In Vitro Muscarinic Activity of Spiromuscarones and Related Analogs

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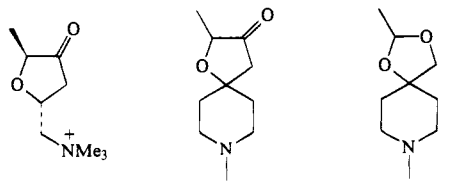
The cholinergic hypothesis of Alzheimer's disease suggests that cholinergic agonists may have therapeutic potential for treating the attendant memory deficits of the disease. As part of a program aimed at preparing metabolically stable, nonquaternary analogs of muscarone, 1-oxa-2,8-dimethyl-8-azaspiro[4.5]decan-3-one, **2a**, and related analogs have been synthesized and their *in vitro* muscarinic activity evaluated. The synthetic strategy in the formation of the 1-spiro[4.5]decan-3-one ring system of **2a** involved cyclization of the diol **4** in the presence of Nafion-Hg. The spiromuscarone **2a** was found to displace [^{3}H]Oxo-M binding with a K_i value of 7 nM. Affinities of the oxime and hydrazone analogs of **2a** were lower than **2a**. The compounds in these series were partial muscarinic agonists as demonstrated by stimulation of phosphatidyl inositol hydrolysis assay, with **2a** showing the highest intrinsic activity (60% as compared with carbachol). The results from this study indicate that an exo double bond at the C-3 position is essential for the receptor binding.

Introduction

Alzheimer's disease (AD), the most common cause of dementia in the elderly, is a neurodegenerative disorder characterized clinically by progressive loss of memory and neuropathologically by the presence of senile plaques and neurofibrillary tangles in regions of neuronal loss.[1c] Although the etiology of AD remains to be determined, there are certain neurochemical abnormalities and pathological changes in AD brains. Among the most profoundly affected are the cholinergic neurons found in basal forebrain and cortical and hippocampal brain regions involved in cognitive functions. The selective loss in cholinergic neurochemical markers together with pharmacological evidence in humans and animals that cholinomimetic agents can improve cognition while cholinergic antagonists impair memory functions forms the basis of the cholinergic hypothesis of AD.[1] The hypothesis suggests that enhancement of cholinergic transmission would alleviate the short memory loss exhibited by AD patients.

On the basis of this hypothesis, a great deal of effort has been made to design and develop centrally active muscarinic agonists or agents which can improve cholinergic function as a means of treating Alzheimer's disease.[2,3a,3b] One potential therapeutical target is the M_1 subtype muscarinic receptor which is located primarily on postsynaptical, noncholinergic neurons and which remains intact despite of progressive cholinergic neuronal loss.[1] Although numerous naturally occurring compounds such as muscarine, muscarone, and acetylcholine (a neurotransmitter) are potent muscarinic agonists, they are not therapeutically useful due to their inability to penetrate the blood–brain barrier.[2] On the other hand, tertiary amines such as arecoline, pilocarpine (both are natural products), and oxotremorine are proven to be centrally active.[2,3] However, the latter three compounds are weak partial agonists, and their clinical efficacy has been difficult to demonstrate.[3c] Recently many synthetic, metabolically stable, and nonquaternary muscarinic agonists have been published in the literature.[3] Several of the synthetic compounds are being evaluated in clinical trials.[3d-f]

Muscarone **1**, a stable and potent muscarinic agonist with some nicotinic agonist activity,[2b] was chosen as a model compound for rational drug design. One approach to design a centrally active muscarone analog is to reconstruct the (trimethylammonio)methyl side chain of muscarone as a piperidine to yield the conformationally semirigid spiromuscarone **2a** (1-oxa-2,8-dimethyl-8-azaspiro[4.5]decan-3-one). The resulting tertiary amine is related to the acetal analog **3**, a potent muscarinic agonist.[4] Instead of having a labile acetal group as shown in **3**, compound **2a** has a rather stable chemical moiety, 3-ketofuran, and therefore is expected to be as metabolically stable as the parent muscarone.



1 2 3

Molecular Modeling

The proposed interaction of muscarinic ligand and receptor requires a cationic head and two hydrogen binding sites.[6] As both muscarone and the acetal **3** bind well to muscarinic receptors, the ether oxygen and the carbonyl group in both molecules must serve as acceptors for hydrogen binding to the receptor. It is likely that the carbonyl group in **2a** is equivalent to the oxygen atom at the 3-position of **3** in terms of 3-dimensional space or hydrogen-bonding property. Using molecular modeling,[5] the lowest energy conformations of **2a** and **3** were calculated. Two minimized molecules, **2a** and **3a**, are shown to have good overlap (RMS 0.0318) when the nitrogen atom, the C-5 atom, and the ether oxygen (at the 1-position) of furan were superimposed.

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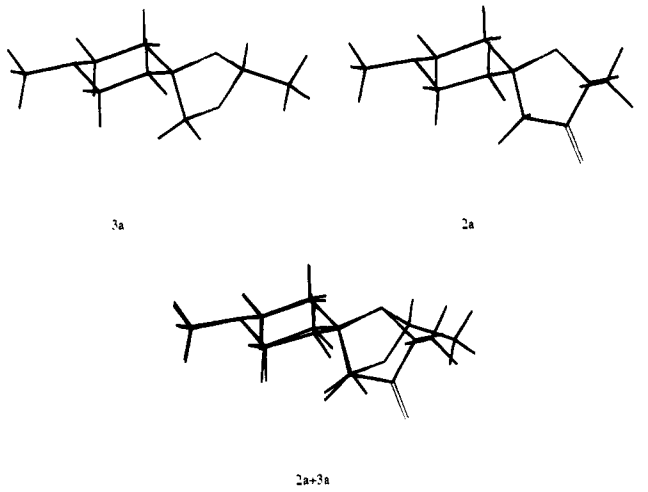
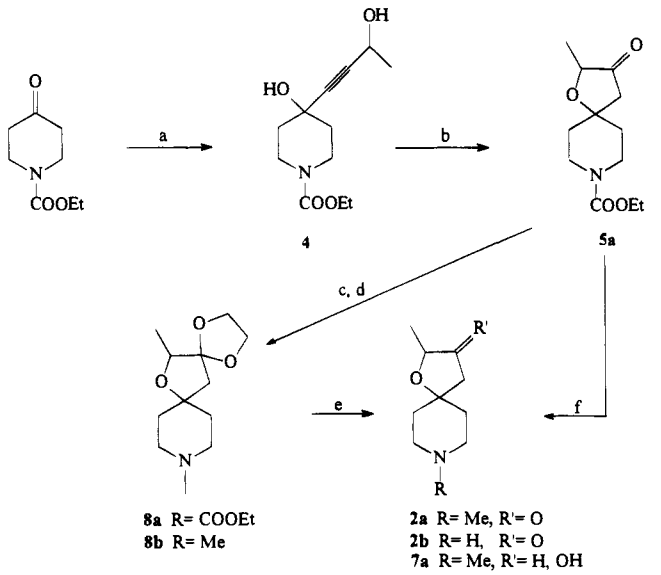


Figure 1. Superimposition of the lowest energy conformations **2a** and **3a**.

Scheme 1

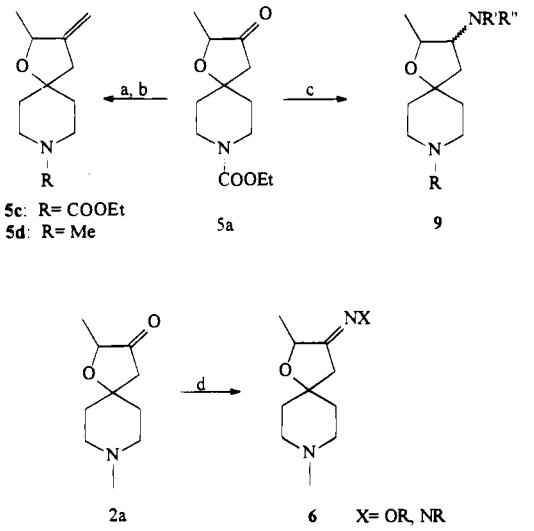


[a] (a) MeCH(OH)C≡CH, *n*-BuLi/THF, −78 °C; (b) Nafion-Hg; 67% yield; (c) $HOCH_2CH_2OH$/TsOH/Δ; (d) Vitride; (e) HCl/Δ; (f) (1) Vitride, (2) $(COCl)_2$/DMSO/$(i\text{-}Pr)_2NEt$.

Synthesis

The general strategy employed in the synthesis of the spiromuscarone **2a** consisted of construction of an 1-oxaspiro[4.5]decane from cyclization of a 4-substituted *N*-carbethoxypiperidine. Synthesis of **2a** was carried out as shown in Scheme 1. Alkylation of *N*-carbethoxy-4-piperidone with the dianion of 3-hydroxy-1-butyne yielded the diol **4** (68% yield). On the basis of the cyclization conditions of 2-butyne-1,4-diol derivatives to 4,5-dihydro-3(2*H*)-furanones,[7] the protected spirofuranone **5a** was obtained regiospecifically from the diol **4** in the presence of Nafion-Hg.[7] The corresponding 4-furanone **15a** (see Scheme 4) was only isolated in a small quantity when a large-scale synthesis of **5a** was performed. Formation of the spirofuranone ring of **2a** from 4-(3-hydroxy-1-butynyl)-1-(ethoxycarbonyl)-4-piperidinol proved to be a more efficent route than the procedure described by Tsukamoto et al.[8] Ketalization of **5a** with ethylene glycol in the presence of *p*-toluenesulfonic acid gave the ketal **8a** which was reduced with Vitride to the ketal amine **8b**. Hydrolysis of **8b** with HCl afforded the ketone **2a**. Although this is a three-step synthesis, i.e., protection−reduction−deprotection process, the overall yield from **5a** was 82%. Alternatively the reduction of **5a** to **7a** (see Table 1) with Vitride followed by the Swern oxidation gave **2a** in 38% yield (purified). This route, the two-step conversion of **5a** to **2a**, tended to give a lower yield and is apparently not better than the previous three-step one, as the reduction also produced the corresponding secondary amine, which is unstable. Hydrolysis of **5a** with concentrated HCl yielded the secondary amine **2b**. Similar to the preparation of the ketal amine **8b**, the thioketal amine **8c**[8a] (Table 1) was obtained from treating **2a** with ethylene dithiol in the presence of borontrifluoride etherate in 28% yield.

