C(8) Substituted 1-Azabicyclo[3.3.1]non-3-enes and C(8) Substituted 1-Azabicyclo[3.3.1]nonan-4-ones: Novel Muscarinic Receptor Antagonists

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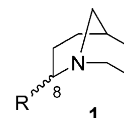
Expedient syntheses of C(8) substituted 1-azabicyclo[3.3.1]non-3-enes and C(8) substituted 1-azabicyclo[3.3.1]nonan-4-ones are reported to begin with 2,5-disubstituted pyridines. Catalytic reduction of the pyridine to the piperidine followed by treatment with ethyl acrylate and Dieckmann cyclization gave diastereomeric mixtures of C(8) substituted 3-ethoxycarbonyl-4-hydroxy-1-azabicyclo[3.3.1]non-3-enes, which were separable by chromatography. We found that the catalytic reduction (PtO_2, H_2) procedure provided the *cis*-substituted piperidine but that pyridine reduction was accompanied by competitive cleavage of the C(2) pyridyl substituent. Accordingly, an alternative route was devised that afforded a diastereomeric mixture of the *cis*- and *trans*-2,5-disubstituted piperidine. Treatment of the substituted pyridine with *m*-CPBA gave the pyridine *N*-oxide, which was reduced to the piperidine by sequential reduction with ammonium formate in the presence of Pd−C followed by $NaBH_3CN$. Addition of ethyl acrylate completed the synthesis of the substituted piperidine. The overall four-step reaction gave higher yields (57%) than the two-step procedure (13%) with little cleavage of the C(2) pyridyl substituent. Acid decarboxylation of the bicyclo[3.3.1]non-3-enes provided the C(8) substituted 1-azabicyclo[3.3.1]nonan-4-ones. Structural studies revealed diagnostic ^{13}C NMR signals that permit assignment of the orientation of the C(8) substituent. Pharmacological investigations documented that 3-ethoxycarbonyl-4-hydroxy-1-azabicyclo[3.3.1]non-3-enes efficiently bind to the human M_1−M_5 muscarinic receptors and function as antagonists. We observed that *exo*-8-benzyloxymethyl-3-ethoxycarbonyl-4-hydroxy-1-azabicyclo[3.3.1]non-3-ene (**3**) displayed the highest affinity, exhibiting K_i values at all five muscarinic receptors that were approximately 10−50 times lower than carbachol and approximately 30−230 times lower than arecoline. Receptor selectivity was observed for **3**. Compound **3** contained two different pharmacophores found in many muscarinic receptor ligands, and preliminary findings indicated the importance of both structural elements for maximal activity. Compound **3** serves as a novel lead compound for further drug development.

The pathophysiological processes under the influence of muscarinic ligands have attracted the attention of medicinal chemists and medical practitioners for many years.[1−4] Recent studies have linked muscarinic receptors to Alzheimer's and Parkinson's diseases, schizophrenia, urinary incontinence, irritable bowel syndrome, chronic obstructive pulmonary disease, and the perception of pain.[1,2] Reports have shown [3.2.1]-, [2.2.2]-, and [2.2.1]-bicyclic amines bind muscarinic receptors with high affinity.[4] Notable examples include the muscarinic receptor antagonist LY316108/NNC11-2192[4b] ([3.2.1]-bicyclic amine) and the agonists talsaclidine[4d] ([2.2.2]-bicyclic amine) and CI-1017[4c] ([2.2.1]-bicyclic amine). By comparison, few [3.3.1]-bicyclic amines have been examined despite early findings of activity.[1b,c,5−8] Efforts have been hampered by lack of general procedures for synthesis of this ring system and the accessibility of the synthetic precursors. Studies in our laboratories required the preparation of derivatives of 8-substituted aza-bridged [3.3.1]-bicyclic amine **1**. In this investigation, we combined older, more recent, and new synthetic methodologies for the preparation of **1**. X-ray and ^{13}C NMR spectroscopic studies are coupled to provide useful structural tools for the stereochemical assignments of [3.3.1]-bicyclic amine ring substituents. We report the pharmacological activity of these compounds against the human M_1−M_5 muscarinic receptors and document their potent activity against these receptors. Evidence for receptor selectivity was observed.



Results and Discussion

Synthesis. Our studies required the synthesis of C(8) substituted methylene-[3.3.1]-bicyclic amines **2**−**6**. In 1952, Sternbach and Kaiser reported that Dieckmann cyclization of the diethyl ester of 3-carboxypiperidine-1-propionic acid (**13a**, Scheme 1) provided **2** and that **2**

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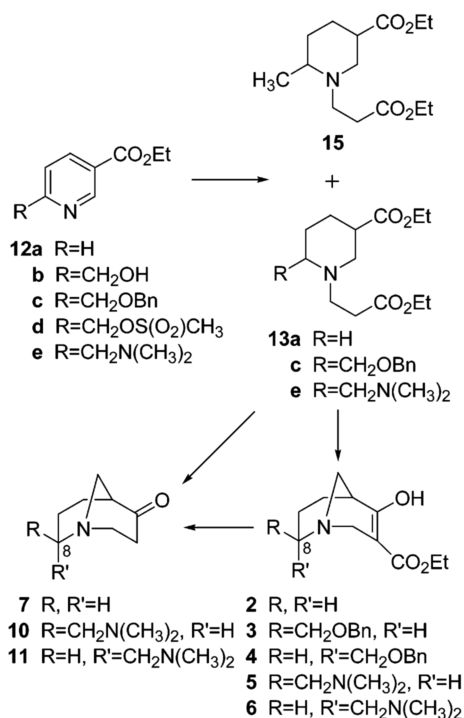
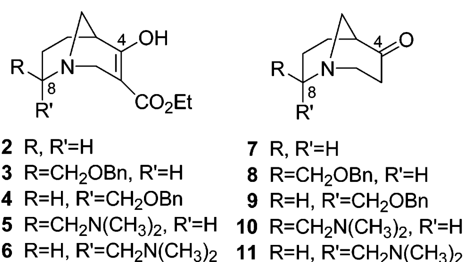
Scheme 1. Synthesis of Aza-Bridged [3.3.1]-Bicyclic Amines



underwent acid decarboxylation to give 1-azabicyclo-[3.3.1]nonan-4-one hydrochloride (**7**) in 23% overall yield.[5a] We are unaware of other general methods for this ring system. Accordingly, we adopted the Dieckmann route[9−11] and first coupled it with a more recent procedure for the synthesis of 2,5-disubstituted piperidines from the corresponding pyridine[12−14] (Scheme 1).

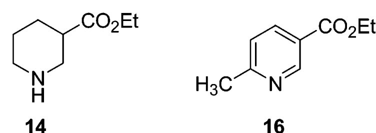


Synthesis of 2,5-disubstituted pyridines **12c**−**e** was straightforward. Treatment of **12b**[15] with NaH and benzyl bromide gave ether **12c** in 75% yield. Compound **12e** was prepared in two steps. First, **12b** was converted to mesylate **12d** in situ, and then the mesylate group was displaced by dimethylamine to give **12e** in 82% yield.

Several pyridine-reductive methods have been reported.[12−14,16] For C(2) substituted methylene pyridines, piperidine formation is accompanied by competitive reduction of the C(2) methylene substituent. We examined several methods and found, for **12c** and **12e**, that PtO_2, H_2, and acid[12−14] provided the desired piperidines.

The crude piperidines prepared from **12c** and **12e** were heated (80 °C) with ethyl acrylate to give the Dieckmann cyclization precursors **13c** and **13e**, respectively. The combined yields for these two steps ranged from 13 to 51%. For the preparation of **13a**, we used commercially available ethyl 3-piperidinecarboxylate (**14**) rather than reduce ethyl nicotinate (**12a**). For **12c**, nearly 50% of the product mixture consisted of the byproduct **15** (C(2) reduction) after treatment with ethyl acrylate.

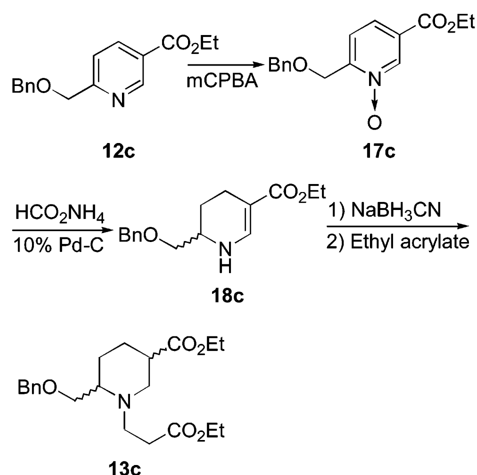
The catalytic reduction of **12c** and **12e** proceeded stereoselectively to give **13c** and **13e** as a single diastereomer after the Michael addition with ethyl acrylate while the C(2) methyl piperidine **15** byproduct was generated as an approximate 1:1 diastereomeric mixture. The ^{13}C NMR provided information concerning piperidine **13** and **15** structures. For **15**, we observed diagnostic signals for the C(2) (δ 53.5, 55.1) and the C(5) (δ 41.2, 42.2) carbons in the *cis*- and *trans*-products. We have assigned the upfield set (δ 41.2, 53.3) to the *cis*-adduct and the corresponding downfield pair (δ 42.2, 55.1) to the *trans*-isomer on the basis of established patterns.[17] This finding permitted us to tentatively assign as the sole adduct for the PtO_2-H_2 reduced products **13c** (δ 40.6, 57.6) and **13e** (δ 40.1, 55.5) as the *cis*-isomers. Further evidence for this assignment came from an independent synthesis of both *cis*- (δ 40.6, 57.6) and *trans*-**13c** (δ 41.9, 59.4) (see below). The stereoselectively of the PtO_2-H_2 reduction for **12c** and **12e** suggested that the C(2) substituent complexed with the PtO_2 surface, promoting pyridine reduction from one side. We tested this hypothesis. Catalytic reduction (PtO_2-H_2, H^+) of ethyl 2-methyl-5-pyridine carboxylate (**16**), followed by treatment with ethyl acrylate, gave **15** as a 1:0.2 diastereomeric mixture.



Cyclization of **13a**, **13c**, and **13e** proceeded under stringent conditions (*t*-BuOK, 110 °C, 1 h)[5−8] to give **2**,[5a] **3** and **4**, and **5** and **6**, respectively. Acid decarboxylation (concd aqueous HCl, 100 °C, 14 h)[5a] of **2**, **5**, and **6** gave the corresponding 1-azabicyclo[3.3.1]nonan-4-ones **7**,[5a] **10**, and **11**, respectively. Use of these conditions for the preparation of **8** and **9** led to complex product mixtures. Improved yields of **7**,[5a] **10**, and **11** were achieved with the Dieckmann cyclization and acid decarboxylation steps but without characterizing the intermediate esters **2**, **5**, and **6**, respectively.

