
23	$2.30 \pm 0.13 \times 10^{-7}$	1.09	4	$9.84 \pm 1.50 \times 10^{-6}$	0.79	4	42.78
8	$1.56 \pm 0.22 \times 10^{-6}$	0.97	4	$1.48 \pm 0.25 \times 10^{-5}$	0.90	4	8.97

group, which contains the acetyl choline fragment N–C–C–O locked in a rigid framework.

An examination of the data in Table II reveals a striking rank order of potency in the four closely related compounds **30** > **22** > **16** > **12** (5-endo > 5-exo > 6-endo > 6-exo). A molecular modeling/mechanics study was undertaken to provide a structural rationale for the relative activities of these four compounds. The rigid ring systems and fixed hydroxyl epimers of this series of compounds made them well-suited for a molecular modeling study. Previous modeling[3,4] suggested that the muscarinic pharmacophore consists of three primary features: an anionic site (most likely a carboxylate), a hydrogen-bond donor site, and a hydrophobic site. The relative position of the anionic site can be used to explain the observed enantio- and regioselectivity of the interaction of azaprophen and atropine ring systems with this receptor.[4] Initial molecular modeling studies also suggested that a 2-azabicyclo[2.2.1]heptane ring system could also provide a suitable backbone for compounds with significant muscarinic activity.

Modeling the azaprophen and 2-azabicyclo[2.2.1]heptane ring systems suggested the muscarinic antagonist pharmacophore model illustrated in Figure 1. The first alignment rule of this pharmacophore requires that the rings of the [3.2.1] and [2.2.1] systems be arranged so as to permit interaction of the nitrogen atom with the receptor anionic site. A position for a hydrogen-bond donor that could interact with the carbonyl oxygen of the ester side chains of these compounds was then located by performing conformational searches on the three compounds and recording the location of the carbonyl oxygen atom and oxygen lone pair vectors. A hydrogen-bond donor site was identified by this method that would permit an efficient hydrogen-bonding interaction with both low-energy conformations of azaprophen and the 5-endo-OH compound **30**. This potential hydrogen-bond donor site is shown in Figure 1. A low-energy conformer of the 5-exo compound **22** also can hydrogen bond with a donor atom at this site, but the angle and distance are consistent with a somewhat weaker hydrogen bond for this epimer.

With the hydrogen-bonding conformers of azaprophen, **30**, and **22** aligned to the anionic site and hydrogen-bonding donor site, it can be seen that the lipophilic side chains of each compound are shifted to a common region. This result suggests the location of a third lipophilic binding site within the muscarinic receptor.

The three-site muscarinic pharmacophore model described above can be applied to explain the observed rank order of potency of the 2-azabicyclo[2.2.1]heptane compounds. First, low-energy conformations of azaprophen and **30** can easily interact with all three sites. Compound **22** can also interact with all three sites, but this requires a higher energy conformation, and the geometry of the carbonyl oxygen to hydrogen-bond-donor site is not ideal. The 6-substituted series of compounds (**12** and **16**) can interact with the anionic and hydrogen-bonding sites in a fashion similar to that of the 5-substituted compounds with the exception that the lipophilic side chains on the 6-substituted compounds will be on the side opposite from the proposed lipophilic site. Alternatively, interaction with the lipophilic site is possible for 6-substituted compounds at the cost of adopting higher energy conformations and the loss of the hydrogen-bond interaction. In either mode of binding the 6-substituted compounds would be expected to bind less efficiently to the muscarinic receptor.

In summary, we have developed convenient synthesis of the exo and endo isomers of both 2-azabicyclo[2.2.1]-heptan-5-ol and 2-azabicyclo[2.2.1]heptan-6-ol. The 2,2-diphenylpropionate 5-endo analog, which fits the azapro-

phen pharmacophore better than the other three isomers, was the most potent muscarinic antagonist. The acetates of all four alcohols were weak agonists.

Experimental Section

Synthesis. Melting points were determined on a Thomas Hoover capillary tube apparatus. NMR spectra were recorded on a Bruker WM-250 spectrometer using tetramethylsilane as an internal standard, and J values are in hertz. High resolution mass spectra were obtained on a VG Analytical ZAB E spectrometer. Thin-layer chromatography was carried out on Whatman silica gel 60 TLC plates using $CHCl_3$–MeOH–concentrated NH_4OH (40:9:1) unless otherwise noted. Visualization was accomplished under UV or in an iodine chamber. For column chromatography, a 230–400 mesh silica gel was used. Microanalyses were carried out by Atlantic Microlab, Inc.