Scheme 2



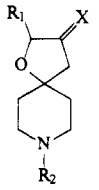
[a] (a) CH_2=$P(Ph)_3$; (b) Vitride; (c) (1) NHRR', (2) Vitride; (d) NH_2OR or NH_2X.

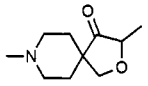
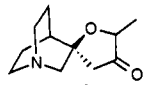
The carbamate ketone **5a** was converted to the olefin carbamate **5c** in 57% yield, treating **5a** with methylenetriphenylphosphorane, formed from methyltriphenylphosphonium bromide with t-BuOK in THF (Scheme 2). Reduction of **5c** with Vitride produced the corresponding amine **5d**. Oximes or related analogs were obtained when the ketone **2a** was reacted with appropriate hydroxyamines or related amines respectively.

The stereochemical outcome of reducing **2a** depends on the reduction method used. Vitride reduction of **5a** or sodium borohydride reduction of **2a** afforded the alcohol **7a** as a mixture of the trans and the cis isomers with the trans being the major isomer (ca. 3:1 ratio of the trans vs the cis isomers based on the NMR spectrum), while the cis isomer **7e** was prepared exclusively from the L-selectride (Aldrich) reduction.[8b] The decoupling experiments by 500 MHz NMR gave additional support for the structural assignment of the trans isomer in **7a**. The result of the sodium borohydride reduction agrees with the literature report.[9a] The ether **7b** (Table 1; ca. 3:1 ratio of the trans vs the cis isomers based on the methine proton at C-3 in the NMR spectrum) was prepared by reducing **5a** with sodium borohydride followed by O-alkylation and the Vitride reduction.

The amino derivative **9** was prepared by reducing the oxime derivatives of **5a** or related compounds with Vitride or reductive amination of **2a** (Scheme 2). The amine **9a** (Table 1) was prepared by reducing the oxime

Table 1. Physical Properties and Pharmacological Activity of Spiromuscar-3-one Derivatives[a]



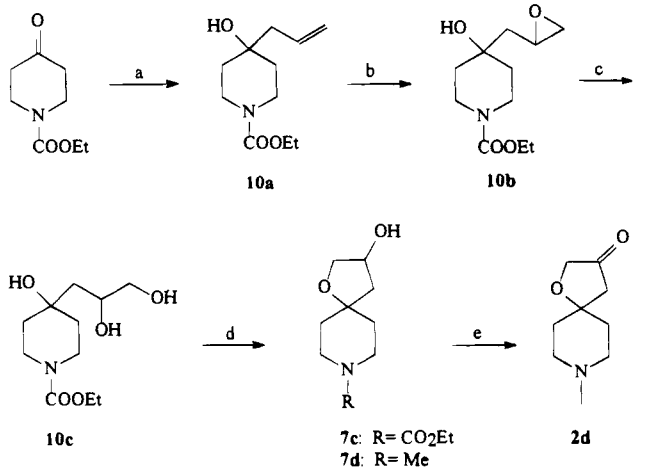
no.	R_1	R_2	X	mp, °C	yield, %	formula	analyses	[^{3}H]Oxo-M K_i, μM	max. PI, %, of carbachol[b]	[^{3}H]NMS K_i, μM	ratio of NMS/ Oxo-M
2a	CH_3	CH_3	=O	139–141	11	$C_{10}H_{17}NO_2{\cdot}C_4H_4O_4$[c]	C,H,N	0.007	60	2.68	380
2b	CH_3	H	=O	150–151	6	$C_9H_{16}NO_2{\cdot}C_4H_4O_4$	C,H,N	0.005	27		
2c	C_2H_5	CH_3	=O	132–133	34	$C_{11}H_{20}NO_2{\cdot}C_4H_4O_4$	C,H,N	0.036	17	2.58	70
2d	H	CH_3	=O	127–129	60	$C_9H_{16}NO_2{\cdot}C_4H_4O_4$	C,H,N	0.032	12	83.3	260
2e	H	H	=O	325	36	$C_8H_{13}NO_2{\cdot}HCl$	C,H,N,Cl	0.340	NA		
2f	CH_3	$(CH_3)_2$	=O	218–220	80	$C_{10}H_{17}NO_2{\cdot}CH_3I$	C,H,N	0.027	30		
3								0.035	36	25.1	711
5a	CH3	$CO_2C_2H_5$	=O	liquid	41	$C_{12}H_{19}NO_4$	C,H,N	175	NA		
5b	H	$CO_2C_2H_5$	=O	liquid	42	$C_{11}H_{17}NO_4$	H,N;C[d]	1000	NA		
5d	CH_3	CH_3	$=CH_2$	188–189	68	$C_{11}H_{19}NO_2{\cdot}HCl$	H,N,Cl;C[e]	0.46	9		
5e	H	CH_3	$=CH_2$	98–100		$C_{10}H_{17}NO_2{\cdot}C_4H_4O_4$	C,H,N	3.07	NA	87.4	28
6a	CH_3	CH_3	=NOH	170–172	62	$C_{10}H_{18}N_2O_2{\cdot}C_4H_4O_4$[c]	C,H;N[f]	0.029	15	1.63	56
6az	CH_3	CH_3	=NOH	122.5–24	18	$C_{10}H_{18}N_2O_2$	C,H,N	0.014	19		
6ae	CH_3	CH_3	=NOH	159–161	30	$C_{10}H_{18}N_2O_2$	C,H,N	0.064	38		
6bz	CH_3	CH_3	$=NOCH_3$	147–150	11	$C_{11}H_{20}N_2O_2{\cdot}C_4H_4O_4$	C,H,N	0.09	24		
6be	CH_3	CH_3	$=NOCH_3$	146–147	31	$C_{11}H_{20}N_2O_2{\cdot}C_4H_4O_4$	C,H,N	0.30	17		
6c	H	CH_3	=NOH	183–184	63	$C_9H_{16}N_2O_2{\cdot}C_4H_4O_4$	C,H,N	0.19	NA		
6d	H	CH_3	$=NOCH_3$	149–152	37	$C_{10}H_{18}N_2O_2{\cdot}C_4H_4O_4$[c]	C,H,N	0.98	NA		
6e	H	CH_3	$=NOCOCH_3$	160–162	30	$C_{11}H_{16}N_2O_3{\cdot}C_4H_4O_4$	C,H;N[g]	0.19	5	44.8	236
6f	CH_3	CH_3	$=NOCOC_2H_5$	116–118	9	$C_{13}H_{22}N_2O_3{\cdot}C_4H_4O_4$	C,H,N	0.077	20		
6g	H	CH_3	$=NOCOC_2H_5$	136–137	14	$C_{12}H_{20}N_2O_3{\cdot}C_4H_4O_4$	C,H,N	0.30	<5	38.1	126.7
6h	H	CH_3	=NOCOPh	147–150	28	$C_{16}H_{20}N_2O_3{\cdot}C_4H_4O_4$	H,N;C[h]	0.91	NT	34	37.4
6i	CH_3	CH_3	$=NOCOCHMe_2$	133–136	4	$C_{14}H_{24}N_2O_3{\cdot}C_4H_4O_4$	C,H,N	0.09	16		
6j	CH_3	CH_3	$=NNHCOCH_3$	164–167	50	$C_{12}H_{21}N_3O_2{\cdot}C_4H_4O_4$	C,H,N	0.40	30		
6k	CH_3	CH_3	$=NNHCO_2C_2H_5$	137–139	67	$C_{13}H_{23}N_3O_3$	C,H,N	0.40	19		
6l	CH_3	CH_3	$=NNHCONH_2$	204–206	15	$C_{11}H_{20}NO_2{\cdot}C_4H_4O_4$	C,H,N	0.80	26		
6m	CH_3	CH_3	$=NOCONMe_2$	165–169	12	$C_{17}H_{27}N_3O_3{\cdot}C_4H_4O_4$c	C,H,N	0.55	12		
7a	CH_3	CH_3	OH, H[m]	oil	99	$C_{10}H_{19}NO_2$	H,N;C[i]	2.71	NA	141	52
7b	CH_3	CH_3	OCH_3, H[m]	oil	84	$C_{11}H_{21}NO_2$	H,N;C[j]	1.47	NA	39.9	27
7c	H	CH_3	OH, H	228–229	30	$C_9H_{17}NO_2{\cdot}HCl$	C,H,N,Cl	8.54	NA	734	86
7d	H	CH_3	$OCOCH_3$, H	122–123	19	$C_{11}H_{19}NO_3{\cdot}C_4H_4O_4$[c]	C,H,N	3.5	NA	98.7	28
7e	CH_3	CH_3	OH(cis), H[m,n]	oil	59	$C_{10}H_{19}NO_2{\cdot}HCl$	C,H,N	8.2	NA	430	52
8b	CH_3	CH_3	$-OCH_2CH_2O-$	105–108	81	$C_{12}H_{21}NO_3{\cdot}C_4H_4O_4$	C,H,N	1.81	IA	36.8	20
8c	CH_3	CH_3	$-SCH_2CH_2S-$	115–116	28	$C_{12}H_{21}NO\,S_2{\cdot}C_4H_4O_4$	C,H,N	0.13	IA	1.47	10
9a	CH_3	CH_3	NH_2, H[m]	115–120	51	$C_{10}H_{20}N_2O{\cdot}2C_4H_4O_4$	C,H,N	4.0	NA		
9b	CH_3	CH_3	NHCOMe, H[m]	115 dec	19	$C_{12}H_{22}N_2O_2{\cdot}C_4H_4O_4$[b]	H,N;C[k]	3.03	8		
9c	CH_3	CH_3	$NHCO_2Et$, H[m]	oil	22	$C_{13}H_{24}N_2O_3$	H,N;C[l]	0.65	<5		
9d	CH_3	CH_3	$N(CH_3)_2$, H	127–130	30	$C_{12}H_{24}N_2O{\cdot}2C_4H_4O_4$	C,H,N	1.80	NA		
9e	H	CH_3	NH_2, H	132–136	0.6	$C_9H_{18}N_2O{\cdot}2C_4H_4O_4$	C,H,N	1.50	NA		
20				134–136	39	$C_{10}H_{17}NO_2{\cdot}C_4H_4O_4$	C,H,N	0.55	6		
22				200 dec	46	$C_{11}H_{16}NO_2Cl$	C,H,N,Cl[p]	0.030	9		
24	arecoline							0.0074	28	7.12	960
25	aceclidine							0.016	23	8.26	516
26	pilocarpine							0.026	37	3.67	141
27	(−)-muscarone							0.00076	88[o]	0.70	930