The competitive reductive cleavage of the C(2) pyridyl substituent hampered our synthetic efforts to produce **2**−**7**, **10**, and **11**. We sought an alternative route to the Dieckmann precursor **13**. In Scheme 2 we outline a four-step procedure for *cis*- and *trans*-**13c** that proceeded with little cleavage of the appended benzyl unit, a group sensitive to reduction. Beginning with **12c**, *m*-CPBA oxidation gave the pyridine *N*-oxide **17c**. Ammonium formate reduction in the presence of Pd−C[18] gave the conjugated enamidoester (76% yield) **18c**[19] and the corresponding debenzylated compound (10% yield). Treatment of **18c** with $NaBH_3CN$ completed the reduction and gave **13c** after treatment with ethyl acrylate. The overall yield of **13c** from **12c** was 57% compared to the 13% yield obtained in Scheme 1. ^{13}C NMR analysis indicated that the ratio of *cis*- to *trans*-**13c** was approximately 0.16:1, respectively. Dieckmann cyclization

Scheme 2. Improved Synthesis of **13c**



of the *cis*- and *trans*-mixture **13c** gave **3** and **4** (69% yield) in an approximate 1:1 ratio. The relative percentage of **3** and **4** was comparable to that observed for *cis*-**13c**, indicating that the C(5) carbon epimerized under the basic Dieckmann cyclization conditions.

Structure Analysis. The identities of Dieckmann products **2**−**6** and the decarboxylated adducts **7**, **10**, and **11** were determined by IR, ^{1}H NMR, ^{13}C NMR, and mass spectroscopic measurements. The stereochemical orientation of the C(8) substituent was assigned on the basis of the X-ray crystallographic analysis of **10**, a comparison of the ^{1}H and the ^{13}C NMR within the data set, and the use of diagnostic ^{13}C NMR patterns for cyclic structures.

Figure 1 shows the ORTEP X-ray crystallographic diagram for **10**, indicating that the C(8) dimethylaminomethylene unit is *exo* to the [3.3.1]-bicyclic ring system. Of note, this compound crystallized as the monohydrochloride salt. Structural identification of **10** permitted us to assign the C(8) configuration for the isomeric acid decarboxylated salt **11** as *endo* and to confidently predict the structures of the corresponding Dieckmann adducts **5** and **6**. Further evidence for these assignments came from an assessment of the NMR data set (Table 1). Comparison of the ^{1}H and ^{13}C NMR resonances for **2**−**7**, **10**, and **11** revealed distinctive ^{13}C NMR chemical shift values for the C(2) and C(9) resonances. We observed that the C(2) chemical shift peak for the *exo*-adducts **3**, **5**, and **10** resonated 9.2−10.1 ppm downfield from the C(2) signal in *endo*-isomers **4**, **6**, and **11**. Correspondingly, the C(9) resonance in **3**, **5**, and **10** appeared 6.9−8.0 ppm upfield from the same signal in **4**, **6**, and **11**. Similar ^{13}C NMR chemical shift differences have been reported for the methyl and the C(3) methylene resonances in substituted methylcyclohexanes[20] and for ring substituents within 2-azabicyclo-[3.3.1]nonanes.[21] In agreement with our proposed ^{1}H NMR assignments, NOESY experiments on the Dieckmann products **3**−**6** showed the expected correlation (Supporting Information). For **3**, we observed a steric interaction between the C(8) methine hydrogen and the C(2) methylene protons, whereas for the *endo*-isomer **4** we noted interactions between the C(8) methine hydrogen and the C(9) and C(8)CH_2 methylene protons. Similar NOESY correlations were detected for the isomeric pair **5** and **6**.

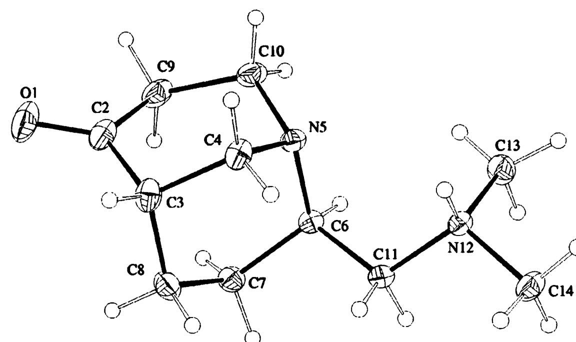
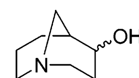


Figure 1. ORTEP X-ray crystallographic diagram for **10**.

Pharmacological Analysis. The structural correspondence of **2**−**7**, **10**, and **11** with bicyclic amines previously[4b−d] shown to interact with muscarinic receptors prompted us to test the pharmacological activity of these compounds. An earlier study reported that 1-azabicyclo[3.3.1]nonan-4-ol (**19**) displayed antispasmodic activity.[5b] Muscarinic receptors are distributed both centrally and peripherally and are recognized to have important roles in cognitive function, central control of movement, peripheral control of gastrointestinal functions, and bronchodilation.[1] To date, five muscarinic receptor subtypes have been characterized and cloned at the molecular level (m_1−m_5), and four of these (m_1−m_4) have been pharmacologically classified as M_1−M_4 on the basis of their response to selective antagonists.[1]



We chose to initially study the Gq/phospholipase C-coupled M_1 muscarinic receptor and the Gi/adenylyl cyclase-coupled M_2 muscarinic receptor in our pharmacological analyses. [^{3}H]QNB was utilized as a radioligand in competition binding assays with membranes obtained from Sf9 insect cells expressing either the human M_1 or M_2 muscarinic receptors (Figure 2). The Dieckmann products **2**−**6** exhibited the highest apparent affinities of the compounds tested. Compounds **7**, **10**, and **11** were not included in subsequent experiments because of their weak affinities for the muscarinic receptors.

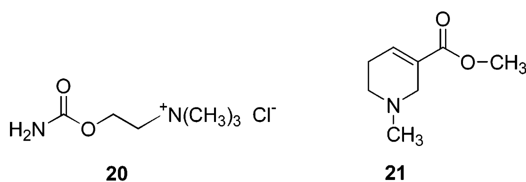
Further studies were carried out with compounds **2**−**6**, using membranes prepared from COS-7 cells independently expressing each of the five human muscarinic receptors.[22] The calculated K_i values determined for compounds **2**−**6** in multiple experiments are presented in Table 2. Only upper limits of activity could be estimated for compounds **5** (at M_1, M_3, M_4, and M_5) and **6** (at M_1, M_2, M_4, and M_5). Within the Dieckmann products, the C(8) benzyloxymethylene compounds **3** and **4** displayed increased affinity compared with that of their C(8) dimethylaminomethylene counterparts **5** and **6**. Furthermore, we found that the C(8) *exo*-isomers were largely more potent than the C(8) *endo*-isomers and that this difference approached 50-fold ($p < 0.01$) and varied with molecules and receptors (i.e., **3** vs **4** at the M_2 and M_4 receptors). Compound **3** displayed the highest affinity, exhibiting K_i values at all five musca-

Table 1. Key NMR Assignments of **2**–**7**, **10**, and **11**

compd	R	R′	^{1}H NMR^a C(2)HH′	C(8)H	C(8′)HH′	C(9)HH′	^{13}C NMR^b C(2)	C(7)	C(8)	C(9)
2	H	H	3.24 (d, 16.8) 3.73 (d, 16.8)	2.90–2.96 (m)		2.89 (d, 13.0) 2.96 (d, 13.0)	49.5	19.1	55.2	51.4
3	CH_2OBn	H	3.33 (d, 16.8) 3.89 (d, 16.8)	3.01–3.02 (m)	3.52 (dd, 6.5, 9.3) 3.65 (dd, 7.5, 9.3)	2.75 (d, 13.4) 3.07 (d, 13.4)	52.1	19.4	60.1	46.3
4	H	CH_2OBn	3.37–3.51 (m)	3.04–3.13 (m)	3.37–3.51 (m)	3.01 (d, 13.2) 3.08 (d, 13.2)	42.5	21.7	61.5	53.2
5	$CH_2N(CH_3)_2$	H	3.31 (d, 16.8) 3.93 (d, 16.8)	2.90–2.94 (m)	2.23 (dd, 6.8, 12.5) 2.70 (dd, 8.4, 12.5)	2.77 (d, 13.6) 3.09 (d, 13.6)	52.5	20.9	58.8	45.9
6	H	$CH_2N(CH_3)_2$	3.35 (dd, 1.0, 17.2) 3.55 (d, 17.2)	2.91–2.97 (m)	2.12 (dd, 7.5, 12.7) 2.45 (dd, 6.3, 12.7)	3.03 (br dt, 1.3, 13.0) 3.12 (dt, 2.3, 13.0)	42.4	24.1	60.3	53.9
7	H	H	3.08–3.23 (m)	3.08–3.23 (m)		3.29–3.39 (m)	51.2	21.9	53.3	53.9
10	$CH_2N(CH_3)_2$	H	3.27–3.33 (m) 3.41–3.50 (m)	3.08–3.12 (m)	2.26 (dd, 6.8, 12.4) 2.72 (dd, 8.4, 12.4)	2.91 (d, 13.9) 3.32 (d, 13.9)	53.8	23.2	56.8	48.0
11	H	$CH_2N(CH_3)_2$	3.20–3.26 (m) 3.34–3.39 (m)	3.20–3.26 (m)	2.13–2.34 (m) 2.72–2.77 (m)	3.20–3.26 (m)	44.6	26.2	58.1	56.0

^{a} The number in each entry is the chemical shift value (δ) observed in ppm followed by multiplicity of the signal and the coupling constant (s) in Hz. The spectra were recorded at 600 MHz (**3**, **4**), 500 MHz (**2**, **5**, **6**, **10**, **11**), and 300 MHz (**7**). The solvent used was $CDCl_3$. ^{b} The number in the entry is the chemical shift value (δ) observed in ppm relative to the solvent peak. The spectra were recorded at 150 MHz (**2**, **3**), 125 MHz (**4**–**6**, **10**, **11**), and 75 MHz (**7**). The solvent used was $CDCl_3$.

rinic receptors that were approximately 10–50 ($p < 0.01$) times lower than carbachol (**20**) and approximately 30–230 times lower ($p < 0.01$) than arecoline (**21**). Receptor selectivity also was observed with compound **3**, which exhibited a 10-fold greater affinity ($p < 0.01$) for the M_2 receptor over the M_3 receptor. Lower degrees of selectivity between receptor subtypes were observed for other compounds (e.g., compounds **2** and **4** at M_4 receptor versus M_5 receptor).