***exo*-2-Benzyl-2-azabicyclo[2.2.1]heptan-6-ol (6) and *exo*-2-Benzyl-2-azabicyclo[2.2.1]heptan-5-ol (7).** To a stirred solution of 2-benzyl-2-azabicyclo[2.2.1]hept-5-ene[10] (5, 10.2 g, 0.058 mol) in 115 mL of dry THF at 0 °C under an atmosphere of N_2 was added dropwise a 1 M solution of BH_3·THF complex (115 mL, 0.115 mol), and stirring was continued for 2 h at 0–10 °C. The excess BH_3 was destroyed by careful addition of THF–H_2O. A 3 N NaOH solution (15 mL) was added followed by dropwise addition of 50% hydrogen peroxide (15 mL), and the mixture was kept at 35–40 °C for 1.5 h with stirring. After cooling to room temperature, 5 g of potassium carbonate was added, and the THF was removed under reduced pressure. The remaining solution was extracted with methylene chloride, and the extracts were washed with water. The organic extract was dried with anhydrous sodium sulfate, filtered, and concentrated to give 12 g of crude product. Flash column chromatography, eluting with CH_2Cl_2–$CHCl_3$–MeOH–NH_4OH (150:40:9:1) gave 8.1 g (69%) of 6: TLC (CH_2Cl_2–$CHCl_3$–MeOH–NH_4OH, 50:40:9:1) R_f 0.4; ^{1}H NMR (free amine) (250 MHz, $CDCl_3$) δ 1.27–1.35 (m, 1), 1.6 (m, 2), 1.77–1.85 (m, 2), 2.3 (d, J = 9.0, 1), 2.3 (m, 1), 2.46–2.52 (m, 1), 3.0 (m, 1), 3.67 (s, 2), 4.06 (m, 1), 7.18–7.35 (m, 5).

Compound 6 was characterized as its hydrochloride salt: mp 164–165 °C; ^{1}H NMR (CD_3OD) (HCl salt) δ 1.53–1.58 (m, 1), 1.87–2.09 (m, 3), 2.73–2.9 (m, 2), 3.27–3.39 (m, 1), 3.71 (s, 1), 4.18 (s, 1), 4.24 (s, 2), 4.51–4.54 (m, 1), 7.44–7.66 (m, 5). Anal. Calcd for $C_{13}H_{18}ClNO$: C, H, N.

Further elution of the column gave compound 7: TLC CH_2Cl_2–$CHCl_3$–CH_3OH–NH_4OH (50:40:9:1) R_f 0.27; ^{1}H NMR (250 MHz) δ 1.15–1.25 (m, 1), 1.6 (m, 2), 1.95–1.99 (d, J = 9.8, 1), 2.1–2.3 (m, 2), 2.73 (dd, J = 10, 4.3, 1), 3.2 (m, 1), 3.55 (s, 2), 3.6–3.68 (m, 1), 7.17–7.38 (m, 5).

2-Benzyl-2-azabicyclo[2.2.1]heptan-6-one (13). To a stirred solution of DMSO (7.5 g, 0.0966 mol) in 50 mL of CH_2Cl_2, was added dropwise a solution of trifluoroacetic anhydride (15.21 g, 0.072 mol) in 30 mL of CH_2Cl_2 at −65 °C. After 10 min a solution of amino alcohol 6 (9.8 g, 0.0483 mol) in 30 mL of CH_2Cl_2 was added dropwise and stirred for 30 min at that temperature. To this reaction mixture was added Et_3N (40 mL), and the mixture was stirred for 1 h while being allowed to slowly warm to room temperature. The reaction was quenched with 200 mL of H_2O and extracted with CH_2Cl_2 (3 × 500 mL). The organic extract was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give 9 g of crude product. Purification by flash column chromatography, eluting with 30% EtOAc in hexane, gave 6 g (80%) of amino ketone 13: TLC (CH_2Cl_2–$CHCl_3$–CH_3OH–NH_4OH, 50:40:9:1) R_f 0.78; ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.9–2.3 (m, 5), 2.66 (m, 1), 3.18–3.8 (m, 4), 7.2–7.4 (m, 5).

This product was used in the next step without further purification.