[a] Melting points are not corrected. Purified yield was given here and calculated based on the last step reaction. $C_4H_4O_4$ stands for maleic acid unless otherwise noted in the molecular formula. Elemental analyses were within 0.4% of the original unless otherwise noted. [b] Percent of maximum stimulation of phosphotidyl inositol (PI) turnover by an agonist in rat hippocampal tissue as compared to the carbachol response; NA = not aavailable; IA = inactive. [c] Fumarate. [d] C: calcd for $C_{11}H_{17}NO_4$ containing 1.84% CH_2Cl_2, 57.33; found, 56.63. [e] C: calcd, 60.68; found, 60.18. [f] N: calcd, 8.91; found, 9.55. [g] N: calcd (adjusted for 1.20% H_2O and 0.24% EtOAc), 8.06; found, 6.92. [h] C: calcd, 59.25; found, 58.72. [i] C: calcd (adjusted for 6.04% H_2O), 60.92; found, 61.71. [j] C: calcd, 66.30; found, 65.74. [k] C: calcd (adjusted for 0.62 equiv of fumaric acid and 0.66 equiv of H_2O), 56.07; found, 55.53. [l] C: calcd (adjusted for 1.42% H_2O and 7.34% CH_2Cl_2), 56.61; found, 55.98. [m] Ratios of the *trans* and the *cis* isomers are 3/1 for **7a** and **7b**, pure *cis* for **7e**, 2/1 for **9a**, **9b**, and **9d**, >95% for **9c**. [n] This sample used for testing was a resolved cis alchohol (*S*,*S*). [o] The response was measured at 10 μM. [p] Contained 2.97% H_2O.

6a with Vitride. On the basis of the previous configurational assignment of compounds **7a** and **7b**, **9a** and **9d** were tentatively assigned as a mixture of the trans and the cis isomers in a ratio of 2/1. Similarly, **9b** contained a ratio of 2/1, predominating with the trans isomer, and **9c** was the trans isomer (>95%).

The stereochemistry of oximes such as pairs of **6az** (*Z*-isomer) and **6ae** (*E*-isomer) and **6bz** (*Z*-isomer) and

Scheme 3



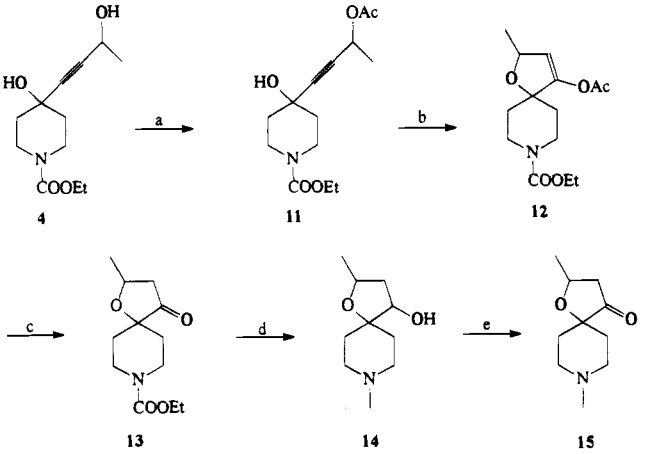
[a] (a) Allylmagnesium bromide; (b) MCPBA; (c) $HClO_4$; (d) *p*-TsCl (excess)/110 °C, 58%; (e) (1) Vitride, 59%, (2) $(COCl)_2$/DMSO/(i-$Pr)_2$NEt, 70%.

6be (*E*-isomer) was determined based on ^{13}C NMR[9b] and the nuclear Overhauser effect in ^1H NMR. The "*Z*" and "*E*" notation refers to the orientation of the oxime oxygen relative to the 2-methyl in the syn and anti sense, respectively. The chemical shifts of the methine or the methyl peak at the 2-position, the methylene protons at the 4-postion in ^1H NMR, and their corresponding carbons in ^{13}C NMR were used to assign the stereochemistry of the oxime (**6az** and **6ae**) relative to 2-methyl group. In the proton NMR the 2-methine of the *Z*-isomer resonates at lower field (4.75 ppm) than that of the corresponding *E*-isomer (4.48 ppm). As expected, the chemical shift of the methine carbon (71.03) in **6az** in the ^{13}C NMR is at the higher field than that of the *E*-isomer (72.14), whereas the 4-methylene carbon reverses their chemical shift order (40.39 ppm for *Z*- and 37.82 ppm for *E*-isomers). In the case of the hydrazone **6j**, only one isomer (the *E*-isomer) was isolated and detected. To determine the configuration, standard decoupling experiments were performed. A moderate NOE enhancement in ^1H NMR was observed between the 4-CH_2 and the NH of the =NNHCOMe.

Following a procedure for making 1-oxaspiro[4.5]-decan-3-one,[10] the desmethyl analog (**2d**) of **2a** was prepared via the triol **10c**, as shown in Scheme 3. Reaction of *N*-carbethoxy-4-piperidone with the Grignard reagent allylmagnesium bromide afforded the alcohol **10a**, which was epoxidized with *m*-chloroperbenzoic acid to give **10b**. Hydrolysis of the epoxide **10b** with perchloric acid gave the triol **10c**. The overall yield for these three steps was 18%. Treatment of **10c** with an excess of *p*-tosyl chloride in pyridine at 110 °C produced the cyclized alcohol **7c** in 58% yield. Compound **7c** was reduced by Vitride followed by the Swern oxidation to **2d**.

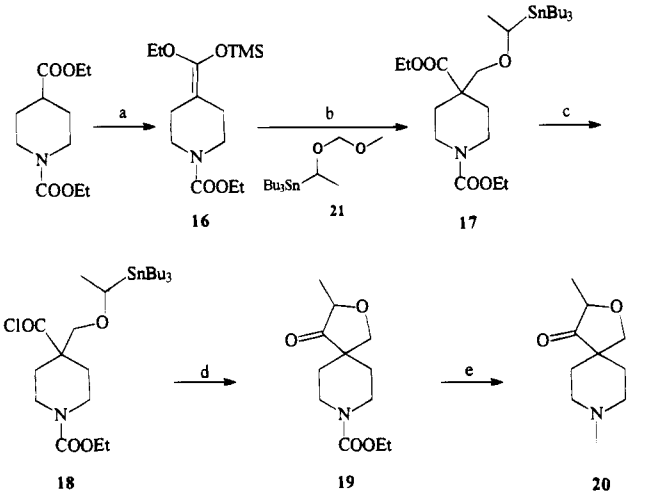
The 4-ketospirofuran **15** was prepared starting from **4** (see Scheme 4). Selective monoacetylation of **4** followed by Ag(I)-catalyzed rearrangement and cyclization produced the enol acetate **12** in excellent yield (89%),[7] which was hydrolyzed with LiOH to the ketone **13** (77% yield). Both the ketone and the *N*-carbethoxy group of **13** were simultaneously reduced in the presence of Vitride to **14** (59% yield). The alcohol **14** was shown by NMR to be a mixture of the trans and the cis isomers in a ratio of 7:1, and the trans isomer was assumed to

Scheme 4



[a] (a) Acetic anhydride, 100%; (b) $AgClO_4$/80 °C, 89%; (c) LiOH, 77%; (d) Vitride, 54%; (e) $(COCl)_2$/DMSO/(i-$Pr)_2$NEt, 64%.

Scheme 5



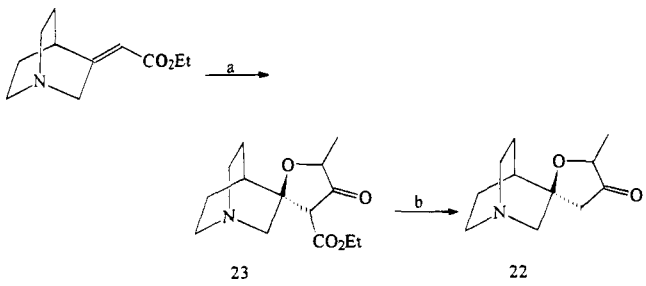
[a] (a) LDA/THF/−78 °C, Me_3SiCl, 71%; (b) **21**, $TiCl_4$/−78 °C, 23–29%; (c) KOH/aqueous MeOH, $(COCl)_2$/DMF, 70%; (d) $Pd(PPh_3)_4$, 86%; (e) Vitride, $(COCl)_2$/DMSO/i-Pr_2Net, 39%.

the major isomer based on the previous argument for **7a**. The Swern oxidation of **14** completed the synthesis of **15**.

Spirofuranone **20** was prepared by the method of Linderman et al.[11a] according to Scheme 5. Formation of the enolate of ethyl 1-(ethoxycarbonyl)piperidine-4-carboxylate using lithium diisopropylamide followed by trapping of the anion with trimethylsilyl chloride gave the silyl ketene acetal **16** in good yield (71%). Compound **16** was condensed with alkoxystannane **21** (prepared by the procedure of W. C. Still[11b]), using titanium tetrachloride as the Lewis acid, to provide ester **17** in 23–29% yield. Conversion of the ester function to acid chloride **18** (70% yield) set the stage for the crucial ring closure via transmetalation using palladium tetrakis(triphenylphosphine), yielding the spirofuranone **19** in excellent yield (86%). Reduction to the tertiary amino alcohol followed by Swern oxidation then provided the desired furanone **20** in 39% yield as its maleate salt.