Documentation of relatively high affinity interaction of compounds **2**–**4** at the M_1–M_5 receptor subtypes in the radioligand binding assays led us to examine their activities in functional assays of muscarinic receptor action. Because the M_1, M_3, and M_5 receptors couple through Gq to activate phospholipase C-β (PLC-β), these receptors were independently expressed in COS-7 cells, and PLC-β activity was measured. The same assay was performed with the Gi-coupled M_2 and M_4 receptors. However, the chimeric G protein Gαq/i was cotransfected along with these receptors to allow signal transduction from these Gi-linked receptors to Gq-activated PLC-β.

As illustrated in Figure 3, 3 μM carbachol promoted a 4–6-fold increase in [^{3}H]inositol phosphate accumulation in COS-7 cells transfected with various muscarinic receptor subtypes. In contrast, no effect of compounds **2**, **3**, and **4** on [^{3}H]inositol phosphate accumulation was observed at 100 μM concentration. A comparable result was observed in Sf9 cells expressing the M_1 muscarinic receptor[23] (Supporting Information Figure 1). Thus, these three analogues do not exhibit agonist activity at any of the muscarinic receptor subtypes. In contrast, analogues **2**, **3**, and **4** inhibited the capacity of 3 μM of carbachol to activate phospholipase C, confirming that these compounds are antagonists of all muscarinic receptor subtypes. Similar results were obtained with these compounds in studies of the M_2 muscarinic receptor, examining its capacity to promote [^{35}S]GTPγS-binding in membranes from Sf9 insect cells expressing the M_2 muscarinic receptor[24] (Supporting Information Figure 2). Taken together, our studies illustrate that analogues **2**–**4** are antagonists of the M_1 through M_5 receptors and do not exhibit any partial agonist activities at any of these five receptors under the conditions studied.

Compound **3** exhibited the greatest affinity for the M_2 receptor, being 3-fold greater ($p < 0.01$) for the M_2 receptor than for either the M_1 or the M_4 receptors, 7-fold greater ($p < 0.01$) than for the M_5 receptor, and 10-fold greater ($p < 0.01$) than for the M_3 receptor. In recent years, M_2 and M_4 selective antagonists have been promoted for the treatment for movement disorders, dementia, cardiac disorder, and pain.[2,25] Also, selective presynaptic M_2 muscarinic antagonists enhance acetylcholine levels in vitro as well as in vivo, suggesting their use for the treatment of degenerative disorders associated with impaired cholinergic functions, such as Alzheimer's disease.[4d,26] Accordingly, **3** serves as a useful lead compound for further optimization.

The biological activities observed with these bicyclic amines showed that muscarinic receptor activity depended on ring size and nitrogen position.[1–4] Analysis of the composite data has revealed structural elements important for receptor binding. Two of these pharmacophores are embedded in the chemical neurotransmitter, acetylcholine chloride (**22**), and the muscarinic

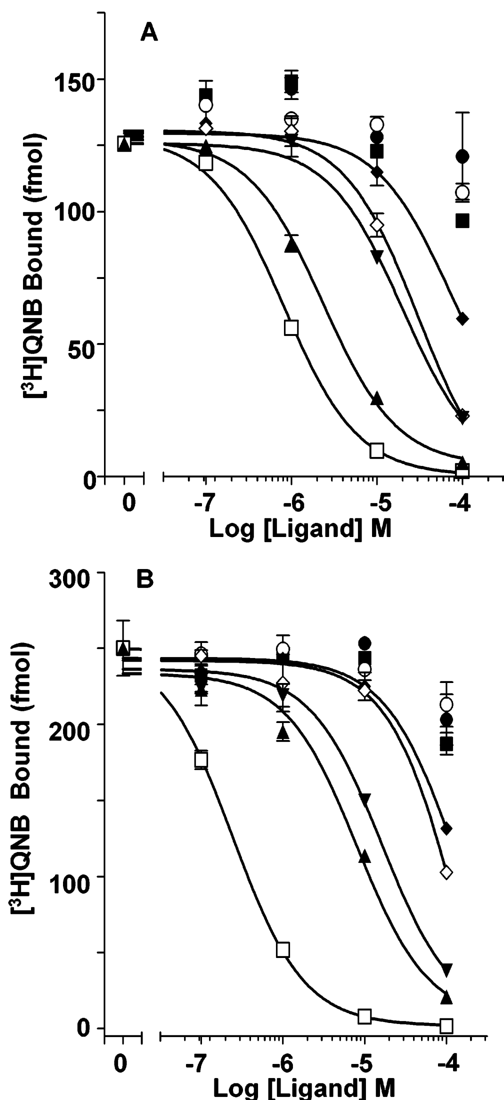


Figure 2. Competition binding assay at the human M_1 and M_2 receptors: Increasing amounts of the indicated analogue were incubated with [^{3}H]QNB and Sf9 insect cell membranes expressing either the M_1 (A) or M_2 (B) muscarinic receptor. Data are shown for compounds **2** (▲), **3** (□), **4** (▼), **5** (◇), **6** (◆), **7** (○), **10** (●), and **11** (■). The data shown are typical curves of three independent experiments carried out in duplicate for both M_1 and M_2 receptors. Bars represent the range of duplicate values.

agonist, arecoline (**21**),[1] and these structural units are highlighted in Figure 4.

Our findings indicate the apparent importance of both pharmacophores shown in Figure 4 for maximal bioactivity for C(8) substituted 1-azabicyclo[3.3.1]non-3-enes. We observed that the parent Dieckmann product **2** showed appreciable binding to all five muscarinic receptor subtypes. Significantly, **2** contains *only* the structural element found in arecoline (**21**). Furthermore, altering this pharmacophore to give **7** led to a loss of binding. Our data set also showed that the structural unit associated with acetylcholine (**22**) may have influenced muscarinic receptor binding for Dieckmann products **2**–**6**. In particular, we observed that incorporating an *exo*-C(8) benzyloxymethylene unit to give **3** led to an increase in muscarinic receptor binding compared with that of **2**, most significantly at the M_2 receptor where an increase of 20-fold ($p < 0.01$) was observed. Substituting the C(8) benzyloxymethylene unit in **3** with a dimethylaminomethylene group to give **5** led to lower activities than **2** at the M_1–M_5 receptor subtypes. Finally, we observed that the geometrical orientation of the C(8) unit was key to efficient binding. In most cases, the *exo*-isomer (**3**, **5**) displayed increased binding affinity compared with the *endo*-product (**4**, **6**). Significantly, our incorporation of two structurally unique pharmacophores within a single muscarinic receptor ligand differs from two recent reports[27,28] describing bivalent[29] muscarinic agonists where two identical 1,2,5-thiadiazole derivatives were linked to provide a novel series of potent agents.[27]

Conclusions

Our studies have documented that C(8) substituted 1-azabicyclo[3.3.1]non-3-ene analogues efficiently bind to muscarinic M_1–M_5 receptors, and functional assays showed that these compounds serve as antagonists at these receptors. Selectivity for the M_2 receptor was observed for **3**. Investigations are in progress to determine if these findings can be generalized to other aza-bridged [3.3.1]-bicyclic amines.

Experimental Section

General. FT-IR spectra were run on a Mattson Galaxy Series FT-IR 5000 spectrometer. ^{1}H and ^{13}C NMR were recorded on Varian VXR 300, Bruker DRX 500, Varian INOVA 500, Varian INOVA 600, and Bruker AMX 600 SR-1 NMR instruments. Low- and high-resolution (CI) mass spectral investigations were conducted at the University of Texas at Austin by Dr. M. Moini. The low-resolution mass spectra were run on a Finnegan MAT-TSQ-70 instrument, and the high-resolution mass spectra were recorded on a Micromass ZAB-E spectrometer. Microanalyses were provided by Atlantic Microlab, Inc. (Norcross, GA).

Synthesis. Ethyl 2-Benzyloxymethyl-5-pyridinecarboxylate (12c). NaH (144 mg, 60% in mineral oil, 3.6 mmol) was slowly added to an anhydrous DMF solution (6 mL) of **12b**[15] (543 mg, 3.0 mmol) and BnBr (429 μL, 3.6 mmol) at room temperature, the slurry was stirred (30 min), and H_2O (10 mL) was added. The reaction mixture was extracted with EtOAc (3 × 20 mL), and the combined extracts were dried (Na_2SO_4) and concentrated in vacuo. Purification of the concentrated residue by column chromotography (EtOAc/hexanes = 1/5) gave **12c** (610 mg, 75%) as a yellow oil: $R_f = 0.65$ (EtOAc/hexanes = 1/1); IR (neat) 3031, 2981, 2860, 1721, 1598, 1379, 1281, 1112 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.40 (t, $J = 7.2$ Hz, 3 H), 4.40 (q, $J = 7.2$ Hz, 2 H), 4.67 (s, 2 H), 4.74 (s, 2 H), 7.24–7.42 (m, 5 H), 7.59 (d, $J = 8.1$ Hz, 1 H), 8.30 (dd, $J = 1.5$, 8.1 Hz, 1 H), 9.15 (br s, 1 H); ^{13}C NMR ($CDCl_3$) 14.4, 61.5, 72.9, 73.3, 120.7, 125.1, 127.9, 128.0 (2 C), 128.6 (2 C), 137.8, 137.9, 150.4, 163.2, 165.4 ppm; MS (+CI) 272 [M + 1]$^+$; M_r (+CI) 272.128 97 [M + 1]$^+$ (calcd for $C_{16}H_{18}NO_3$, 272.128 67).