***endo*-2-Benzyl-2-azabicyclo[2.2.1]heptan-6-ol (14).** Amino ketone 13 (5.7 g, 0.028 mol) was dissolved in 140 mL of dry THF and cooled to −78 °C. To this solution was added a 1 M solution of L-Selectride (71 mL, 0.071 mol) dropwise. The reaction was stirred at that temperature for 3 h. The reaction mixture was warmed to 0 °C and quenched with 3 N NaOH (30 mL) followed by 30% H_2O_2 (15 mL). After stirring at room temperature for 2 h, the reaction mixture was concentrated in vacuo to remove THF and extracted with CH_2Cl_2. The organic extract was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give 6 g of crude product. The crude product was purified by flash chromatography, eluting with CH_2Cl_2–$CHCl_3$–MeOH–NH_4OH (150:40:9:1) in CH_2Cl_2 to give 5.4 g (95%) of amino alcohol 14: TLC (CH_2Cl_2–$CHCl_3$–CH_3OH–NH_4OH, 50:40:9:1) R_f 0.5; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.0 (dt, 1, J = 13, 3), 1.3 (d, 1, J = 10.4), 1.6–1.7 (m, 1), 1.77–1.88 (m, 1), 2.36–2.42 (m, 2), 2.77 (d, 1, J = 9), 3.1 (m, 1), 3.61 (d, 1, J = 13), 3.79 (d, 1, J = 13), 3.98 (dt, 1, J = 9.4, 3), 7.2–7.36 (m, 5).

The hydrochloride salt was recrystallized from methanol–ether: mp 175–176 °C. Anal. Calcd for $C_{15}H_{17}ClNO$·0.75H_2O: C, H, N.

***endo*-2-Methyl-2-azabicyclo[2.2.1]heptan-6-ol (15).** To a solution of amino alcohol 14 (7 g, 0.035 mol) in 150 mL of methanol was added 2.2 g of 10% Pd/C under a nitrogen atmosphere, followed by ammonium formate (11 g). The resulting mixture was refluxed for 1 h, cooled to room temperature, filtered through Celite, and concentrated to 50 mL. To this concentrated solution was added 1.5 g of 10% Pd/C and paraformaldehyde (10 g). The resulting mixture was stirred under H_2 atmosphere for 12 h, filtered through Celite and concentrated to give 4 g of crude product. Purification by flash column chromatography, eluting with $CHCl_3$–CH_3OH–NH_4OH (40:9:1) gave 4.0 g (91%, two steps) of amino alcohol 15: TLC ($CHCl_3$–CH_3OH–NH_4OH, 40:9:1) R_f 0.3; ^{1}H NMR (free amine) (90 MHz, $CDCl_3$) δ 0.9–1.1 (m, 1), 1.2–1.35 (m, 1), 1.5–2.0 (m, 3), 2.2–2.4 (m, 1), 2.39 (s, 3), 2.8–3.0 (m, 2), 3.9–4.2 (m, 2); ^{1}H NMR (fumarate, gummy solid) (60 MHz, CD_3OD) δ 1.2–1.4 (m, 1), 1.9–2.35 (m, 3), 2.7–2.85 (m, 1), 2.9 (s, 3), 2.9–3.15 (m, 1), 3.35–3.65 (m, 1), 3.8–3.9 (m, 1), 4.3–4.55 (m, 1), 6.7 (s, 2).

This product was used to prepare esters 16 without further purification.

2-(Carbobenzyloxy)-2-azabicyclo[2.2.1]hept-5-ene (19). Cyclopentadiene (120 g, 1.82 mol), ammonium chloride (291.8 g, 5.46 mol) in 1 L of H_2O, and 37% formaldehyde solution (221 mL, 2.73 mol) were mixed together. The resulting heterogeneous mixture was stirred at room temperature for 36 h. The reaction mixture was then neutralized with solid Na_2CO_3 and cooled to 0 °C. To this mixture were added benzyl chloroformate (300 g, 1.76 mol) and a solution of Na_2CO_3 in 1 L of H_2O with mechanical stirring at such a rate that the addition of Na_2CO_3 was completed just after that of benzyl chloroformate. After the addition was completed, the stirring was continued for 2 h at 0 °C. The reaction mixture was diluted with 1 L of H_2O and extracted with CH_2Cl_2 (4 × 1 L). The organic extract was dried over anhydrous Na_2SO_4, filtered, and concentrated to give 360 g of crude product which was purified in small amounts as needed. Thus, 30 g of the crude product was purified by flash chromatography, eluting with 1:1 ethyl acetate–hexane, to give 22.5 g (65%) of pure 19: TLC (ethyl acetate–hexane, 1:1) R_f 0.6; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.53–1.58 (m, 2), 2.6–2.72 (m, 1), 3.19–3.2 (m, 1), 3.36–3.41 (m, 1), 4.61–4.8 (m, 1), 5.11–5.25 (m, 2), 6.27–6.38 (m, 2), 7.25–7.39 (m, 5).

This product was used in the next step without further purification.