Synthesis of the 3-quinuclidylspirofuranone **22**[8] was accomplished in 46% yield starting from 3-(carbethoxymethylene)quinuclidine[17] by the method of Tsukamoto et al.[8] (Scheme 6), while **22** could not be prepared as shown in Scheme 1. The Michael addition of the

Scheme 6



23 22

[a] (a) MeCH(OH)COOEt/NaH/DMF; (b) 1 N HCl.

ethyl lactate anion with 3-(carbethoxymethylene)quinuclidine[17] followed by the Dieckmann cyclization produced the keto ester **23** which was hydrolyzed and decarboxylated in refluxing 1 N HCl to give **22**.

Biochemistry

Binding. The affinity of all compounds to muscarinic receptors was determined by their ability to displace [^{3}H]-*N*-methylscopolamine (NMS), an antagonist, and [^{3}H]oxotremorine-M (Oxo-M), an agonist, in rat brain membranes.[13] The ratio of NMS/Oxo-M affinity constants was employed to predict the ability of agonists to stimulate phosphatidyl inositol (PI) turnover at muscarinic receptors. Full agonists possessed a ratio of >4000. Partial agonists such as pilocarpine and RS-86 had intermediate NMS/Oxo-M ratios (100–150). A second group of compounds including oxotremorine and arecoline had somwhat higher ratios (500–1400). Muscarinic antagonists displayed affinity ratios of 1–3.[13]

Functional Assay. Stimulation of phosphatidyl inositol hydrolysis *in vitro*,[14b,c] in rat hippocampal slices, an effect which is mainly mediated through M_1 receptors,[15] was used as a direct measure of intrinsic activity.[13]

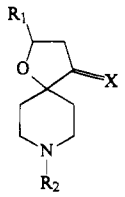
Results and Discussion

The pharmacological complexity of mAchRs (muscarinic acetylcholine receptor) has long indicated not only the existence of multiple receptor subtypes, but also that each subtype is distinct in the cellular and physiological function of acetylcholine which it carries out. MAchRs belong to the family of G-protein-coupled receptors where the efficacy of G-protein coupling takes place at a site different from that of the ligand–receptor binding.[12] Muscarinic receptor binding assays are useful in drug discovery. However, their limitation lies in that they only provide information about receptor affinity, but not relative efficacy. To circument this problem, Freeman et al.[13] used the affinity ratio of [^{3}H]NMS/[^{3}H]-Oxo-M bindings of a muscarinic ligand in rat cerebral cortex as an index to predict its efficacy.

Using the [^{3}H]NMS/[^{3}H]Oxo-M-derived affinity ratio, full and partial agonists could, in most instances, be distinguished from compounds with little or no intrinsic activity. For example, compounds **8b** and **8c** (Table 1), with affinity ratios of 20 and 10, were inactive, while compounds such as arecoline **24**, aceclidine **25**, and the acetal **3** (Table 1) with ratios of 960, 516, and 711 produced measurable increases of 28%, 23%, and 36%, respectively, in PI hydrolysis. However, contrary to the published work[13] the Oxo-M/NMS affinity ratio was not predictive when attempting to make quantitative comparisons of intrinsic activity among partial agonists in our hands. This is illustrated in the following examples. Compound **2a** with a ratio of 380 was found to have greater intrinsic activity than arecoline, while compounds **6e** and **2c** (Table 1, ratio = 236 and 70, respectively) displayed low intrinsic activity (5% and 17%, respectively). Furthermore, Oxo-M/NMS affinity ratios and measured intrinsic activities of aceclidine **25** and pilocarpine **26** were actually reversed (Table 1). We therefore used direct measures of intrinsic activity as the basis for comparing relative efficacies of partial agonists.

While the eutomer of muscarone **27** (Table 1) is a full muscarinic agonist (88% increase in phosphatidyl inositol (PI) turnover at 10 μM), the newly designed semirigid spiromuscarone **2a** was shown to be a partial agonist (60% in efficacy). This suggests that the decrease in activity might be due to either the steric factor or the change of the cationic center, i.e., a quaternary nitrogen vs the tertiary nitrogen. However, the affinity of **2a** was same as that of arecoline (K_i in the Oxo-M: 7 vs 7.4 nM, respectively) and was better than that of **3** (K_i = 35 nM). The former was also more efficacious than the later two compounds in stimulation of PI hydrolysis (60% vs 28% for arecoline and 36% for **3**). This demonstrates that the 3-ether oxygen of **3** can be substituted with a carbonyl group. This conversion not only retained the muscarinic activity but also enhanced both the potency and M_1 efficacy. When a bulkier group, e.g., **2c** (R_1 = Et), is substituted at the C-2 position of the spiro ring system, the affinity as well as intrinsic activity decreased as compared with the parent **2a**, indicating a steric effect in this position. Similar observations have been well-documented in the literature where an introduction or extention of one carbon unit at certain positions of an agonist leads to an antagonist, a partial agonist, or a weaker partial agonist.[16a,b] On the other hand, when the C-2 methyl of **2a** was replaced with a hydrogen, i.e., compound **2d**, both *in vitro* activities (K_i = 32 nM; PI = 12%) were also reduced and were the same as those of **2c** (Table 1). The affinity of compound **2e** (R_1, R_2 = H) was 10-fold lower than those of **2c** and **2d**. These facts suggest that the size of the substituent at the C-2 position of 1-oxa-8-azaspiro[4.5]decan-3-one is critical for its activity and the 2-methyl group appears to be optimal for the muscarinic activity. The secondary amine analog **2b** exhibited a similar Oxo-M affinity (K_i = 5 nM) to that of the corresponding tertiary amine **2a**, but the intrinsic activity of this compound was only 27% of the carbachol response. A similar trend was reported for oxadiazoles.[16c] The quaternary ammonium salt **2f** (Table 1) exhibited an affinity K_i of 27 nM for the Oxo-M binding and an M_1 efficacy of 30%. Surpringly, the *in vitro* activity of **2f** was not as good as that of the corresponding tertiary amine **2a**. Traditionally, muscarinic agonists bearing a quaternary nitrogen are far better than the corresponding tertiary amines in terms of affinity and efficacy, as ligands are required to have a good cationic center to bind to the aspartate residue of the receptor.[2,16b] *N*-Methyl substitution in the piperidine produced the best M_1 efficacy whereas the secondary amine sometimes improved the potency (**2b** vs **2a**; also see **15b** vs **15a** in Table 2). Protection of the piperidine nitrogen as carbamates such as **5a** and **5b** (R_2 = COOEt, Table 1) resulted in loss of potency in

Table 2. Physical Properties and Pharmacological Activity of Spiromuscar-4-one Derivatives[a]



no.	R_1	R_2	X	mp, °C	yield, %	formula	analyses	K_i, μM [^{3}H]Oxo-M	K_i, μM [^{3}H]NMS	ratio of NMS/Oxo-M
14	CH_3	CH_3	OH,[d] H	161–168	35	$C_{10}H_{19}NO_2 \cdot {}^2/_3C_4H_4O_4$[b]	C,H,N	330	30	11
15a	CH_3	CH_3	=O	128–130	23	$C_{10}H_{17}NO_2 \cdot C_4H_4O_4$	C,H,N	2.5	33	13
15b	CH_3	H	=O	167–169	73	$C_9H_{15}NO_2 \cdot HCl$	H,N;C[c]	0.64	71	110

[a] Melting points are not corrected. $C_4H_4O_4$ stands for maleic acid unless otherwise noted in the molecular formula. Elemental analyses were within 0.4% of the original unless otherwise noted. [b] Fumarate. [c] C: calcd (adjusted for 1.39% H_2O), 51.83; found, 51.35. [d] *Trans*/*cis*: 7/1.

the Oxo-M assay. This was an expected outcome because the requisite basic nitrogen no longer exists in the molecule.

The second study on the structure–activity relationship (SAR) was undertaken to evaluate the importance of the carbonyl group, a polarizable bond capable of acting as a hydrogen-bonding acceptor. Replacing the carbonyl group with a nonpolarizable olefin produced compounds **5d** and **5e** (Table 1) which displayed low affinity (K_i = 0.46 and 3.07 μM, respectively) and M_1 intrinsic activity (9% for **5d**). Similar structural modification has been performed on muscarone, i.e., the replacement of the carbonyl group with an olefin, leading to similar results.[18a,b] The reduction in activity could be attributed to inability of an olefin bond to form a hydrogen bond with the postulated serine residue.[6] Similar to the findings in spirofuranones (**2a**, **2d**, and **2e** in Table 1), the desmethyl analog **5e** displayed lower affinity than that of **5d**.[19] This structural modification revealed the importance of a carbonyl group in the receptor binding, which is possibly acting as a binding site.

The results shown above suggested the importance to retain a polar sp^2-hybridized group in the molecule for further SAR studies. One of the directions, then, was to replace the carbonyl group with different polar groups such as oximes, hydrazones, and imines. To this end, several oximes and hydrazones were prepared. The oxime **6a** (Table 1), a mixture of *Z*- and *E*-isomers, was found to be a weak partial agonist with a K_i = 29 nM (Oxo-M), a low ratio of NMS/Oxo-M (56), and a 15% stimulation of PI hydrolysis. The *E*-isomer **6ae** of this oxime **6a** exhibited a better efficacy (38%) than that of *Z*-isomer **6az** (19%); however, the *Z*-isomer (14 nM) had better affinity than the *E*-isomer (64 nM) in the Oxo-M binding. Upon converting parent oximes to methyl oxime ethers, **6be** and **6bz**, the PI hydrolysis for the *Z*-isomer **6bz** (24%) remained essentially unchanged while binding affinities (90 and 300 nM for the *Z*- and *E*-isomers, respectively) for both isomers and the intrinsic activity for the *E*-isomer (17%) decreased. Several oxime esters were also synthesized and tested to evaluate if an additional polar group would enhance the activity. Similar activity profiles to those of **6a** were observed for the oxime esters (**6f** and **6i** in Table 1) with 2-methyl substitution. As discussed in the previous SAR, the desmethyl oxime esters (**6c**, **6d**, **6e**, **6g**, and **6h**) again produced lower affinity and efficacy than the parent oxime **6a** or its corresponding 2-methyl oxime esters. The oxime amide **6m** (X = $OCONMe_2$, Table 1) demonstrated a weak partial agonist profile with a weak binding to Oxo-M (K_i = 0.55 μM). While three hydrazones (**6j–l** in Table 1) exhibited low affinity (0.4–0.8 μM), these compounds showed moderate intrinsic activity (19–30%). This study demonstrates that replacement of the carbonyl group in **2a** with an imine bond attenuates the muscarinic activity of **2a**. Some efforts were made to prepare some simple imines; however, they were found to be too unstable chemically and were not pursued further.