Ethyl 2-Dimethylaminomethyl-5-pyridinecarboxylate (12e). Methanesulfonyl chloride (186 μL, 2.4 mmol) was added dropwise to a cooled (0 °C) CH_2Cl_2 solution (8 mL) containing **12b**[15] (362 mg, 2.0 mmol) and triethylamine (334 μL, 2.4 mmol) under Ar, and then the solution was stirred at 0–5 °C (1 h). CH_2Cl_2 (10 mL) was added to the reaction, and the organic phase was successively washed with a saturated aqueous $NaHCO_3$ solution (10 mL) and brine (2 × 10 mL), dried (Na_2SO_4), filtered, and evaporated to give mesylate **12d** (508 mg, 98%). Compound **12d** was combined with a 2 M dimethylamine THF solution (10 mL, 20 mmol) and triethylamine (279 μL, 2.0 mmol) in EtOH (15 mL) and heated at 80 °C (15 h). The reaction solution was concentrated in vacuo and the residue taken up in CH_2Cl_2 (10 mL). The organic solution was washed with H_2O (2 × 10 mL) and brine (2 × 10 mL), dried (Na_2SO_4), filtered, and evaporated. The crude product was purified by

Table 2. Binding Affinities of **2**−**6** at Human Muscarinic Receptors

compd	hM_1 receptor $K_i^{a,b}$	hM_2 receptor $K_i^{a,b}$	hM_3 receptor $K_i^{a,b}$	hM_4 receptor $K_i^{a,b}$	hM_5 receptor $K_i^{a,b}$
2	1.2 ± 0.1	1.6 ± 0.2	1.5 ± 0.1	2.6 ± 0.3	0.98 ± 0.06
3	0.25 ± 0.01[c]	0.08 ± 0.01	0.84 ± 0.07	0.27 ± 0.02	0.56 ± 0.03
4	6.6 ± 0.5	4.1 ± 0.1	7.5 ± 0.6	11 ± 1	3.0 ± 0.5
5	>100[d]	86 ± 30	>100[d]	>100[d]	>100[c,d]
6	>100[d]	>100[d]	37 ± 9	>100[d]	>100[c,d]
20	12 ± 5	1.3 ± 0.3	20 ± 4	7.6 ± 0.4	4.3 ± 0.7
21	29 ± 1	2.4 ± 0.4	43 ± 5	60 ± 30	56 ± 30[c]

[a] K_i values were calculated according to the formula

$$K_i = \frac{IC_{50}}{1 + \frac{[[^3H]QNB]}{K_D}}$$

where IC_{50} is the concentration of the competing analogue that inhibited [^{3}H]QNB binding by 50%, [[^{3}H]QNB] is the concentration of [^{3}H]QNB in the binding assay, and K_D is the K_D of [^{3}H]QNB. The K_D determined for [^{3}H]QNB for hM_1 was 83.9 pM, hM_2 was 56.7 pM, hM_3 was 249 pM, hM_4 was 93.7 pM, and hM_5 was 176 pM. Values are means ± SEM (n = 3) unless otherwise indicated. [b] In μM. [c] The value is the average of two determinations. [d] K_i value was not calculated for this compound.

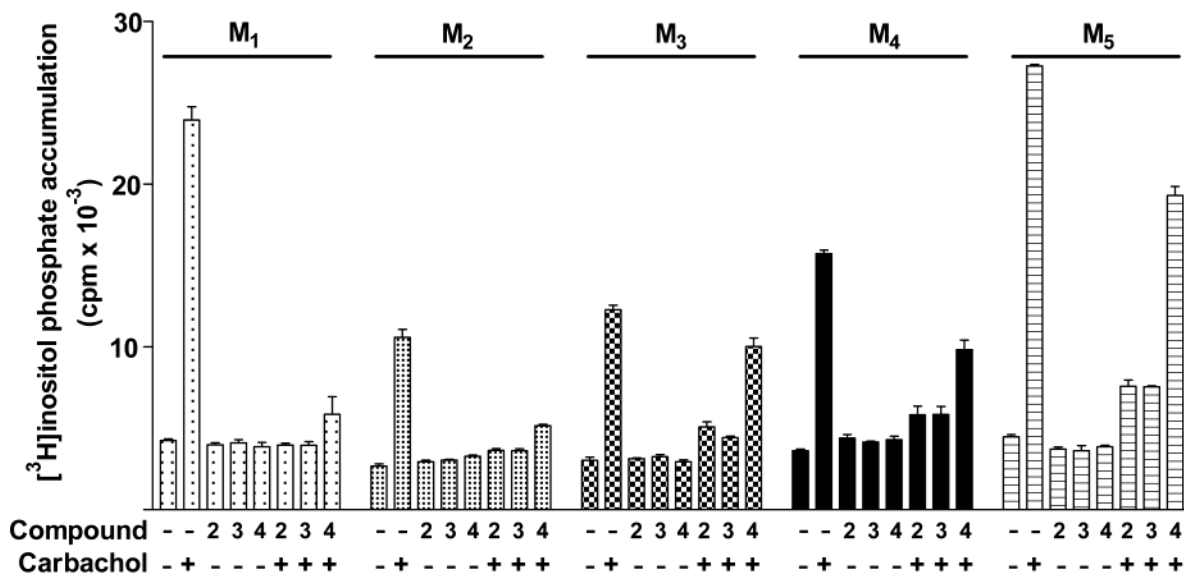


Figure 3. Quantitation of functional activity at the human M_1−M_5 receptors. The accumulation of [^{3}H]inositol phosphates in COS-7 cells expressing the M_1, M_3, or M_5 receptor alone or the M_2 or M_4 receptor coexpressed with chimeric Gαq/i was measured in response to 3 μM carbachol and/or 100 μM compound **2**, **3**, or **4**. The data shown are the result of an experiment performed in triplicate.

PTLC (EtOAc) to give **12e** (342 mg, 82%) as a yellow oil: R_f = 0.17 (EtOAc/hexanes = 3/1); IR (neat) 2976, 2821, 2776, 1723, 1599, 1459, 1376, 1282, 1117 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.41 (t, J = 7.2 Hz, 3 H), 2.31 (s, 6 H), 3.65 (s, 2 H), 4.41 (q, J = 7.2 Hz, 2 H), 7.51 (d, J = 7.8 Hz, 1 H), 8.27 (dd, J = 2.1, 7.8 Hz, 1 H), 9.16 (d, J = 2.1 Hz, 1 H); ^{13}C NMR (CDCl$_3$) 14.2, 45.5 (2 C), 61.2, 65.4, 122.4, 124.7, 137.3, 150.3, 163.5, 165.2 ppm; MS (+CI) 209 [M + 1]$^+$; M_r (+CI) 209.129 93 [M + 1]$^+$ (calcd for $C_{11}H_{17}N_2O_2$, 209.129 00).

Ethyl 1-[2-(Ethoxycarbonyl)ethyl]-3-piperidinecarboxylate (13a).[7] Ethyl 3-piperidinecarboxylate (**14**) (3.14 g, 20 mmol) was dissolved in ethyl acrylate (2.50 g, 25 mmol) and stirred at 80 °C (12 h), and then the reaction was concentrated in vacuo and purified by PTLC (EtOAc/hexanes = 3/1) to give **13a** (4.64 g, 90%) as a pale yellow oil: R_f = 0.51 (EtOAc/hexanes = 3/1); IR (neat) 2944, 2806, 1735, 1455, 1376, 1309 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.17−1.22 (m, 6 H), 1.35−1.51 (m, 2 H), 1.62−1.68 (m, 1 H), 1.83−1.89 (m, 1 H), 1.95−2.02 (m, 1 H), 2.11−2.18 (m, 1 H), 2.40−2.50 (m, 3 H), 2.62−2.70 (m, 3 H), 2.91 (dd, J = 1.8, 8.1 Hz, 1 H), 4.03−4.12 (m, 4 H); ^{13}C NMR (CDCl$_3$) 14.3 (2 C), 24.7, 26.9, 32.5, 41.9, 53.5, 53.9, 55.3, 60.3, 60.4, 172.6, 174.1 ppm; MS (+CI) 258 [M + 1]$^+$; M_r (+CI) 258.170 30 [M + 1]$^+$ (calcd for $C_{13}H_{24}NO_4$, 258.170 53).

Ethyl 2-Benzyloxymethyl-1-[2-(ethoxycarbonyl)ethyl]-5-piperidinecarboxylate (13c). To an EtOAc solution (0.9 mL) of **12c** (271 mg, 1.0 mmol) was added 2 M ethereal HCl (3.6 mL), and then the solvent was removed in vacuo, the residual **12c**·HCl salt was dissolved in MeOH (5.4 mL), and PtO_2 (14 mg) was added. The mixture was hydrogenated under 1 atm of H_2 (1 h). The catalyst was filtered, and the solvent was removed in vacuo. The residue was dissolved in aqueous 1 N NaOH (3.5 mL) and extracted with EtOAc (3 × 15 mL). The organic extracts were combined, dried (Na_2SO_4), concentrated, dissolved in ethyl acrylate (1.1 mL, 10 mmol), and stirred at 80 °C (20 h). The reaction was concentrated to dryness, and the residue was separated by PTLC (EtOAc/hexanes = 2/3) to give **13c** (48 mg, 13%) and **15** (32 mg, 12%), respectively.

Compound 13c: clear oil; R_f = 0.57 (EtOAc/hexanes = 1/1); IR (neat) 2978, 2935, 2862, 1731, 1454, 1372, 1181 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 1.24 (t, J = 7.2 Hz, 3 H), 1.25 (t, J = 7.2 Hz, 3 H), 1.64−1.77 (m, 4 H), 2.43−2.54 (m, 3 H), 2.66 (dd, J = 3.9, 12.2 Hz, 1 H), 2.82−2.97 (m, 4 H), 3.45 (dd, J = 5.9, 9.6 Hz, 1 H), 3.66 (dd, J = 5.4, 9.6 Hz, 1 H), 4.12 (q, J = 7.2 Hz, 4 H), 4.47 (1/2 AB, J = 12.2 Hz, 1 H), 4.52 (1/2 AB, J = 12.2 Hz, 1 H), 7.27−7.34 (m, 5 H); ^{13}C NMR (CDCl$_3$) 14.1, 14.2, 23.3, 25.9, 33.1, 40.6, 50.1, 50.3, 57.6, 60.1, 60.2, 69.3, 73.2, 127.4, 127.5 (2 C), 128.3 (2 C), 138.3, 172.7, 174.1 ppm; MS (+CI) 378 [M + 1]$^+$; M_r (+CI) 378.228 63 [M + 1]$^+$ (calcd for $C_{21}H_{32}NO_5$, 378.228 05).

Compound 15[8] (*cis*- and *trans*-mixture): yellow oil; R_f = 0.44 (EtOAc/hexanes = 1/1); IR (neat) 2974, 2924, 2814,

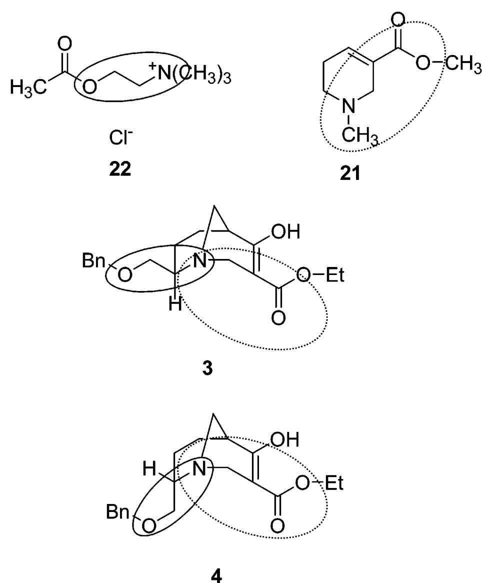


Figure 4. Pharmacophore elements present in **3**, **4**, **21**, and **22**.