2-(Carbobenzyloxy)-5,6-epoxy-2-azabicyclo[2.2.1]heptane (20). To carbamate 19 (22 g, 0.096 mol) in 300 mL of CH_2Cl_2 at 0 °C was added MCPBA (19.9 g, 0.115 mol) in one portion, and the resulting mixture was stirred for 2 days at room temperature. At the end of the stirring, the reaction mixture was filtered and concentrated under reduced pressure to about 200 mL. The concentrated reaction mixture was diluted with 600 mL of ether and washed with 3 N NaOH (3 × 200 mL). The organic extract was dried over anhydrous Na_2SO_4, filtered, and concentrated to give 22 g (93%) of epoxide 20. The product was used for the next reaction without purification: TLC (ethyl acetate–hexane, 1:1) R_f 0.47; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.1–1.17 (m, 1), 1.6–1.7 (m, 1), 2.78–2.79 (m, 1), 3.0–3.1 (m, 1), 3.22–3.45 (m, 3), 4.07–4.17 (m, 1), 5.0–5.2 (m, 2), 7.32–7.35 (m, 5).

***exo*-2-Methyl-2-azabicyclo[2.2.1]heptan-5-ol (21).** To a slurry of LAH (13.6 g, 0.359 mol) in 350 mL of dry THF was added epoxy carbamate 20 (22 g, 0.09 mol) with stirring and external cooling. After the addition was completed, the reaction mixture was stirred at room temperature for 4 h and then refluxed overnight. The reaction mixture was cooled to 0 °C, diluted with 300 mL of ether, and quenched by dropwise addition of 13.6 mL of H_2O, 13.6 mL of 15% NaOH, and 40 mL of H_2O in that order. After stirring for 1 h, the mixture was filtered, dried (Na_2SO_4), and concentrated. The crude product thus obtained was purified

by flash chromatography, eluting with CH_2Cl_2–$CHCl_3$–CH_3OH–NH_4OH (150:40:9:1) followed by $CHCl_3$–CH_3OH–NH_4OH (40:9:1) to give 4.5 g (39%) of **21**: TLC ($CHCl_3$–CH_3OH–NH_4OH, 40:9:1) R_f 0.075; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.2–1.3 (m, 1), 1.57–1.72 (m, 2), 1.94 (d, 1, J = 10), 2.12–2.3 (m, 5), 2.7 (dd, 1, J = 10, 4.3), 3.1 (m, 1), 3.65 (m, OH), 3.8–3.82 (m, 1).

This product was used to prepare esters **22** and **23** without further purification.

***exo*-2-(Carbobenzyloxy)-2-azabicyclo[2.2.1]heptan-5-ol (24).** To mercuric acetate (40.4 g, 0.127 mol) in 127 mL of water was added 100 mL of THF. The yellow suspension thus formed was cooled to 0 °C, and carbamate **19** (29 g, 0.127 mol) was added dropwise. After the addition was over, the reaction mixture was allowed to warm to room temperature and stirred for 12 h. After cooling to 0 °C, 127 mL of 3 N NaOH followed by 127 mL of 0.5 M $NaBH_4$ in 3 N NaOH was added to the mixture. The mercury was allowed to settle, and the supernatant liquid decanted and extracted with ether. The ether extract was dried over anhydrous Na_2SO_4 and concentrated to give the crude product which contained a mixture of 5-*exo*- and 6-*exo*-alcohols (**24** and **25**, respectively) in 2:1 ratio. Purification by flash chromatography eluting with 50% ethyl acetate in hexane gave 8 g of 6-*exo*-alcohol **25** in the first fraction, a mixture of 5-*exo*- and 6-*exo*-alcohols in the second fraction, and 17 g of 5-*exo*-alcohol **24** in the final fraction (combined yield 86%): TLC (EtOAc–hexane, 1:1) R_f 0.2; ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.25–2.2 (m, 2), 2.31–2.45 (m, 1), 2.65–2.9 (m, 2), 3.1–3.33 (m, 1), 3.85–4.0 (m, 1), 4.15–4.35 (m, 1), 5.1 (s, 1), 7.2–7.3 (m, 5).

Amino alcohol **24** was used to prepare ketone **26** without further purification.

2-(Carbobenzyloxy)-2-azabicyclo[2.2.1]heptan-5-one (26). Carbamate alcohol **24** (16.5 g, 0.067 mol) was dissolved in 300 mL of acetone and cooled to 0 °C. Jones reagent (80 mL) was added to the mixture dropwise until an orange color persisted. The reaction mixture was warmed to room temperature, stirred for 3 h, cooled to 0 °C, made basic with saturated $NaHCO_3$, and extracted with ether (3 × 300 mL). The ethereal extract was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give 15 g of the crude product. Purification by flash column chromatography, eluting with 50% ethyl acetate in hexane gave 10.2 g (62% yield) of the carbamate ketone (**26**): TLC (EtOAc–hexane, 1:1) R_f 0.33; ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.9–2.35 (m, 4), 2.9 (m, 1), 3.35–3.6 (m, 2), 4.65–4.75 (m, 1), 5.15 (m, 2), 7.4 (s, 5). Anal. Calcd for $C_{14}H_{15}NO_3$: C, H, N.