The other way to mimic the polarity of the carbonyl group is to make use of the amino or hydroxy group. This structural modification would further provide information about whether the double bond or π-electrons are necessary for the activity. Reduction of the ketone **2a** with vitride produced the muscarine-like compound **7a** (Table 1) which contained 75% of the trans isomer, a configuration similar to that of muscarine. While muscarine is a full agonist (the NMS/Oxo-M ratio = 4200, 87% in PI),[13] both **7a** and **7e** displaced [^{3}H]Oxo-M poorly in the receptor binding assay (K_i = 2.71 μM). Similar affinity (1.42–8.54 μM) was found for all the hydroxy derivatives (**7b–d** in Table 1). In the amine series, the derivatized analogs (**9b–d**, Table 1) appeared to have better affinity (K_i = 3.03, 0.65, and 1.80 μM, respectively) than the the parent amine (**9a**, K_i = 4 μM). However, their intrinsic activity, based on compounds tested, was weak. An unusual observation was that the desmethyl compound **9e** (K_i = 1.5 μM) exhibited better binding than **9a**, although both affinities were low. Two ketal derivatives **8b** and **8c** (Table 1) were also tested for muscarinic activity. Although the thio analog **8c** is 14 times better than the oxygen analog **8b** in the Oxo-M binding, both were found to have low NMS/Oxo-M ratios (10 and 20, respectively) and to be inactive in the functional assay. These results confirmed that a polarizable double bond is essential for the muscarinic activity of the 1-oxa-8-azaspiro[4.5]decan-3-one ring system.

To gain more insight about ligand–receptor interaction in terms of binding sites, relocation of the ketone from the 3-position to the 4-position was made. A significant drop (K_i = 2.5 μM; 359-fold decrease) in the binding affinity was found for the resulting compound **15a** (Table 2). The secondary amine **15b** (K_i = 0.64 μM) is more potent than **15a** and exhibited the highest NMS/

Oxo-M ratio in this table. The alcohol derivative **14** (Table 2) was totally inactive in the Oxo-M binding.

Other modifications made were (1) replacing piperidine with a bulkier quinuclidine ring, compound **22**[8] (Table 1), to see if receptors would allow the extra volume present and (2) interconverting the oxygen atom for the carbonyl and vice versa, compound **20** (Table 1), or substituting the 2-ether oxygen of **3** with a carbonyl group to explore the electrostatic interaction as well as spatial distance between ligand and receptor. The quinuclidine analog **22** showed good affinity (K_i = 0.030 μM for the Oxo-M), while **20** only weakly bound to the receptors (K_i = 0.55 μM). Both compounds had low efficacy in stimulating PI turnover (6% for **20** and 9% for **22**), however. Apparently the size of the quinuclidine ring greatly reduced the muscarinic efficacy but only slightly lowered the affinity. The poor muscarinic profile of **20** further indicates that replacement of a 2-ether oxygen with a carbonyl group results in a significant loss of the activity.

As compound **2a** was proven to be the best compound in this SAR study, further profiling of this compound was made.[21] A comparison of the apparent K_i to displace [^{3}H]pirenzepine, a selective M_1 antagonist, and [^{3}H]NMS[22] (3.7 and 5.7 μM, respectively) revealed that the compound is not a selective agonist. This was further confirmed by an M_2 functional assay[20] in which **2a** exhibited a full agonist response (EC_{50} = 12.5 μM) in inhibiting cAMP formation in heart membranes as compared to carbachol. In *in vivo* models the ketone was shown to lower rectal temperature in the hypothermia test[23] and to demonstrate a robust effect in improving the memory of old rats and scopolamine-impaired middle-aged rats in a T-maze working memory model.[24] The current trend in the design and synthesis of muscarinic agonists for Alzheimer's disease has focused on the development of a centrally acting M1-selective agonist, as traditional muscarinic agonists have little or no muscarnic receptor subtype selectivity, thus limiting their clinical usefulness due to side effects associated with stimulation of the M_2 and M_3 muscarnic receptors.[3d,16,25] Consequently the design of subtype-selective agonists are being actively pursued in our laboratories.

In summary, the present study revealed that **2a** is the most potent and efficacious compound among our spirofuranone derivatives synthesized. The compound is a nonselective and centrally acting muscarinic agonist. The partial agonist activity was optimum with a 2-methyl substituent in the furanone ring. The 3-keto functionality is critical for good activity, and a basic nitrogen is essential for binding to receptors.

Experimental Section

The melting points were determined in capillary tubes on a Thomas-Hoover apparatus and are uncorrected. NMR spectra were determined in the indicated solvent mostly on a Brucker AC 200 and sometimes on a Brucker AMX500 NMR spectrometer at the ambient operating temperature with tetramethylsilane (TMS) as the internal standard for proton spectra unless otherwise stated. Chemical shifts are given in ppm, and coupling constants are in hertz. Splitting patterns are designated as follows: s, singlet; br s, broad singlet; d, doublet; t, triplet; q, quartet; m, multiplet. Infrared spectra were recorded on a Nicolet MX-1 Fourier transform infrared spectrophotometer. Elemental analyses were performed internally and were within +0.4% of the theoretical value when indicated by symbols of the element unless otherwise noted. Mass spectra were recorded by Hewlett-Packard HP 5988A quadrupole mass spectrometer using desorption chemical ionization (CI) or extrel quadrupole using electron impact (EI) probe at 70 eV.

4-(3-Hydroxy-1-butynyl)-1-(ethoxycarbonyl)-4-piperidinol (4). A solution of 3-butyn-2-ol (42 g, 0.6 mol) in dry THF (800 mL) under nitrogen was cooled with a dry ice/acetone bath. *n*-Butyllithium (390 mL of 2.5 M and 230 mL of 1.5 M) in hexane was added rapidly dropwise. The suspension became gelatinous, and another 800 mL of dry THF was added. The reaction mixture was stirred at approximately −78 °C for 1 h and then at 0 °C for 20 min. The reaction mixture was cooled to −78 °C, and 1-(ethoxycarbonyl)-4-piperidinone (104 g, 0.607 mol) in 300 mL of dry THF was added rapidly dropwise. The reaction mixture was allowed to warm to room temperature and then stirred overnight. The reaction mixture was cooled in an ice bath, diluted with THF (1.5 L), and decomposed with saturated NH_4Cl, and the THF layer was washed three times with 200 mL of saturated ammonium chloride. The aqueous layers were back-washed with THF (200 mL × 3). The organic layers were combined, dried over $MgSO_4$, and evaporated to give an oil. A one-fifth portion of the oil was purified by flash chromatography on silica gel, and elution with 30% ethyl acetate/ether afforded the desired product diol (19.7 g; 68% yield): ^{1}H NMR ($CDCl_3$) 4.55 (q, 1H, C*H*OH), 4.15 (q, 2H, OC*H*$_2$Me), 4.5−2.8 (m, 6H, 2 OH and CH_2NCH_2), 1.8 (m, 4H, $OC(CH_2)_2$), 1.5 (d, 3H, HOCC*H*$_3$), 1.3 (t, 3H, C*H*$_3$CH$_2$); IR (neat) 3340, 2240, 1680 cm^{-1}.

8-(Ethoxycarbonyl)-2-methyl-1-oxa-8-azaspiro[4.5]-decan-3-one (5a). The diol **4** (19.7 g, 0.082 mol) was dissolved in ethanol (82 mL) and water (7.4 g, 0.41 mol). Mercury/Nafion NR50 (41 g), prepared according to the method reported by Saimoto et al.,[7c] was added, and the slurry was stirred in a closed flask for 3 days. The resin was filtered off and washed with methylene chloride. The combined filtrates were evaporated. The residue was dissolved as far as possible in ether and filtered through a bed of silica gel to clarify the solution. The filtrate was evaporated to give the ketone as a yellow oil (13.3 g). The oil (7.5 g) was purified by column chromatography eluting with 3:1 hexane:ethyl acetate. This gave a colorless oil (4.54 g, 41% yield): ^{1}H NMR ($CDCl_3$) 4.15 (q, 2H, CH_2Me), 4.0 (q, 1H, CHO), 3.7 (m, 2H, NCH_2), 3.49 (m, 2H, NCH_2), 2.35 (m, 2H, CH_2C=O), 2.0−1.4 (m, 4H, $OC(CH_2)_2$), 1.2 (2t, 6H, 2 × CH_3); IR (neat) 1760, 1700, 1650 cm^{-1}. Anal. ($C_{12}H_{19}NO_4$) C, H, N.

8-(Ethoxycarbonyl)-2-methyl-1-oxa-8-azaspiro[4.5]-decan-3-one Ethylene Ketal (8a). The carbamate−ketone **5a** (23 g, 0.0963 mol), ethylene ketal (50 mL), and *p*-toluenesulfonic acid (1 g) were suspended in toluene (500 mL) and refluxed for 3 h while water and ethylene glycol were collected in a Dean−Stark trap. The solution was cooled and washed with water, and the aqueous layer was backwashed with chloroform. The combined solvent layers were dried and concentrated to give the title ketal as a thick oil (25.8 g).

2,8-Dimethyl-1-oxa-8-azaspiro[4.5]decan-3-one Ethylene Ketal (8b). The carbamate−ketal **8a** (10 g, 0.035 mol) in THF (50 mL) was added at room temperature during 0.5 h to a solution of Vitride (0.105 mol) in THF (150 mL). The reaction mixture was stirred for 1 h and then decomposed by the addition of 20 mL of 20% aqueous THF and then additional water until a clear supernatant layer formed. The solvent layer was separated and evaporated *in vacuo*. The residue was chromatographed on silica gel and eluted with 20% MeOH/CH_2Cl_2 to give the dimethyl ethylene ketal as a thick oil (1414 g). A sample of the oil (0.5 g) was converted to the maleate salt in ether and crystallized from methylene chloride/ether to give the salt (0.24 g), mp 105−108 °C.