1735, 1455, 1377 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.00 (d, J = 6.6 Hz, 1.4 H), 1.10 (d, J = 6.6 Hz, 1.6 H), 1.23–1.28 (m, 6 H), 1.40–2.01 (m, 4 H), 2.18–2.31 (m, 1 H), 2.42–2.59 (m, 4 H), 2.67–2.88 (m, 2 H), 3.03–3.10 (m, 1 H), 4.08–4.17 (m, 4 H); ^{13}C NMR ($CDCl_3$) 13.7 (2 C), 14.2 (2 C), 19.9 (2 C), 23.1 (2 C), 27.4 (2 C), 32.7, 33.8, 41.2, 42.2, 48.8, 49.6, 49.8, 53.3, 54.1, 55.1, 60.2 (2 C), 60.3 (2 C), 172.8 (2 C), 174.1 (2 C) ppm; MS (+CI) 272 [M + 1]$^+$; M_r (+CI) 272.186 63 [M + 1]$^+$ (calcd for $C_{14}H_{26}NO_4$, 272.186 18).

Ethyl 2-Dimethylaminomethyl-1-[2-(ethoxycarbonyl)-ethyl]-5-piperidinecarboxylate (13e). With the same procedure employed for the preparation of **13c**, **12e** (335 mg, 1.6 mmol), 2 M ethereal HCl (6 mL), PtO_2 (24 mg), and ethyl acrylate (1.7 mL, 16 mmol) gave **13e** (259 mg, 51%) and **15** (52 mg, 12%) following PTLC purification (10% MeOH–$CHCl_3$).

Compound 13e: reddish-yellow oil; R_f = 0.37 (10% MeOH–$CHCl_3$); IR (neat) 2945, 2818, 1731, 1457, 1376, 1182 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.18 (t, J = 7.2 Hz, 3 H, CH_2CH_3), 1.19 (t, J = 7.2 Hz, 3 H, CH_2CH_3), 1.56–1.70 (m, 4 H, C(3)H_2, C(4)-H_2), 2.15 (s, 6 H, $N(CH_3)_2$), 2.26–2.48 (m, 5 H, C(5)H, NCH_2CH_2, $(CH_3)_2NCH_2$), 2.60–2.91 (m, 5 H, C(2)H, C(6)H_2, NCH_2CH_2), 4.05 (q, J = 7.2 Hz, 2 H, CH_2CH_3), 4.07 (q, J = 7.2 Hz, 2 H, CH_2CH_3), the ^{1}H NMR assignments were consistent with the COSY spectrum; ^{13}C NMR ($CDCl_3$) 14.0 (CH_2CH_3), 14.1 (CH_2CH_3), 22.8 (C(3)), 25.5 (C(4)), 33.5 (NCH_2CH_2), 40.1 (C(5)), 46.0 ($N(CH_3)_2$), 49.3 (C(6)), 49.7 (NCH_2CH_2), 55.4 (C(2)), 57.7 ($(CH_3)_2NCH_2$), 60.1 (2 C, CH_2CH_3), 172.6 (C(O)), 174.2 (C(O)) ppm, the assignments were consistent with the DEPT and HMQC spectra; MS (+CI) 315 [M + 1]$^+$; M_r (+CI) 315.229 49 [M + 1]$^+$ (calcd for $C_{16}H_{31}N_2O_4$, 315.228 38).

Compound 15 (*cis*- and *trans*-mixture): R_f = 0.75 (10% MeOH–$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.00 (d, J = 6.6 Hz, 2 H), 1.10 (d, J = 6.6 Hz, 1 H), 1.21–1.28 (m, 6 H), 1.44–2.00 (m, 4 H), 2.21–2.30 (m, 1 H), 2.42–2.59 (m, 4 H), 2.67–2.88 (m, 3 H), 4.08–4.17 (m, 4 H).

Ethyl 2-Benzyloxymethyl-5-pyridinecarboxylate *N*-Oxide (17c). A $CHCl_3$ solution (22 mL) of *m*-chloroperbenzoic acid (3.72 g, 16.6 mmol) was gradually added to an ice-cooled (0–5 °C), stirred $CHCl_3$ solution (17 mL) of **12c** (4.50 g, 16.6 mmol). The reaction mixture was stirred (4 h), during which the mixture was allowed to come to room temperature. The reaction mixture was successively washed with aqueous 0.5 N NaOH (20 mL) and H_2O (20 mL), and the $CHCl_3$ layer was dried (Na_2SO_4) and concentrated in vacuo. Purification of the concentrated residue by column chromatography (EtOAc/hexanes = 3/1) gave **17c** (4.00 g, 84%) as yellowish white solid: mp 50–52 °C; R_f = 0.19 (EtOAc/hexanes = 2/1); IR 3047, 2987, 2859, 1726, 1465, 1386, 1301, 1230, 1118 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 1.39 (t, J = 7.2 Hz, 3 H), 4.40 (q, J = 7.2 Hz, 2 H), 4.73 (s, 2 H), 4.77 (s, 2 H), 7.27–7.42 (m, 5 H), 7.78 (d, J = 8.1 Hz, 1 H), 8.04 (d, J = 8.1 Hz, 1 H), 8.71 (s, 1 H); ^{13}C NMR (CD_3OD, 75 MHz) 14.4, 63.4, 67.1, 74.7, 125.1, 129.1 (2 C), 129.2 (2 C), 129.6, 129.9, 130.0, 138.9, 141.0, 155.1, 164.1 ppm; MS (+CI) 288 [M + 1]$^+$; M_r (+CI) 288.123 95 [M + 1]$^+$ (calcd for $C_{16}H_{18}NO_4$, 288.123 58). Anal. ($C_{16}H_{17}NO_4$): C, H, N.

Ethyl (2*R*,*S*)-2-Benzyloxymethyl-1,2,3,4-tetrahydro-5-pyridinecarboxylate (18c). Dry ammonium formate (9.24 g, 146.5 mmol) was added to a solution of **17c** (4.21 g, 14.7 mmol) containing 10% Pd–C (1.45 g) in anhydrous MeOH (130 mL) under an atm of Ar. The reaction mixture was stirred (17 h) at room temperature, and the mixture was filtered. The filtrate was evaporated in vacuo, and the residue was triturated with EtOAc (100 mL). The insoluble solid was filtered, and the filtrate was concentrated in vacuo. The residue was purified by column chromatography (EtOAc/hexanes = 1/2) to give benzyl compound **18c** (3.08 g, 76%) and the corresponding debenzylated compound (271 mg, 10%), respectively.

Compound 18c: white solid; mp 64–66 °C; R_f = 0.60 (EtOAc/hexanes = 2/1); IR (KBr) 3384, 2943, 2857, 1661, 1616, 1490, 1353, 1303, 1207, 1099 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.25 (t, J = 7.2 Hz, 3 H), 1.43–1.55 (m, 1 H), 1.76–1.85 (m, 1 H), 2.23–2.45 (m, 2 H), 3.30–3.57 (m, 3 H), 4.13 (q, J = 7.2 Hz, 2 H), 4.53 (s, 2 H), 4.81–4.82 (m, 1 H), 7.26–7.40 (m, 5 H), 7.46 (d, J = 6.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) 14.6, 19.6, 23.6, 49.9, 58.9, 73.2, 73.3, 95.9, 127.7, 127.9 (2 C), 128.5 (2 C), 137.7, 141.9, 168.6 ppm; MS (+CI) 276 [M + 1]$^+$; M_r (+CI) 276.160 31 [M + 1]$^+$ (calcd for $C_{16}H_{22}NO_3$, 276.159 97). Anal. ($C_{16}H_{21}NO_3$): C, H, N.

Debenzylated Compound: colorless oil; R_f = 0.16 (EtOAc/hexanes = 2/1); IR (neat) 3384, 2940, 2871, 1731, 1660, 1615, 1454, 1369, 1204, 1110 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.25 (t, J = 7.2 Hz, 3 H), 1.44–1.56 (m, 1 H), 1.75–1.84 (m, 1 H), 2.05–2.42 (m, 2 H), 3.34–3.72 (m, 4 H), 4.11 (q, J = 7.2 Hz, 2 H), 5.32–5.34 (m, 1 H), 7.50 (d, J = 6.0 Hz, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) 14.6, 19.5, 23.3, 51.9, 59.0, 65.8, 96.2, 142.1, 168.7 ppm; MS (+CI) 186 [M + 1]$^+$; M_r (+CI) 186.113 01 [M + 1]$^+$ (calcd for $C_9H_{16}NO_3$, 186.113 02). Anal. ($C_9H_{15}NO_3 \cdot 0.4\ H_2O$): C, H, N.

Ethyl 2-Benzyloxymethyl-1-[2-(ethoxycarbonyl)ethyl]-5-piperidinecarboxylate (13c). To a HOAc solution (23 mL) of **18c** (2.62 g, 9.52 mmol) was slowly added a MeOH solution (23 mL) of $NaBH_3CN$ (718 mg, 11.42 mmol). The solution was stirred at room temperature (3 h) and concentrated in vacuo. The dried residue (1 h) was treated with ethyl acrylate (10.3 mL, 95.2 mmol) and triethylamine (1.59 mL, 11.4 mmol) at 80–85 °C (17 h). The reaction mixture was concentrated in vacuo and the residue taken up in EtOAc (70 mL). The mixture was washed with H_2O (2 × 50 mL), dried (Na_2SO_4), filtered, and evaporated. The crude residue was purified by column chromatography (EtOAc/hexanes = 1/2) to give **13c** (3.20 g, 89%, the ratio of *cis*/*trans* = 0.16:1) as yellow oil. The piperidine adduct consisted of a mixture of *cis*- and *trans*-diastereomers, respectively, that was separable by PTLC (EtOAc/hexanes = 1/1).

***cis*-Diastereomer 13c** (0.45 g, 13%)**:** yellow oil; R_f = 0.56 (EtOAc/hexanes = 1/1); IR (neat) 2978, 2935, 2862, 1731, 1454, 1372, 1181 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.24 (t, J = 7.2 Hz, 3 H), 1.25 (t, J = 7.2 Hz, 3 H), 1.64–1.77 (m, 4 H), 2.43–2.54 (m, 3 H), 2.66 (dd, J = 3.9, 12.2 Hz, 1 H), 2.82–2.97 (m, 4 H), 3.45 (dd, J = 5.9, 9.6 Hz, 1 H), 3.66 (dd, J = 5.4, 9.6 Hz, 1 H), 4.12 (q, J = 7.2 Hz, 4 H), 4.47 (1/2 ABq, J = 12.2 Hz, 1 H), 4.52 (1/2 ABq, J = 12.2 Hz, 1 H), 7.27–7.34 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75 MHz) 14.1, 14.2, 23.3, 25.9, 33.1, 40.6, 50.1, 50.3, 57.6, 60.1, 60.2, 69.3, 73.2, 127.4, 127.5 (2 C), 128.3 (2 C), 138.3, 172.7, 174.1 ppm; MS (+CI) 378 [M + 1]$^+$; M_r (+CI) 378.228 63 [M + 1]$^+$ (calcd for $C_{21}H_{32}NO_5$, 378.228 05). Anal. ($C_{21}H_{31}NO_5$): C, H, N.