***endo*-2-(Carbobenzyloxy)-2-azabicyclo[2.2.1]heptan-5-ol (27).** Carbamate ketone **26** (10.2 g, 0.0416 mol) was dissolved in 80 mL of dry THF and cooled to −78 °C. To this solution was added a 1 M solution of L-Selectride (62 mL, 0.062 mol) dropwise and then stirred at that temperature for 3 h. The reaction mixture was warmed to 0 °C and quenched with 3 N NaOH (40 mL) followed by 30% H_2O_2 (20 mL), and stirred at room temperature for 1.5 h. The reaction mixture was saturated with NaCl and extracted with ether. The ethereal extract was dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to give 12 g of product **27**: TLC (EtOAc–hexane, 1:1) R_f 0.11; ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.3–1.7 (m, 3), 1.8–2.15 (m, 1), 2.5–2.65 (m, 1), 3.0–3.3 (m, 2), 3.77 (d, 1, J = 10.5), 4.1–4.5 (m, 2), 5.1 (s, 2), 7.2–7.4 (m, 5).

This product was used in the next step without further purification.

***endo*-2-Methyl-2-azabicyclo[2.2.1]heptan-5-ol (28).** To a slurry of LAH (4.74 g, 0.125 mol) in 150 mL of dry THF cooled to 0 °C was added dropwise carbamate alcohol **27** (12 g, 0.0416 mol) in 100 mL of THF. After the addition was over, the reaction mixture was warmed to room temperature and stirred for 5 h, diluted with ether, cooled to 0 °C, and quenched with 4.75 mL of H_2O, 4.75 mL of 3 N NaOH, and 14.5 mL of H_2O in that order. The resulting mixture was stirred at room temperature for 30 min, filtered, dried over anhydrous Na_2SO_4, and concentrated to give 8 g of crude product. Purification by flash column chromatography, eluting with $CHCl_3$–MeOH–NH_4OH (40:9:1), gave 4.2 g (80%) of amino alcohol **28**.

The hydrochloride salt was prepared by treatment with HCl–ether followed by recrystallization from methanol–ethyl acetate: mp >200 °C (dec); TLC ($CHCl_3$–CH_3OH–NH_4OH, 40:9:1) R_f 0.07; ^{1}H NMR (90 MHz) δ 1.3–2.0 (m, 4), 2.3–2.6 (m, 2), 2.33 (s, 3), 3.0–3.15 (m, 2), 3.33 (br s, 1), 4.1–4.3 (m, 1). Anal. Calcd for $C_7H_{14}ClNO$: C, H, N.

Preparation of Esters. General Procedure A. A mixture of the corresponding amino alcohol (1.2 mmol), triethylamine (2.4 mmol), DMAP (catalytic amount), and acetic anhydride (1.8 mmol) or 2,2-diphenylpropionyl chloride (1.8 mmol) in CH_2Cl_2 (5 mL) was stirred at room temperature overnight. The reaction mixture was quenched with saturated $NaHCO_3$ (5 mL), stirred for 0.5 h, extracted with CH_2Cl_2 (3 × 50 mL), and dried (Na_2SO_4). The residue, after removal of the solvent, was purified on a silica gel column using CH_2Cl_2–$CHCl_3$–CH_3OH–NH_4OH (150:40:9:1) as eluent to give the pure esters. Analytical samples were prepared as appropriate acid salts. The physical characteristics are reported in Table V.

Method B. A solution of the appropriate *N*-benzyl amino esters in MeOH (10 mL/mmol of amino ester) with ammonium formate (5 mmol/mmol of amino ester) and Pd/C (150–200 mg/mmol of amino ester) was gently heated to reflux for 1 h. The cooled mixture was filtered through a Celite pad. Paraformaldehyde (9–10 mmol/mmol of amino ester) and Pd/C catalyst (150–220 mg/mmol) were added to the filtrate, and hydrogenation was continued overnight. The catalyst was removed by filtration through Celite, the filtrate was concentrated, and the residue was purified by column chromatography. Elution with a solvent system of CH_2Cl_2–$CHCl_3$–CH_3OH–NH_4OH (150:40:9:1) gave the *N*-methyl acetates. The physical characteristics are listed in Table V.