2,8-Dimethyl-1-oxa-8-azaspiro[4.5]decan-3-one Maleate (2a). The ketal amine **8b** (14.4 g, 0.0634 mol) dissolved in 1.25 N HCl (100 mL) was heated at 50 °C for 4 h. The reaction mixture was cooled and basified with saturated aqueous Na_2CO_3 solution, and the precipitated base was extracted into chloroform. The chloroform solution was dried and the solvent evaporated *in vacuo* to give the ketone as an oil (13.35 g). A sample of the oil (4.46 g) was converted to the

maleate salt in ether. The precipitated salt was recrystallized twice from ethyl acetate to give the title product (3.11 g): mp 137.5–139 °C; ^{1}H NMR (DMSO-d_6) 8.6 (bs, 2H, COOH, exchangeable with D_2O), 6.1 (s, 2H, C=CH), 4.15 (q, 1H, CHO), 3.3 (bs, 4H, CH_2NCH_2), 2.8 (s, 3H, NCH_3), 2.6 (q, 2H, CH_2C=O), 1.9 (m, 4H, $OC(CH_2)_2$), 1.2 (d, 3H, CH_3); IR (KBr) 2500, 1762, 1617, 1576 cm^{-1}; MS *m/z* 183 (M^+). Anal. ($C_{14}H_{21}NO_6$) C, H, N.

Alternative Method for Preparing 2,8-Dimethyl-1-oxa-8-azaspiro[4.5]decan-3-one (2a). (a) *trans*- and *cis*-3-Hydroxy-2,8-dimethyl-1-oxa-8-azaspiro[4.5]decane (7a). The ketone **5a** (10.2 g, 0.042 mol) was dissolved in dry THF (375 mL) under nitrogen and cooled in an ice bath. Vitride (57 mL, 0.021 mol), diluted with THF (450 mL), was added dropwise, and the reaction mixture was stirred at room temperature overnight. The reaction mixture was cooled with an ice bath and decomposed with water added dropwise. After gas evolution ceased, the bath was removed and enough water was added to give a pasty white precipitate. The THF was decanted, and the precipitate was washed with THF. The THF layers were combined, dried, and stripped. The crude was purified by flash silica chromatography using 5, 10, and 20% MeOH in chloroform (NH_3). The title compound was isolated as an oil (6.8 g; 89% yield). On the basis of the NMR spectrum, the product was a 3:1 ratio of the trans vs the cis isomers: ^{1}H NMR (500 mHz) 4.14 [bs, 1/4 H, *CH*(OH), cis isomer], 3.94 (m, 1 H, *CH*Me for cis and *CH*(OH) for trans), 3.87 (m, 3/4 H for trans), 2.1–2.8 (m, 4H, CH_2NCH_2), 2.35 (s, 3H, NCH_3), 1.8–2.05 (m, 4H, CH_2CCH_2), 1.73 [bs, 2H, CH(OH)C*H*$_2$], 1.26 (d, 3H, $OCCH_3$, cis), 1.25 (d, 3H, $OCCH_3$, trans).

(b) 2,8-Dimethyl-1-oxa-8-azaspiro[4.5]decan-3-one Fumarate (2a). Oxalyl chloride (6.1 g, 4.8 mmol) was dissolved in dry CH_2Cl_2 (720 mL), placed under N_2, and cooled to −60 °C with a dry ice/acetone bath. Dry DMSO (7.4 g, 95 mmol) in dry CH_2Cl_2 (150 mL) was added dropwise. The reaction mixture was stirred for 10 min. The alcohol **7a** (8.0 g, 44 mmol) in CH_2Cl_2 (360 mL) was added in slow drops, the temperature being maintained below −60 °C. The reaction mixture was stirred for 20 min and then was treated dropwise with diisopropylethylamine (28.3 g, 219 mmol). The bath was removed and the reaction mixture allowed to warm somewhat. The solution was treated with distilled water (720 mL) in rapid drops. A little saturated Na_2CO_3 was added to the mixture to ensure a basic pH, and the layers were separated. The aqueous layer was extracted four times with CH_2Cl_2. The organic layers were dried with Na_2SO_4 and stripped. Purification by silica flash chromatography, using 2–20% MeOH in $CHCl_3$ (NH_3), gave the title product as a yellow oil (3.4 g; 42% yield). The amine (2 g) was dissolved in ether and treated with an equivalent amount of fumaric acid dissolved in ether. The precipitated salt was filtered and dried to give the title compound (1.15 g), mp 139–141 °C.

***cis*-3-Hydroxy-2,8-dimethyl-1-oxa-8-azaspiro[4.5]decane (7e).** L-Selectride (1 M in THF, 60 mL, 0.06 mol) was slowly added to a solution of 5.40 g (0.03 mol) of the ketone **2a** in 150 mL of dry THF at −78 °C, and the temperature was maintained below −70 °C. After the addition, the reaction mixture was stirred at −78 °C for 1/4 h and then at 0 °C for 1/4 h. Acetone (30 mL) was added to the reaction mixture. The resulting mixture was stirred at room temperature overnight, a small amount of water was added to decompose a trace of borane, and the mixture was evaporated to give a viscous liquid. Purification by silica flash chromatography, using 5% MeOH in CH_2Cl_2 (NH_3), gave 3.5 g (59%) of the cis alcohol: ^{1}H NMR 4.14 [bs, 1H, C*H*(OH)], 3.94 (m, 1H, C*H*Me), 3.60 (bs, 1H, OH), 2.35 (s, 3H, NCH_3), 2.1–2.8 (m, 4H, CH_2NCH_2), 1.8–2.05 (m, 4H, CH_2CCH_2), 1.73 [t, 2H, CH(OH)C*H*$_2$], 1.26 (d, 3H, $OCCH_3$).

2-Methyl-1-oxa-8-azaspiro[4.5]decan-3-one Maleate (2b). The carbamate ketone **5a** (5 g, 20.7 0.0207 mol) was dissolved in concentrated HCl (34 mL) and water (16 mL) and heated at reflux for 2.5 h. Toluene (125 mL) was added, and the water was removed by azeotroping. The toluene was evaporated *in vacuo*. The residue thus obtained was purified by flash column chromatography, eluting to 3–10% methanol (ammoniated) in chloroform. The isolated product was taken up with ethyl acetate and filtered. The filtrate was treated with maleic acid (0.58 g) in ethyl acetate and evaporated. The residue was dissolved in boiling 2-propanol and stored in the freezer for several days. A tan solid (0.35 g) was collected and washed with cold 2-propanol: mp 150–151 °C; ^{1}H NMR (DMSO-d_6) 8.6 (bs, 2H, COOH, exchangeable with D_2O), 6.1 (s, 2H, C=CH), 4.1 (q, 1H, CHO), 3.2 (bs, 4H, CH_2NCH_2), 2.6 (d, 2H, C*H*$_2$C=O), 1.9 (m, 4H, $OC(CH_2)_2$), 1.3 (d, 3H, CH_3); IR (KBr) 2500, 1750 cm^{-1}; CIMS $(M + 1)^+$ 170. Anal. ($C_{13}H_{20}NO_6$) C, H, N.

3-Methoxy-2,8-dimethyl-1-oxa-8-azaspiro[4.5]decane (7b). (a) 8-(Ethoxycarbonyl)-3-hydroxy-2-methyl-1-oxa-8-azaspiro[4.5]decane. To a cooled (0 °C) solution of the carbamate ketone **5a** (10.13 g, 42 mmol) in absolute EtOH (50 mL) under nitrogen was added dropwise a suspension of sodium borohydride (0.7 g, 18.5 mmol). The reaction mixture was then allowed to warm up to room temperature for 2 h. The solution was cooled with ice bath, treated with 2.5 N HCl (28 mL), and evaporated. The residue was dissolved in water and extracted with chloroform three times. The extracts were combined and dried (Na_2SO_4), giving 10.4 g of a yellow oil (100% crude yield).

(b) 8-(Ethoxycarbonyl)-3-methoxy-2-methyl-1-oxa-8-azaspiro[4.5]decane. Sodium hydride (2.1 g of 60% NaH oil dispersion) was washed several times with petroleum ether and then added to a cooled (0 °C) solution of the crude hydroxycarbamate (11.4 g, 47 mmol), obtained from the above reaction, in dry THF (450 mL) in the presence of nitrogen. A solution of methyl iodide (33.4 g, 0.235 mol) in dry THF (75 mL) was added dropwise at 0 °C to the anion solution. The reaction mixture was then allowed to warm up to room temperature overnight, cooled with an ice bath, treated with water (20 mL), and diluted with brine. The organic layer was separated, and the aqueous layer was extracted with THF once. The residue obtained from the dried organic layers was purified by silica flash chromatography and eluted with ether to give 8.4 g (69% yield).

(c) 3-Methoxy-2,8-dimethyl-1-oxa-8-azaspiro[4.5]decane (7b). Similar to the method used to prepare **7a**, the methoxycarbamate (8.4 g in 100 mL of THF, 33 mmol), obtained from the step b, was reduced with Vitride (100 mmol) to afford the title compound (5.5 g, 84% yield): ^{1}H NMR ($CDCl_3$) 3.8–4.2 (m, 1H, MeC*H*O), 3.18 (m, 1/4H, C*H*OMe, cis isomer), 3.47 (m, 3/4H, C*H*OMe, trans isomer], 3.36 (s, OCH_3, trans isomer), 3.35 (s, OCH_3, cis isomer), 2.06 (m, 4H, CH_2NCH_2), 2.22 (s, 3H, NCH_3), 1.5–2.0 (m, 6H, C*H*$_2$C=O, $OC(CH_2)_2$), 1.23 (d, CH_3, trans isomer), 1.21 (d, CH_3, cis isomer); IR (KBr) 2838, 2794, 2764 cm^{-1}; CIMS $(M + 1)^+$ 198. Anal. ($C_{11}H_{21}NO_2$) H, N; C: calcd, 66.30; found, 65.74.