***trans*-Diastereomer 13c** (2.75 g, 76%)**:** yellow oil; R_f = 0.51 (EtOAc/hexanes = 1/1); IR (neat) 2936, 2863, 1730, 1455, 1370, 1183 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.24 (t, J = 7.2 Hz,

3 H), 1.25 (t, $J = 7.2$ Hz, 3 H), 1.35−1.50 (m, 2 H), 1.69−1.77 (m, 1 H), 2.01−2.04 (m, 1 H), 2.28−2.58 (m, 5 H), 2.83−2.92 (m, 1 H), 3.07−3.19 (m, 2 H), 3.43 (dd, $J = 4.2$, 9.9 Hz, 1 H), 3.52 (dd, $J = 4.5$, 9.9 Hz, 1 H), 4.11 (q, $J = 7.2$ Hz, 4 H), 4.50 (1/2 ABq, $J = 12.4$ Hz, 1 H), 4.54 (1/2 ABq, $J = 12.4$ Hz, 1 H), 7.24−7.37 (m, 5 H); ^{13}C NMR ($CDCl_3$, 75 MHz) 14.2 (2 C), 26.9, 28.7, 30.6, 41.6, 48.9, 54.1, 59.4, 60.2 (2 C), 72.7, 73.3, 127.6, 127.7 (2 C), 128.3 (2 C), 138.1, 172.7, 174.0 ppm; MS (+CI) 378 [M + 1]$^+$; M_r (+CI) 378.229 05 [M + 1]$^+$ (calcd for $C_{21}H_{32}NO_5$, 378.228 05). Anal. ($C_{21}H_{31}NO_5$): C, H, N.

***exo*-8-Benzyloxymethyl-3-ethoxycarbonyl-4-hydroxy-1-azabicyclo[3.3.1]non-3-ene (3) and *endo*-8-Benzyloxymethyl-3-ethoxycarbonyl-4-hydroxy-1-azabicyclo[3.3.1]non-3-ene (4) from *cis*- and *trans*-13c.** A suspension of *t*-BuOK (842 mg, 7.5 mmol) in anhydrous toluene (5 mL) was heated to reflux (1 h), and then an anhydrous toluene solution (1.5 mL) of the *cis*- and *trans*-**13c** (944 mg, 2.5 mmol) was added (20 min). The reaction mixture was heated to reflux (3 h). The reaction mixture was concentrated in vacuo and the residue taken up in H_2O (10 mL) and neutralized (pH 7−8) with concentrated aqueous HCl. The mixture was extracted with $CHCl_3$ (3 × 20 mL), dried (Na_2SO_4), filtered, and evaporated. The crude mixture was purified by column chromatography (2.5% MeOH/$CHCl_3$) to give *exo*-compound **3** (297 mg, 36%) and *endo*-compound **4** (277 mg, 33%) as yellow oils, respectively.

Compound 3: yellow oil; $R_f = 0.52$ (5% MeOH−$CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.29 (t, $J = 7.2$ Hz, 3 H), 1.43−1.45 (m, 1 H), 1.71−1.81 (m, 3 H), 2.16 (br s, 1 H), 2.73 (d, $J = 13.5$ Hz, 1 H), 2.99−3.02 (m, 1 H), 3.05 (d, $J = 13.5$ Hz, 1 H), 3.33 (d, $J = 16.7$ Hz, 1 H), 3.51 (dd, $J = 7.5$, 9.3 Hz, 1 H), 3.64 (dd, $J = 6.6$, 9.3 Hz, 1 H), 3.89 (d, $J = 16.7$ Hz, 1 H), 4.21 (q, $J = 7.2$ Hz, 2 H), 4.52 (d, $J = 12.3$ Hz, 1 H), 4.59 (d, $J = 12.3$ Hz, 1 H), 7.23−7.34 (m, 5 H), 11.89 (br s, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) 14.1, 19.2, 22.6, 31.5, 46.1, 52.0, 60.0, 60.1, 70.6, 72.9, 98.8, 127.3, 127.4 (2 C), 128.1 (2 C), 138.2, 170.7, 171.8 ppm.

Compound 4: yellow oil; $R_f = 0.30$ (5% MeOH−$CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.30 (t, $J = 7.2$ Hz, 3 H), 1.31−1.41 (m, 2 H), 1.74−1.86 (m, 1 H), 1.91−1.98 (m, 1 H), 2.23 (br s, 1 H), 3.02−3.17 (m, 3 H), 3.31−3.57 (m, 4 H), 4.12−4.26 (m, 2 H), 4.53 (d, $J = 12.6$ Hz, 1 H), 4.59 (d, $J = 12.6$ Hz, 1 H), 7.27−7.36 (m, 5 H), 11.84 (br s, 1 H); ^{13}C NMR ($CDCl_3$, 75 MHz) 14.2, 21.8, 27.6, 32.0, 42.5, 53.3, 60.2, 61.5, 71.9, 73.1, 99.3, 127.5, 127.7 (2 C), 128.3 (2 C), 138.3, 170.7, 172.2 ppm.

3-Ethoxycarbonyl-4-hydroxy-1-azabicyclo[3.3.1]non-3-ene (2). A suspension of *t*-BuOK (505 mg, 4.5 mmol) in anhydrous toluene (4.5 mL) was refluxed (1 h), and then an anhydrous toluene solution (1.5 mL) of **13a** (386 mg, 1.5 mmol) was added (20 min). The reaction mixture was refluxed for an additional 3 h and then cooled. EtOH (2 mL) was added, and the reaction was filtered (Celite pad) and concentrated in vacuo. The residue was purified by PTLC (5% MeOH−$CHCl_3$) to give **2** (127 mg, 40%) as a yellow oil: $R_f = 0.21$ (5% MeOH−$CHCl_3$); IR (neat) 2932, 2857, 1656, 1292, 1207 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.25 (t, $J = 7.1$ Hz, 3 H, CH_2CH_3), 1.55−1.64 (m, 2 H, C(7)H_2), 1.65−1.73 (m, 1 H, C(6)*H*H′), 1.84−1.86 (m, 1 H, C(6)H*H*′), 2.19 (br s, 1 H, C(5)H), 2.89 (d, $J = 13.0$ Hz, 1 H, C(9)*H*H′), 2.90−2.96 (m, 2 H, C(8)H_2), 2.96 (d, $J = 13.0$ Hz, 1 H, C(9)H*H*′), 3.24 (d, $J = 16.8$ Hz, 1 H, C(2)*H*H′), 3.73 (d, $J = 16.8$ Hz, 1 H, C(2)H*H*′), 4.17 (q, $J = 7.1$ Hz, 2 H, CH_2CH_3), 11.83 (br s, 1 H, C(4)OH), the ^{1}H NMR assignments were consistent with the COSY spectrum; ^{13}C NMR ($CDCl_3$, 150 MHz) 14.2 (CH_2CH_3), 19.1 (C(7)), 26.6 (C(6)), 32.2 (C(5)), 49.5 (C(2)), 51.4 (C(9)), 55.2 (C(8)), 60.2 (CH_2CH_3), 99.3 (C(3)), 170.6 (C(O)), 172.1 (C(4)) ppm, the assignments were consistent with the DEPT, HMQC, and HMBC spectra; MS (+CI) 212 [M + 1]$^+$; M_r (+CI) 212.128 08 [M + 1]$^+$ (calcd for $C_{11}H_{18}NO_3$, 212.128 67). Anal. ($C_{11}H_{17}NO_3$): C, H, N.

***exo*-8-Benzyloxymethyl-3-ethoxycarbonyl-4-hydroxy-1-azabicyclo[3.3.1]non-3-ene (3) and *endo*-8-Benzyloxymethyl-3-ethoxycarbonyl-4-hydroxy-1-azabicyclo[3.3.1]non-3-ene (4) from *cis*-13c.** With the same procedure employed for the preparation of **2**, *t*-BuOK (29 mg, 0.25 mmol) and *cis*-**13c** (32 mg, 0.09 mmol) in anhydrous toluene (1 mL) gave **3** (4.5 mg, 16%) and **4** (4.0 mg, 14%) following PTLC purification (5% MeOH−$CHCl_3$).

Compound 3: yellow oil; $R_f = 0.52$ (5% MeOH−$CHCl_3$); IR (neat) 2933, 2863, 1734, 1656, 1370, 1293, 1209 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.31 (t, $J = 7.1$ Hz, 3 H, CH_2CH_3), 1.45−1.47 (m, 1 H, C(7)*H*H′), 1.72−1.81 (m, 3 H, C(7)H*H*′, C(6)H_2), 2.17 (br s, 1 H, C(5)H), 2.75 (d, $J = 13.4$ Hz, 1 H, C(9)*H*H′), 3.01−3.02 (m, 1 H, C(8)H), 3.07 (d, $J = 13.4$ Hz, 1 H, C(9)H*H*′), 3.33 (d, $J = 16.8$ Hz, 1 H, C(2)*H*H′), 3.52 (dd, $J = 6.5$, 9.3 Hz, 1 H, BnOC*H*H′), 3.65 (dd, $J = 7.5$, 9.3 Hz, 1 H, BnOCH*H*′), 3.89 (d, $J = 16.8$ Hz, 1 H, C(2)H*H*′), 4.23 (q, $J = 7.1$ Hz, 2 H, CH_2CH_3), 4.54 (d, $J = 12.2$ Hz, 1 H, PhC*H*H′O), 4.60 (d, $J = 12.2$ Hz, 1 H, PhCH*H*′O), 7.28−7.35 (m, 5 H, Ph), 11.86 (br s, 1 H, C(4)OH), the ^{1}H NMR assignments were consistent with the COSY and NOESY spectra; ^{13}C NMR ($CDCl_3$, 150 MHz) 14.2 (CH_2CH_3), 19.4 (C(7)), 22.7 (C(6)), 31.6 (C(5)), 46.3 (C(9)), 52.1 (C(2)), 60.1 (C(8)), 60.2 (CH_2CH_3), 70.7 (BnOCH_2), 73.1 (PhCH_2O), 99.9 (C(3)), 127.0, 127.5 (2 C), 127.6 (2 C), 138.3 (Ph), 170.9 (C(O)), 171.9 (C(4)) ppm, the assignments were consistent with the DEPT, HMQC, and HMBC spectra; MS (+CI) 331 [M]$^+$; M_r (+CI) 331.179 07 [M]$^+$ (calcd for $C_{19}H_{25}NO_4$, 331.178 36). Anal. ($C_{19}H_{25}NO_4$): C, H, N.