***exo*-2-Benzyl-2-azabicyclo[2.2.1]heptan-6-ol 2,2-Diphenylpropionate (Method C).** To a mixture of diethyl azodicarboxylate (5.06 g, 0.029 mol) and 2,2-diphenylpropionic acid (6.58 g, 0.029 mol) in THF (30 mL) was added dropwise a solution of amino alcohol **6** (5.9 g, 0.029 mol) and triphenylphosphine (7.62 g, 0.029 mol) in THF (60 mL) and stirred for 48 h at room temperature. The mixture was diluted with ether (100 mL), and the precipitate that formed was filtered off. The filtrate was concentrated and purified on a silica gel column. Elution with CH_2Cl_2–$CHCl_3$–CH_3OH–NH_4OH (150:40:9:1) gave 4.5 g (38%) of **10**. The physical characteristics are listed in Table V.

Cell Cultures. Transfected Chinese hamster ovary cell (CHO) lines specifically expressing rat m_1 or m_3 muscarinic receptor subtypes were employed.[15] Cells were cultured in monolayers in 100 mm culture dishes containing 90% medium nutrient mixtures F-12 (Ham's) 10% fetal bovine serum, 2 mM L-glutamine, 50 μunits/mL and 50 μg/mL streptomycin, in a humidified atmosphere of 95% O_2–5% CO_2. Geneticin (50 μg/mL) was added to the medium. Cells were subcultured from 1:5 to 1:10 and used when confluent by day 2 or 3.

Preparation of Tissues and Cell Cultures for Radioligand Binding. Microsomal fractions of rat heart and rat brain were prepared as described previously by our laboratory.[4,16] In brief, male Sprague–Dawley rats (weight 250–300 g) were killed by decapitation. Rat heart and brain minus brain stem and cerebellum were removed. Rat brain portions were minced with scissors in 15 volume/g wet weight of ice-cold Tris (pH 7.2, 25 °C) buffer and homogenized with 10 passes of a motor-driven (TRI-R-Stirrer) glass–Teflon homogenizer at setting 7. Rat heart homogenate was prepared in a similar fashion, save that it was first homogenized in a Brinkman Polytron at setting 7 for 5 s. The m_1- or m_3-receptor-expressing CHO cell lines were suspended in ice-cold 50 mM Tris buffer and homogenized with a motor-driven glass–Teflon homogenizer at setting 5 with 10 passes.

The homogenates were centrifuged at 1100*g* for 20 min, and the supernatant was recentrifuged at 45000*g* for 45 min at 4 °C. The resultant pellets were homogenized in 50 mM ice-cold Tris buffer at a concentration of 20–30 μg, 120–150 μg, 5–8 μg, and 10–15 μg per 5 mL binding assay volume for brain, heart, and m_1-

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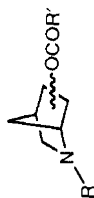
(16) Jim, K.; Bolger, G. T.; Triggle, D. J.; Lambrecht, G. Muscarinic Receptors in Guinea Pig Ileum. A Study by Agonist–^{3}H-labelled Antagonist Competition. *Can. J. Physiol. Pharmacol.* **1982**, *60*, 1707-1714.