2,8-Dimethyl-1-oxa-8-azaspiro[4.5]decanone Ethylene Thioketal Maleate (8b). To a cold (0 °C) solution of ketone **2a** (3.0 g, 16.4 mmol) in CH_2Cl_2 (60 mL) was added ethanedithiol (3.03 g, 32.2 mmol) followed by BF_3·OEt_2 (12 mL) keeping the temperature below 9 °C. the solution was stirred cold for 1 h and poured into 20% aq NaOH (180 mL), the insolubles were filtered off through Celite, and the cake was washed with water then EtOAc. The aqueous layer was washed with an additional two portions of EtOAc, The extracts were dried ($MgSO_4$), and the solvent was evaporated. The residue was purified by filtering through a pad of NH_3 deactivated silica gel eluting with $CHCl_3$–MeOH 20:1 then 10:1, affording 1.8 g (28%) of the title compound as an oil. The maleate had mp (EtOAc–Et_2O) 115–116 °C (lit.[8] mp 114–115 °C).

2,8-Dimethyl-3-methylene-1-oxa-8-azaspiro[4.5]decane Hydrochloride (5d). (a) 8-(Ethoxycarbonyl)-2-methyl-3-methylene-1-oxa-8-azaspiro[4.5]decane (5c). To a suspension of methyltriphenylphosphonium bromide (4.67 g, 13.1 mmol) in THF (120 mL) was added t-BuOK (1.47 g, 13.1 mmol) in one portion, and the mixture was stirred under nitrogen at room temperature overnight. A solution of the carbamate ketone **5a** (3.0 g, 12.4 mmol) in THF (10 mL) was added dropwise to the above ylide solution, and the stirring continued for 4 h. The reaction mixture was quenched with saturated ammonium chloride. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The combined layers were dried ($MgSO_4$) and evaporated to give a

residue. The residue was purified by flash column chromatography through silica gel, eluting with hexane/ethyl acetate (3:1), giving 1.70 g (57% yield) of an oil, an olefin.

(b) 2,8-Dimethyl-3-methylene-1-oxa-8-azaspiro[4.5]decane Hydrochloride (5d). Similar to the preparation of **5a** to **7a**, the olefin (1.60 g, 6.69 mmol) thus obtained was reduced with Vitride (5.6 mL, 20.1 mmol) in 25 mL of THF to give the title compound: 0.46 g; mp 187.5–188.5 °C; ^{1}H NMR ($CDCl_3$) 5.0 (dt, 1H, C=CH), 4.90 (dt, 1 H, C=CH), 4.45 (bq, 1H, C*H*Me), 3.5–2.95 (m, 4H, CH_2NCH_2), 2.75 (s, 3H, NCH_3), 2.45 (bs, 2H, CH_2C=O), 2.5–2.2 (4d, 2H, $OCCH_2$), 1.80 (bt, 2H, $OCCH_2$), 1.25 (d, 3H, CH_3); IR (KBr) 2700–2400, 1660 cm^{-1}; CIMS $(M + 1)^+$ 182. Anal. ($C_{12}H_{19}NO_2Cl$) H, N, Cl; C: calcd, 60.68; found, 60.182.

1-(Ethoxycarbonyl)-4-hydroxy-4-(2-propenyl)piperidine (10a). Allylmagnesium bromide was prepared *in situ* by suspending Mg turnings (21.2 g, 0.87 mol) in dry ether 700 mL and adding allyl bromide (34.8 g, 0.29 mol) gradually until the reaction initiated and then at a sufficient rate to maintain reflux. The reaction mixture was stirred at room temperature for 1.5 h. The reaction mixture was cooled to −15 °C with a methanol/ice bath, and 1-(ethoxycarbonyl)-4-piperidinone (25 g, 0.146 mol) was added in ether (700 mL). The reaction mixture was stirred at room temperature for 4 h and then left overnight. The reaction mixture was cooled with an ice bath while quenching with ammonium chloride (360 mL of a saturated solution diluted to 1440 mL). The reaction mixture was stirred and the phases separated. The aqueous layer was extracted once more with ether, and the combined organic layers were washed with brine, dried, and stripped. Purification by flash chromatography on silica and elution with $CHCl_3$/NH_3 and then MeOH/$CHCl_3$/NH_3 gave the title compound as a yellow oil (17.2 g).

1-(Ethoxycarbonyl)-4-hydroxy-4-(2,3-epoxypropyl)piperidine (10b). The alcohol **10a** (17.2 g, 0.081 mol) was dissolved in dry CH_2Cl_2 (370 mL) under nitrogen. *m*-Chloroperbenzoic acid (80%, 35 g, 0.16 mol) was added, and the reaction mixture was stirred at room temperature overnight. The white precipitate was removed by suction filtration and washed with CH_2Cl_2. The CH_2Cl_2 was washed with 10% sodium sulfite, and this was extracted once with CH_2Cl_2. The combined organic layers were washed with 10% sodium hydrogen carbonate, and this was extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and stripped. The yellow oil obtained was stored under nitrogen in the freezer. Purification by silica flash chromatography, eluting with MeOH/$CHCl_3$, gave the title compound as a yellow oil (11.1 g): ^{1}H NMR ($CDCl_3$) 4.1 (q, 2H, CH_2Me), 3.95–2.45 (m, 7H, $OCHCH_2$, CH_2NCH_2), 2.1 (m, 2H, C*H*$_2$-epoxide), 1.65 (m, 4H, $OC(CH_2)_2$), 1.20 (t, 3H, CH_3); IR (neat) 3420, 2930, 1670 cm^{-1}.

1-(Ethoxycarbonyl)-4-hydroxy-4-(2,3-dihydroxypropyl)piperidine (10c). The partially purified epoxide **10b** (78.9 g, 0.34 mol) was dissolved in 250 mL of THF and 500 mL of deionized water. Concentrated $HClO_4$ (50 mL) was added, and the reaction mixture was stirred overnight. The solution was cooled with an ice bath and neutralized with saturated aqueous $NaHCO_3$. The suspension was then washed with CH_2Cl_2, and this was backextracted with H_2O. The aqueous layers were stripped. The resulting residue was digested with four portions of methanol. These were combined, diluted with $CHCl_3$, and dried over Na_2SO_4. The solvents were stripped, and the crude was purified by eluting from silica with an ammoniated methanol/$CHCl_3$ gradient. This gave 37.3 g of brown oil or 13% for the three steps from the starting piperidone.

8-(Ethoxycarbonyl)-3-hydroxy-1-oxa-8-azaspiro[4.5]decane (7c). The triol **10c** (5.2 g, 0.021 mol) was dissolved in dry pyridine (60 mL), placed under nitrogen, and cooled with an ice bath. Tosyl chloride (4.8 g, 0.025 mol) was dissolved in pyridine (30 mL) and added dropwise. The reaction mixture was heated at 110 °C. After 5 h another 2.2 g (0.011 mol) of tosyl chloride was added in 20 mL of pyridine at room temperature, and the heating was continued overnight. The pyridine was removed as an azeotrope with three portions of toluene and the residue digested seven times with anhydrous ether. The combined organic extracts were filtered and stripped. Purification on a silica flash column using MeOH/$CHCl_3$/NH_3 gave the title compound as a colorless oil (1.1 g). Further ether digestion of the residue, followed by extraction with ether and purification, gave a further 1.3 g of product.

3-Hydroxy-8-methyl-1-oxa-8-azaspiro[4.5]decane Hydrochloride (7d). The cyclized product **7c** (1.3 g, 5.7 m mol) was dissolved in dry THF (60 mL), placed under nitrogen, and cooled with an ice bath. Vitride (70%, 2.7 mL) in THF (30 mL) was added dropwise and the reaction mixture stirred at room temperature overnight. Three further portions of Vitride (5 mL each) in THF (15 mL each) were added dropwise to the cooled solution, and after each portion the solution was stirred for several hours. The reaction mixture was cooled again and treated with 5% NaOH until evolution of hydrogen ceased. The addition of NaOH was continued at room temperature until a sticky white paste had precipitated. The THF was decanted and suction filtered, and the paste was washed once with THF. The solvent was then dried and stripped, and the paste was washed once with THF. Purification by silica flash column using MeOH/$CHCl_3$ gave a viscous yellow oil (1.1 g) which partially solidified on standing to white needles. The solid was taken up in isopropyl alcohol and the solution cooled and acidified with HCl/ethanol. This gave a white precipitate which was collected and washed with cold ether to yield the title compound as the hydrochloride salt (0.47 g): mp 228–229 °C; ^{1}H NMR (DMSO-d_6) 10.9 (bs, 1H, HCl), 5.0 (bs, 1H, OH), 4.4 (bs, 1H, C*H*OH), 3.8 and 3.6 (m, 2H, CH_2O), 3.1(m, 4H, CH_2NCH_2), 2.7 (s, 3H, NCH_3), 1.9 (m, 4H, $OC(CH_2)_2$), 1.7 (m, 2H, C*H*$_2$CHOH); IR (KBr) 3400, 2720 cm^{-1}; MS $(M+1)^+$ = 172. Anal. ($C_9H_{18}ClNO_2$) C, H, N, Cl.

8-Methyl-1-oxa-8-azaspiro[4.5]decan-3-one Maleate (2d). Oxalyl chloride (1.9 g, 14.3 m mol) was dissolved in dry CH_2Cl_2 (150 mL), placed under N_2, and cooled to −60 °C with a dry ice/acetone bath. Dry DMSO (2.2 g, 29 m mol) in dry CH_2Cl_2 (30 mL) was added in slow drops. The reaction mixture was stirred for 10 min. The free base of **7d** (1.5 g, 8.8 mmol) in CH_2Cl_2 (100 mL) was added in slow drops, the temperature being maintained below −60 °C. The reaction mixture was stirred for 20 min and then treated dropwise with diisopropylethylamine (9.0 g, 67.5 mmol). The bath was removed and the reaction allowed to warm somewhat. The solution was treated with distilled water (150 mL) in rapid drops. The layers were separated, and the aqueous layer was extracted three times with CH_2Cl_2. A little saturated Na_2CO_3 was added, and two more CH_2Cl_2 extractions were done. The organic layers were dried with Na_2SO_4 and stripped. Purification by silica flash chromatography, using MeOH/$CHCl_3$/NH_3 gave the title product as a yellow oil (1.5 g). The yellow oil was taken up in ethyl acetate, the solution was cooled in an ice bath, and maleic acid/ethyl acetate was added. The title compound was obtained as an off-white powder: mp 127–128.5 °C; ^{1}H NMR (DMSO-d_6) 6.1 (s, 2H, C=CH), 4.0 (s, 2H, CH_2O), 3.3 (m, 4H, CH_2NCH_2), 2.8 (d, 3H, NCH_3), 2.6 (q, 2H, CH_2C=O), 1.95 (m, 4H, $OC(CH_2)_2$); IR (KBr) 2675, 1760, 1580 cm^{-1}; CIMS $(M + 1)^+$ 170. Anal. ($C_{13}H_{20}NO_6$) C, H, N.