Compound 4: yellow oil; $R_f = 0.30$ (5% MeOH−$CHCl_3$); IR (neat) 2928, 2858, 1657, 1371, 1301, 1209 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.22 (t, $J = 7.1$ Hz, 3 H, CH_2CH_3), 1.30−1.40 (m, 2 H, C(7)H_2), 1.71−1.78 (m, 1 H, C(6)*H*H′), 1.88−1.91 (m, 1 H, C(6)H*H*′), 2.22 (br s, 1 H, C(5)H), 3.01 (d, $J = 13.2$ Hz, 1 H, C(9)*H*H′), 3.04−3.13 (m, 1 H, C(8)H), 3.08 (d, $J = 13.2$ Hz, 1 H, C(9)H*H*′), 3.37−3.51 (m, 4 H, C(2)H_2, BnOCH_2), 4.06−4.19 (m, 2 H, CH_2CH_3), 4.46 (d, $J = 12.3$ Hz, 1 H, PhC*H*H′O), 4.52 (d, $J = 12.3$ Hz, 1 H, PhCH*H*′O), 7.21−7.28 (m, 5 H, Ph), 11.81 (br s, 1 H, C(4)OH), the ^{1}H NMR assignments were consistent with the COSY and NOESY spectra; ^{13}C NMR ($CDCl_3$, 125 MHz) 14.3 (CH_2CH_3), 21.7 (C(7)), 27.6 (C(6)), 32.0 (C(5)), 42.5 (C(2)), 53.2 (C(9)), 60.2 (CH_2CH_3), 61.5 (C(8)), 71.6 (BnOCH_2), 73.1 (PhCH_2O), 99.3 (C(3)), 127.6, 127.7 (2 C), 128.3 (2 C), 138.3 (Ph), 170.7 (C(O)), 172.1 (C(4)) ppm, the assignments were consistent with the DEPT, HMQC, and HMBC spectra; MS (+CI) 331 [M]$^+$; M_r (+CI) 331.178 13 [M]$^+$ (calcd for $C_{19}H_{25}NO_4$, 331.178 36). Anal. ($C_{19}H_{25}NO_4$): C, H, N.

***exo*-8-Dimethylaminomethyl-3-ethoxycarbonyl-4-hydroxy-1-azabicyclo[3.3.1]non-3-ene (5) and *endo*-8-Dimethylaminomethyl-3-ethoxycarbonyl-4-hydroxy-1-azabicyclo[3.3.1]non-3-ene (6).** With the same procedure employed for the preparation of **2**, *t*-BuOK (41 mg, 0.4 mmol) and **13e** (38 mg, 0.1 mmol) in anhydrous toluene (1.5 mL) gave **5** (4 mg, 12%) and **6** (4 mg, 12%) following PTLC purification (10% MeOH−$CHCl_3$).

Compound 5: yellow oil; $R_f = 0.20$ (10% MeOH−$CHCl_3$); IR (neat) 2935, 2862, 2770, 1655, 1456, 1363, 1294, 1208 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.32 (t, $J = 7.2$ Hz, 3 H, CH_2CH_3), 1.73−1.83 (m, 4 H, C(6)H_2, C(7)H_2), 2.19 (br s, 1 H, C(5)H), 2.23 (dd, $J = 6.8$, 12.5 Hz, 1 H, $(CH_3)_2$NC*H*H′), 2.29 (s, 6 H, N$(CH_3)_2$), 2.70 (dd, $J = 8.4$, 12.5 Hz, 1 H, $(CH_3)_2$NCH*H*′), 2.77 (d, $J = 13.6$ Hz, 1 H, C(9)*H*H′), 2.90−2.94 (m, 1 H, C(8)H), 3.09 (d, $J = 13.6$ Hz, 1 H, C(9)H*H*′), 3.31 (d, $J = 16.8$ Hz, 1 H, C(2)*H*H′), 3.93 (d, $J = 16.8$ Hz, 1 H, C(2)H*H*′), 4.23 (q, $J = 7.1$ Hz, 2 H, CH_2CH_3), 11.87 (br s, 1 H, C(4)OH), the ^{1}H NMR assignments were consistent with the COSY and NOESY spectra; ^{13}C NMR ($CDCl_3$, 125 MHz) 14.7 (CH_2CH_3), 20.9 (C(7)), 22.9 (C(6)), 32.2 (C(5)), 45.9 (C(9)), 46.1 (2 C, N$(CH_3)_2$), 52.5 (C(2)), 58.8 (C(8)), 60.7 (CH_2CH_3), 61.2 ($(CH_3)_2$NCH_2), 99.2 (C(3)), 171.0 (C(O)), 171.8 (C(4)) ppm, the assignments were consistent with the DEPT, HMQC, and HMBC spectra; MS (+CI) 269 [M + 1]$^+$; M_r (+CI) 269.185 87 [M + 1]$^+$ (calcd for $C_{14}H_{25}N_2O_3$, 269.186 52). Anal. ($C_{14}H_{24}N_2O_3$·0.5 $C_6H_5CH_3$·1.6 H_2O): C, H, N.

Compound 6: yellow oil; $R_f = 0.26$ (10% MeOH−$CHCl_3$); IR (neat) 2934, 2864, 2773, 1656, 1457, 1366, 1292, 1208 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.32 (t, $J = 7.2$ Hz, 3 H, CH_2CH_3), 1.28−1.32 (m, 1 H, C(7)*H*H′), 1.40−1.45 (m, 1 H, C(7)H*H*′), 1.77−1.84 (m, 1 H, C(6)*H*H′), 1.92−1.97 (m, 1 H, C(6)H*H*′), 2.12 (dd, $J = 7.5$, 12.7 Hz, 1 H, $(CH_3)_2$NC*H*H′), 2.23 (br s, 1 H,

C(5)H), 2.27 (s, 6 H, $N(CH_3)_2$), 2.45 (dd, J = 6.3, 12.7 Hz, 1 H, $(CH_3)_2NCHH'$), 2.91−2.97 (m, 1 H, C(8)H), 3.03 (br dt, J = 1.3, 13.0 Hz, 1 H, C(9)*H*H′), 3.12 (dt, J = 2.3, 13.0 Hz, 1 H, C(9)H*H′*), 3.35 (dd, J = 1.0, 17.2 Hz, 1 H, C(2)*H*H′), 3.55 (d, J = 17.2 Hz, 1 H, C(2)H*H′*), 4.17−4.30 (m, 2 H, CH_2CH_3), 11.84 (br s, 1 H, C(4)OH), the ^1H NMR assignments were consistent with the COSY and NOESY spectra; ^{13}C NMR ($CDCl_3$, 125 MHz) 14.7 (CH_2CH_3), 24.1 (C(7)), 28.4 (C(6)), 32.5 (C(5)), 42.4 (C(2)), 46.6 (2 C, $N(CH_3)_2$), 53.9 (C(9)), 60.3 (C(8)), 60.7 (CH_2CH_3), 63.8 ($(CH_3)_2NCH_2$), 99.3 (C(3)), 170.7 (C(O)), 172.3 (C(4)) ppm, the assignments were consistent with the DEPT, HMQC, and HMBC spectra; MS (+CI) 269 $[M + 1]^+$; M_r (+CI) 269.185 39 $[M + 1]^+$ (calcd for $C_{14}H_{25}N_2O_3$, 269.186 52). Anal. ($C_{14}H_{24}N_2O_3$·0.4 $C_6H_5CH_3$·1 H_2O): C, H, N.

1-Azabicyclo[3.3.1]nonan-4-one (7)[7,8] from 2. A solution of **2** (21 mg, 0.1 mmol) and concentrated aqueous HCl (1 mL) was refluxed (14 h), and then the solution was basified (30% aqueous KOH) and extracted with CH_2Cl_2 (5 × 10 mL). The combined CH_2Cl_2 extracts were dried (Na_2SO_4) and concentrated in vacuo to give **7** (5 mg, 38%) as a semisolid: R_f = 0.14 (5% MeOH−$CHCl_3$); IR (neat) 2929, 2857, 1697, 1352, 1201 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.64−1.81 (m, 2 H, C(7)H_2), 1.80−1.86 (m, 2 H, C(6)H_2), 2.41 (br s, 1 H, C(5)H), 2.49−2.54 (m, 2 H, C(3)H_2), 3.08−3.23 (m, 4 H, C(2)H_2, C(8)H_2), 3.29−3.39 (m, 2 H, C(9)H_2), the ^1H NMR assignments were consistent with the COSY spectrum; ^{13}C NMR ($CDCl_3$, 75 MHz) 21.9 (C(7)), 27.5 (C(6)), 41.8 (C(3)), 45.4 (C(5)), 51.2 (C(2)). 53.3 (C(8)), 53.9 (C(9)), 212.6 (C(4)) ppm, the assignments were consistent with the DEPT and HMQC spectrum; MS (+EI) 139 $[M]^+$; M_r (+EI) 139.100 15 $[M]^+$ (calcd for $C_8H_{13}NO$, 139.099 71).

1-Azabicyclo[3.3.1]nonan-4-one (7) from 13a. A suspension of *t*-BuOK (1.01 g, 9.0 mmol) in anhydrous toluene (12 mL) was refluxed (1 h), and then an anhydrous toluene solution (2.5 mL) of **13a** (772 mg, 3.0 mmol) was added (20 min). The reaction mixture was refluxed for an additional 3 h, cooled, and extracted with concentrated aqueous HCl (2 × 5 mL). The combined aqueous HCl extracts were refluxed (16 h), and then the solution was basified (30% aqueous KOH) and extracted with CH_2Cl_2 (3 × 10 mL). The combined CH_2Cl_2 extracts were dried (Na_2SO_4) and concentrated in vacuo to give **7** (137 mg, 33%) as a semisolid: R_f = 0.14 (5% MeOH−$CHCl_3$); ^1H NMR ($CDCl_3$, 300 MHz) δ 1.52−1.82 (m, 2 H), 1.90−1.97 (m, 2 H), 2.42 (br s, 1 H), 2.50−2.55 (m, 2 H), 3.10−3.23 (m, 4 H), 3.27−3.43 (m, 2 H).