Table V. Physical Properties of 2-Substituted-2-azabicyclo[2.2.1]heptanol Esters



compd	molecular formula	mp, °C	cryst solv	method of prep.	% yield of free base	position	R'	R	C(O)CH$_3$	NCH$_3$	CCH$_3$	CHOH	aromatics	other
									^{1}H NMR (free base, CDCl$_3$, 250 MHz), δ					
8	$C_{22}H_{25}NO_6$ [a]	134–135	MeOH–EtOAc	A	73	5-exo	CH_3	$CH_2C_6H_5$	2.00 (s)			4.67 (m)		3.54 (s, CH_2Ar)
9	$C_{15}H_{20}ClNO_2$ [b]	179–180	MeOH–Et$_2$O	A	90	6-exo	CH_3	$CH_2C_6H_5$	1.99 (s)			4.97 (m)		3.74 (d, CH_2Ar)
10	$C_{29}H_{30}NO_2$ [e]			C	38	6-exo	$C(CH_3)(C_6H_5)_2$	$CH_2C_6H_5$			1.90 (s)	5.07 (t)	7.45 (m)	3.72 (s, CH_2Ar)
11	$C_{15}H_{21}NO_8$ [c]	119	MeOH–EtOAc	B	82	6-exo	CH_3	CH_3	2.00 (s)	3.10 (s)		4.90 (m)		
12	$C_{24}H_{27}NO_4$ [c]	150–152	MeOH–EtOAc	B	54	6-exo	$C(CH_3)(C_6H_5)_2$	CH_3		2.45 (s)	1.88 (s)	5.07 (m)	7.25 (m)	
16	$C_{28}H_{33}NO_9$ [e] · 0.5 H_2O [d]	139–141	MeOH–EtOAc	A	80	6-endo	$C(CH_3)(C_6H_5)_2$	CH_3		2.66 (s)	1.99 (s)	5.36 (m)	7.25 (m)	
17	$C_{22}H_{25}NO_6$ [a]	136–137	acetone–pentane	A	79	6-endo	CH_3	$CH_2C_6H_5$	2.10 (s)			4.85 (m)	7.28 (m)	3.74 (s, CH_2Ar)
22	$C_{26}H_{29}NO_6$ [c]	190	MeOH–Et$_2$O	A	31	5-exo	$C(CH_3)(C_6H_5)_2$	CH_3		2.81 (s)	1.90 (s)	4.93 (t)	7.27 (m)	
23	$C_{16}H_{21}NO_6$ [a]	174–176	MeOH–EtAc	A	62	5-exo	CH_3	CH_3	2.00 (s)	2.27 (s)		4.67 (m)		
29	$C_{15}H_{23}NO_9$ [d]	100–110	MeOH–EtAc	A	71	5-endo	CH_3	CH_3	2.10 (s)	2.55 (s)		5.05 (m)		
30	$C_{26}H_{29}NO_6$ [c]	164–165	MeOH–Et$_2$O	A	31	5-endo	$C(CH_3)(C_6H_5)_2$	CH_3		2.15 (s)	1.91 (s)	5.05 (m)	7.24 (m)	

[a] 2,3-Dihydroxybenzoate salt. [b] HCl salt. [c] Fumarate salt. [d] Citrate salt. [e] The free amino ester was used without further characterization to prepare 12.

and m$_3$-expressing cells, respectively, for [^{3}H]QNB binding assay. For [^{3}H]Oxo-M binding, rat brain membrane preparations were prepared in the same way in 20 mM HEPES buffer (pH 7.4), and the pellets were washed once and centrifuged at 45000g for 45 min. The resultant pellet was homogenized at a concentration of 30–40 μg per 1 mL binding assay volume. Protein concentrations were measured by the method of Bradford[17] with bovine serum albumin as the standard.

[^{3}H]QNB, [^{3}H]Oxo-M Radioligand Binding. The method established in our laboratory was employed.[4,16] Membrane fractions were incubated with 6.08 × 10^{-11} M [^{3}H]QNB and various concentrations of muscarinic agents in a 5-mL binding assay volume for 60, 90, and 120 min for tissue preparations or m$_1$- or m$_3$-expressing cell membrane preparations, respectively. For [^{3}H]Oxo-M binding assays, 1.14 × 10^{-9} M [^{3}H]Oxo-M was incubated with muscarinic agonists for 60 min in a 1-mL binding assay volume. After incubation, samples were filtered through Whatman GF/B filters and washed twice with 5 mL of ice-cold Tris buffer using a cell harvester (Model M-24R, Brandel Instrument, Gaithersberg, MD). For the [^{3}H]Oxo-M assay, samples were filtered through GF/C filters presoaked in 0.05% polyethylenimine. The radioactivity of filters in 5 mL of scintillation fluid was counted using a scintillation counter at an efficiency of approximately 45%. Nonspecific binding was measured in the presence of 10^{-5} M atropine.

Materials. [^{3}H]QNB (L-[*benzilic*-4,4-^{3}H]quinuclidinyl benzilate, specific activity 32.9 Ci/mmol), [^{3}H]Oxo-M ([*methyl*-^{3}H]-oxotremorine acetate, specific activity 87.5 Ci/mmol) were purchased from Du Pont-New England Nuclear (Boston, MA). Tissue culture media and supplements were obtained from GIBCO Laboratories (Grand Island, NY).

Data Analysis. Radioligand binding data were calculated using a nonlinear curve fitting program (BDATA, CDATA, EMF Software, Knoxville, TN) implemented on an IBM personal computer. K_I values were calculated by the method of Cheng and Prusoff.[18]

Molecular Modeling. Molecular modeling was performed using the SYBYL software package[19] (version 5.41a, version 5.31b5) running on Digital Equipment Corp. microVAX II's and VAX-station 3110 workstations and a Silicon Graphics 4D/310 workstation. An Evans & Sutherland PS 330 graphics workstation and a Macintosh IIcx interfaced to the VAX cluster were used for display and real-time manipulation of three-dimensional molecular models.