***exo*-8-Dimethylaminomethyl-1-azabicyclo[3.3.1]nonan-4-one·HCl (10) from 5.** With the procedure for the synthesis of **7** from **2**, **5** (10 mg, 0.04 mmol) and concentrated aqueous HCl (0.5 mL) gave **10** (3.9 mg, 38%) as a yellow semisolid following PTLC purification (10% MeOH−$CHCl_3$); R_f = 0.08 (10% MeOH−$CHCl_3$); IR (neat) 2932, 2866, 2774, 1700, 1460, 1357, 1277 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.52−1.56 (m, 1 H, C(7)*H*H′), 1.72−1.78 (m, 1 H, C(6)*H*H′), 1.80−1.86 (m, 1 H, C(7)H*H′*), 1.97−2.05 (m, 1 H, C(6)H*H′*), 2.26 (dd, J = 6.8, 12.4 Hz, 1 H, $(CH_3)_2NCHH'$), 2.31 (s, 6 H, $N(CH_3)_2$), 2.39 (br s, 1 H, C(5)H), 2.49 (dd, J = 6.2, 17.5 Hz, 1 H, C(3)*H*H′), 2.62 (ddd, J = 8.9, 10.6, 17.5 Hz, 1 H, C(3)H*H′*), 2.72 (dd, J = 8.4, 12.4 Hz, 1 H, $(CH_3)_2NCHH'$), 2.91 (d, J = 13.9 Hz, 1 H, C(9)*H*H′), 3.08−3.12 (m, 1 H, C(8)H), 3.27−3.33 (m, 1 H, C(2)*H*H′), 3.32 (d, J = 13.9 Hz, 1 H, C(9)H*H′*), 3.41−3.50 (m, 1 H, C(2)H*H′*), the ^1H NMR assignments were consistent with the COSY spectrum; ^{13}C NMR ($CDCl_3$, 125 MHz) 23.2 (C(7)), 24.1 (C(6)), 41.1 (C(3)), 45.2 (C(5)), 46.2 ($N(CH_3)_2$), 48.0 (C(9)), 53.8 (C(2)), 56.8 (C(8)), 62.4 ($(CH_3)_2NCH_2$), 213.1 (C(4)) ppm, the assignments were consistent with the HMQC and HMBC spectra; MS (+CI) 197 $[(M-HCl) + 1]^+$; M_r (+CI) 197.165 81 $[(M-HCl) + 1]^+$ (calcd for $C_{11}H_{21}N_2O$, 197.165 39).

***endo*-8-Dimethylaminomethyl-1-azabicyclo[3.3.1]nonan-4-one·HCl (11) from 6.** With the procedure for the synthesis of **7** from **2**, **6** (12 mg, 0.04 mmol) and concentrated aqueous HCl (0.5 mL) gave **11** (4.3 mg, 42%) as a yellow semisolid following PTLC purification (10% MeOH−$CHCl_3$); R_f = 0.13 (10% MeOH−$CHCl_3$); IR (neat) 2936, 2873, 1694, 1462, 1361, 1219 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.29−1.36 (m, 1 H, C(7)*H*H′), 1.57−1.60 (m, 1 H, C(7)H*H′*), 1.89−1.98 (m, 2 H, C(6)H_2), 2.13−2.34 (m, 3 H, C(3)H_2, $(CH_3)_2NCHH'$), 2.39 (br s, 1 H, C(5)H), 2.45 (s, 6 H, $N(CH_3)_2$), 2.72−2.77 (m, 1 H, $(CH_3)_2NCHH'$), 3.20−3.26 (m, 4 H, C(2)*H*H′, C(8)H, C(9)H_2), 3.34−3.39 (m, 1 H, C(2)H*H′*), the ^1H NMR assignments were consistent with the COSY spectrum; ^{13}C NMR ($CDCl_3$, 125 MHz) 26.2 (C(7)), 27.6 (C(6)), 42.0 (C(3)), 44.6 (C(2)), 45.2 (C(5)), 45.8 ($N(CH_3)_2$), 56.0 (C(9)), 58.1 (C(8)), 62.2 ($(CH_3)_2NCH_2$), 212.5 (C(4)) ppm, the assignments were consistent with the DEPT, HMQC, and HMBC spectra; MS (+CI) 197 $[(M-HCl) + 1]^+$; M_r (+CI) 197.165 74 $[(M-HCl) + 1]^+$ (calcd for $C_{11}H_{21}N_2O$, 197.165 39).

***exo*-8-Dimethylaminomethyl-1-azabicyclo[3.3.1]nonan-4-one·HCl (10) and *endo*-8-Dimethylaminomethyl-1-azabicyclo[3.3.1]nonan-4-one·HCl (11) from 13e.** With the procedure for the synthesis of **7** from **13a**, **13e** (314 mg, 1.0 mmol), *t*-BuOK (337 mg, 3.0 mmol), and anhydrous toluene (6 mL) gave a crude mixture that was heated (85−90 °C, 16 h) with concentrated aqueous HCl (4 mL) and then separated by PTLC (25% MeOH−$CHCl_3$) to give **10** (36 mg, 16%) and **11** (31 mg, 13%), respectively.

Compound 10: yellow semisolid; R_f = 0.08 (10% MeOH−$CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 1.53−1.80 (m, 2 H), 1.80−2.01 (m, 2 H), 2.26 (dd, J = 6.8, 12.4 Hz, 1 H), 2.30 (s, 6 H), 2.37 (br s, 1 H), 2.49 (dd, J = 6.2, 17.5 Hz, 1 H), 2.62 (ddd, J = 8.9, 10.6, 17.5 Hz, 1 H), 2.70 (dd, J = 8.4, 12.4 Hz, 1 H), 2.90 (d, J = 13.9 Hz, 1 H), 3.05−3.10 (m, 1 H), 3.27−3.33 (m, 1 H), 3.32 (d, J = 13.9 Hz, 1 H), 3.40−3.49 (m, 1 H).

Compound 11: yellow semisolid; R_f = 0.13 (10% MeOH−$CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz) δ 1.30−1.61 (m, 2 H), 1.89−1.98 (m, 2 H), 2.10−2.33 (m, 3 H), 2.40 (br s, 1 H), 2.46 (s, 6 H), 2.70−2.75 (m, 1 H), 3.21−3.25 (m, 4 H), 3.35−3.41 (m, 1 H).

X-ray Crystallographic Study of 10. Compound **10** was recrystallized from chloroform-*d*. Crystals of **10** belong to the space group $P2_1/n$ (monoclinic) with a = 10.953 (3) Å; b = 7.864 (2) Å; c = 25.010 (8) Å; V = 2140 Å^3, D_{calcd} = 1.46 Mg·m^{-3}, and Z = 4. Data were collected at −100 °C, and the structure was refined to R_f = 0.053, R_w = 0.063 for 5057 reflections with $I > 3\sigma(I)$.

Pharmacological Analyses

hM_1−hM_2 Expression in Sf9 Insect Cells and Membrane Preparation. Human M_1 and M_2 receptors were expressed in Sf9 insect cells by using the baculovirus expression system. Cells were lysed by nitrogen cavitation, and membranes were collected through differential centrifugation and resuspended in a buffer containing 20 mM HEPES (pH= 8), 250 mM sucrose, 0.1 mM EDTA, and a protease inhibitor cocktail for a final protein concentration of 5−10 mg/mL.

hM_1−hM_5 Expression in COS-7 Cells and Membrane Preparation. COS-7 cells were subcultured in 150 mm dishes to a density of 20 000 cells/cm^2. The cells were transfected with Fugene 6 transfection reagent (according to Roche Molecular Biochemical's specifications) and pcDNA3.1 DNA containing sequence coding for the hM_1, hM_2, hM_3, hM_4, or hM_5 receptor (approximately 20 μg DNA/150 mm dish). Cell lysates were harvested after 24 h by scraping the cells and sonicating in buffer (5 mM Tris (pH 7.5), 1 mM $MgCl_2$, and protease inhibitors). Membranes were isolated by differential centrifugation and resuspended in freezing buffer (20 mM HEPES (pH 8.0), 250 mM sucrose, 0.1 mM EDTA, and protease inhibitors). Binding assays were carried out as described below.

hM_1−hM_5 Binding Assay. Competition binding assays were performed essentially as described[30] in a volume of 2 mL by using 10 μL membrane (50−100 μg protein), 200 pM [^{3}H]-QNB (approximately 20 000 cpm/assay), and various concentrations of competing agents (carbachol, arecoline, and compounds **2**−**7**, **10**, and **11**) in binding buffer (20 mM HEPES (pH 7.5), 150 mM NaCl, 3 mM $MgCl_2$, and 1 mM EDTA). Incubations were for 90 min at 30 °C. The assays were terminated by addition of 4 mL of wash buffer (20 mM Tris (pH 7.5), 150 mM NaCl, and 2 mM $MgCl_2$), quick filtration over Whatman GF/A filters, and two washes with 4 mL of wash

buffer. The filters were placed into scintillation vials with 5 mL of scintillation fluid and radioactivity quantitated.

Inositol Phosphate Accumulation Assay. COS-7 cells were subcultured in 12-well culture dishes to a density of 20 000 cells/cm^2. The cells were transfected with Fugene 6 transfection reagent (according to Roche Molecular Biochemical's specifications) and pcDNA3.1 DNA containing sequence coding for hM_1, hM_2, hM_3, hM_4, or hM_5 receptor (approximately 20 μg DNA/150-mm dish) and either empty pcDNA3.1 (for hM_1, hM_3, and hM_5) or pcDNA3.1 containing sequence coding for the chimeric G protein Gαq/i (for hM_2 and hM_4). Approximately 24 h after the addition of DNA and transfection agent, the inositol lipid pool of cells was radiolabeled by incubating the cells overnight in inositol-free DMEM medium containing 1 μCi of *myo*-[^{3}H]inositol per well. Approximately 12 h postlabeling, cells were preincubated with either drug or vehicle for 5 min. [^{3}H]Inositol accumulation was initiated by addition of LiCl (final concentration of 10 mM) and either vehicle or the indicated drug concentration. The assay was terminated by aspirating the medium and adding 750 μL ice-cold 50 mM formic acid. After 30 min at 4 °C, the samples were neutralized with 250 μL 150 mM NH_4OH. [^{3}H]Inositol phosphates were isolated by ion-exchange chromatography on Dowex AG 1-X8 columns and quantitated by liquid scintillation counting.[23]

Statistical Analysis. Nonlinear regression and paired *t*-test were performed using GraphPad Prism version 3.00 for Windows, GraphPad Software, San Diego, California; www.graphpad.com.

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Supporting Information Available: ^{1}H, ^{13}C NMR spectra for compounds **2**−**7**, **10**, and **11**. NOESY spectra for **3**−**6** and functional assays at the human M_1 and M_2 receptor using Sf9 insect cells. Tables of elemental analyses and mass spectral data for key intermediates and final products. This material is available free of charge via the Internet at http://pubs.acs.org.

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