The published X-ray crystallographic coordinates of azaprophen were used the generate starting structure of azaprophen using the CRYSIN command. The models of **30**, **22**, **16**, and **12** were built from fragments provided by the SYBYL software package. The structures were energy-minimized with the MAXIMIN2 force field. The final structures of atropine, **30**, **22**, **16**, and 12 were overlayed using the SYBYL root-mean-square FIT command. Corresponding pairs of bridgehead and nitrogen atoms in each ring structure were used as the basis for superimposing the models.

Conformational searches were then performed on each molecule with 30° increments of all rotatable bonds in the ester side chains. The positions of suitable hydrogen-bond donor sites for each low-energy conformation were mapped by recording the endpoint of a 2.8 Å vector originating at the carbonyl oxygen atom aligned in the direction of the oxygen lone pairs.

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Registry No. 5, 112375-05-0; 6, 46407-10-7; 7, 140927-00-0; 8, 140926-96-1; 8·2,3-dihydroxybenzoate salt, 140926-97-2; 9, 140927-01-1; 9·HCl, 140926-98-3; 10, 140927-02-2; 11, 50494-47-8; 11·fumarate, 140926-99-4; 12, 140927-03-3; 12·citrate, 140927-18-0;

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(±)-Carbocyclic 5′-Nor-2′-deoxyguanosine and Related Purine Derivatives: Synthesis and Antiviral Properties

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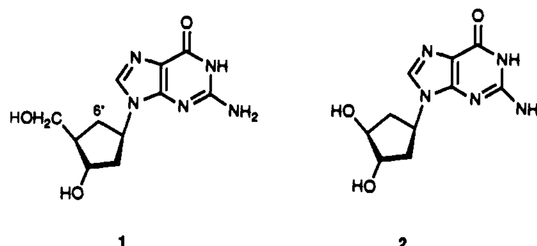
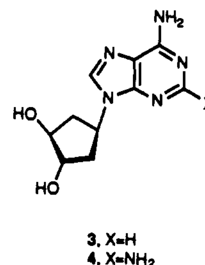
Beginning with 3-cyclopenten-1-ylamine hydrochloride, the 5′-nor derivatives of carbocyclic 2′-deoxyguanosine (**2**), 2′-deoxyadenosine (**3**), and 2,6-diaminopurine 2′-deoxyribofuranoside (**4**) have been prepared. These compounds were evaluated for antiviral potential versus herpes simplex virus, varicella-zoster virus, cytomegalovirus, vaccinia virus, vesicular stomatitis virus, and human immunodeficiency virus and found to lack activity. Also, compounds **2–4** were virtually nontoxic toward the host (human diploid fibroblast ESM and HEL) cells. These biological properties may be due to the inability of **2–4** to be phosphorylated to the requisite nucleotide level that is likely to be necessary for biological activity by correlation to carbocyclic 2′-deoxyguanosine (**1**), which possesses significant antiviral properties as a result of conversion to its 5′-triphosphate derivative.

Racemic[1] and D-carbocyclic[2] 2′-deoxyguanosine (represented as **1**) have shown significant antiviral activity as a result of selective conversion to their 5′-triphosphate derivatives.[3] Recent studies[4-8] focusing on the development of antiviral agents derived from nucleosides lacking the C-5′ carbon prompted a synthesis and evaluation of (±)-**2** as the 5′-nor derivative of **1**. The results of this investigation, which also included the adenine (**3**)[9] and 2,6-diaminopurine (**4**) derivatives, are presented in this report.





Chemistry

A convenient starting material for the synthesis of **2–4** was determined to be (±)-(1α,3β,4α)-3,4-dihydroxycyclopent-1-ylamine (**5**, Scheme I), which was prepared from 3-cyclopenten-1-ylamine hydrochloride (**6**)[10] and stored as the triacyl derivative **10**. To achieve **5**, benzoylation of **6** to **7** was followed by epoxidation to give the diastereomeric mixture of **8** and a small amount of **9**. The ^{1}H NMR data for the benzamide NH and the oxirane protons was used to distinguish **8** and **9**. In that regard, the NH region for **8** (δ 7.3–7.7) is shielded by the *cis*-oxirane oxygen relative to the NH in **9** (δ 8.31), whereas the oxirane protons in **9** (δ 3.54) are shielded by the *cis*-nitrogen when compared to the oxirane protons in **8** (δ 3.57). This cis stereochemistry of **8** is corroborated by an X-ray structural analysis of compound **3**[11] and by similar observations for the products resulting from epoxidation of the benzamide derivative of 2-cyclopenten-1-ylamine[12] and 2-cyclo-

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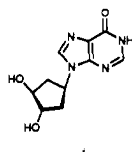

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