4-Acetoxy-8-(ethoxycarbonyl)-2-methyl-1-oxa-8-azaspiro[4.5]-3-decene (12). (a) 1-(Ethoxycarbonyl)-4-hydroxy-4-(3-acetoxy-1-butynyl)piperidine (11). The carbamate diol **4** (3.4 g, 14 mmol) in dry methylene chloride (10 mL) was treated under nitrogen with acetic anhydride (10 mL) and anhydrous pyridine (0.67 g, 8.4 mmol) and stirred at room temperature for 4 h. The solvent and high-boiling materials were evaporated under aspirator initially followed by vacuum pump. The residue was purified by flash column chromatography using 20% ethyl acetate in ether. This gave ~100% yield of the product (3.96 g) as an oil.

(b) 4-Acetoxy-8-(ethoxycarbonyl)-2-methyl-1-oxa-8-azaspiro[4.5]-3-decene (12). 1-(Ethoxycarbonyl)-4-hydroxy-4-(3-acetoxy-1-butynyl)piperidine (14.5 g, 0.051 mol) was combined with dry toluene (150 mL) and silver perchlorate (0.6 g). The reaction vessel was wrapped in foil, and the reaction mixture was heated under nitrogen at 80 °C for 19 h. A further 0.2 g of silver perchlorate were added, and heating was continued for another 5 h. The cooled solution was diluted with methylene chloride (150 mL) and washed with 450 mL

of 10% aqueous ammonium hydroxide. The organic layer was dried and concentrated. The residue was purified by flash chromatography in ammoniated silica gel and eluted with 4% hexane/ether. The enol acetate was isolated: 12.8 g; ^{1}H NMR ($CDCl_3$) 5.80 (d, 1H, C=CH), 5.0 (dq, 1H, C*H*CH_3), 4.20 (q, 2H, C*H*$_2$$CH_3$), 3.2 (m, 4H, CH_2NCH_2), 2.2 (s, 3H, $OCOCH_3$), 1.8 (m, 4H, $OC(CH_2)_2$), 1.5 (t, 3H, $CHCH_3$, overlapped with the multiplets centered at 1.8 ppm); IR (KBr) 1780, 1700 cm^{-1}.

2-Methyl-1-oxa-8-azaspiro[4.5]decan-4-one Hydrochloride (15b). The enol acetate **12** (3.4 g, 0.012 mol) was heated with concentrated HCl (15 mL) in toluene (40 mL) at reflux under Dean–Stark conditions. After 3 h, additional concentrated HCl (10 mL) was added, and heating was continued for 2 h. The toluene was evaporated, the residue was heated at reflux with concentrated HCl (15 mL) for a further 3 h, and toluene was added to remove the water. Evaporation of the toluene afforded a solid which was triturated with ether to remove impurities. The insolubles were dissolved in 2-propanol, treated with charcoal, and filtered, and the filtrate was concentrated to dryness. The solid residue was stirred in ether, and the ether was decanted to leave the title ketone hydrochloride as a solid.

4-Hydroxy-2,8-dimethyl-1-oxa-8-azaspiro[4.5]decane Fumarate (14). (a) 4-(Ethoxycarbonyl)-2-methyl-1-oxa-8-azaspiro[4.5]decan-4-one (13). The enol acetate **12** (12.6 g, 0.044 mol) was dissolved in THF, under nitrogen, and cooled with an ice bath. A solution of lithium hydroxide (1.0 g) in water was added. Water was added until solution was obtained. After 3 h at 0 °C, the reaction mixture was warmed to room temperature and another 2 g of lithium hydroxide was added. The THF phase was separated, and the aqueous phase was extracted fruther with THF. The combined extracts were dried and concentrated, and the residue was purified by chromatography on silica gel and elution with ammoniated hexane/ethanol (3:7). Evaporation of the solvents afforded the title ketone (8.2 g) which was used directly in the next step.

(b) 4-Hydroxy-2,8-dimethyl-1-oxa-8-azaspiro[4.5]decane Fumarate (14). Following essentially the same procedure as in the preparation of **7a**, the carbamate ketone **13** (8.2 g, 0.034 mol) afforded the titled alcohol **14** as an oil (3.4 g). A sample of the oil (1 g) was converted to the fumarate salt by treatment with fumaric acid in ether (124 mL). The precipitated solid was purified by trituration with ethyl acetate: mp 161–168 °C; ^{1}H NMR (DMSO-d_6/D_2O) 4.1 [m, $^1/_8$ H, C*H*(OH)], 3.90 [m, $^7/_8$H, C*H*(OH)].

(c) 2,8-Dimethyl-1-oxa-8-azaspiro[4.5]decan-4-one Maleate (15a). Following the same procedure as described in the conversion of **7a** to **2**, the alcohol **14** (2.4 g, 0.013 mol) was oxidized to **15a** and isolated as maleate salt (0.9 g): mp 128–130 °C; ^{1}H NMR (DMSO-d_6/D_2O) 6.1 (s, 2H, C=CH), 4.4 (m, 1H, C*H*CH_3), 3.7 (HOD), 3.3–3.1 (m, 4H, CH_2NCH_2), 2.7 (s, 3H, NCH_3), 2.6–2.4 (m, 2H, CH_2C=O), 1.8 [m, 4H, $OC(CH_2)_2$], 1.3 (d, 3H, CH_3); IR (KBr) 2500, 1760, 1700, 1620, 1580 cm^{-1}; CIMS (M + 1)$^+$ 184. Anal. ($C_{15}H_{24}NO_6$) C, H, N.

3-Amino-2,8-dimethyl-1-oxa-8-azaspiro[4.5]decane (9a). (a) 8-(Ethoxycarbonyl)-2-methyl-1-oxa-8-azaspiro[4.5]-decan-3-one Oxime. The carbamate ketone **5a** (20 g, 0.083 mol) was dissolved in absolute ethanol (1 L) and combined with triethylamine (11.5 mL) and hydroxylamine hydrochloride (5.7 g). The mixture was stirred at room temperature overnight. The ethanol was evaporated *in vacuo*, and the residue was stirred with ether. The insoluble solids were filtered, and the ether filtrate was evaporated. The residual oxime was purified by chomatography on silica gel and eluted with ammoniated 2–10% MeOH/$CHCl_3$. Evaporation of the solvents afforded the oxime, 11.5 g.

(b) 3-Amino-2,8-dimethyl-1-oxa-8-azaspiro[4.5]decane Maleate (9a). A solution of 8-(ethoxycarbonyl)-2-methyl-1-oxa-8-azaspiro[4.5]decan-3-one oxime (9.2 g, 0.036 mol) from step a in dry THF (180 mL) was added dropwise to a suspension of lithium aluminum hydride (5.5 g, 0.098 mol) and aluminum chloride (0.55 g, 4 mmol) in dry THF (360 mL). The reaction mixture was stirred overnight at room temperature. The reaction mixture was cooled and decomposed by successive addition of water (5.5 mL), 15% aqueous NaOH (5.5 mL), and water (16.5 mL). The solids were filtered off, and the THF filtrate was concentrated *in vacuo*. The residual oil was dissolved in chloroform, dried, and evaporated. The crude amine was purified by chomatography in silica gel and elution with ammoniated 10–40% MeOH/$CHCl_3$. The amine (1 g) was dissolved in hot ethyl acetate and treated with maleic acid (1.3 g). The gummy precipitate was dissolved in hot 2-propanol, and ethyl acetate was added until precipitation began. After 16 h, the precipitated solid was isolated by filtration and dried to give the title product (1.15g): mp 115–120 °C dec; ^{1}H NMR (DMSO-d_6) 8.5 (bs, 2H, NH_2), 6.05 (s, 2H, C=CH), 4.2 (m, 1H, C*H*CH_3), 3.93 and 3.77 (2m, 1H, C*H*NH_2), 3.6–2.6, (m, 6H, CH_2NCH_2, NH_2), 2.7 (s, 3H, NCH_3), 2.4–1.4 [m, 6H, CH_2C=O, $OC(CH_2)_2$], 1.27 (d, 2H, CH_3, trans isomer), 1.20 (d, 1H, CH_3, cis isomer); IR (KBr) 3450, 1800, 1620, 1500 cm^{-1}; CIMS (M + 1)$^+$ 185. Anal. ($C_{15}H_{24}NO_6$·1.54H_2O) C, H, N.

***(E)*-*N*-Acetyl-2,8-dimethyl-1-oxa-8-azaspiro[4.5]decan-3-one Hydrazone (6j).** The ketone **2a** (1.0 g, 5.5 mmol) was combined with acetic hydrazide (0.5 g, 6.7 mmol) and absolute ethanol (50 mL). Ethanolic HCl was added to pH 5, and then the reaction mixture was stirred at room temperature overnight. The ethanol was evaporated *in vacuo*, and the residue was dissolved in $CHCl_3$. The chloroform solution was washed with saturated Na_2CO_3. The $CHCl_3$ layer was evaporated to dryness. The crude product was purified by chromotography on silica gel and eluted with ammoniated 2–20% MeOH/$CHCl_3$ to give 0.86 g of a yellow solid. The solid was dissolved in hot ethyl acetate, and maleic acid (0.42 g) was added. The precipitated solid was filtered to give the title compound (0.98 g): mp 164–167 °C; ^{1}H NMR (DMSO-d_6, 500 MHz) 9.98 and 10.05 (2 s, 1H, NH or OH of two amide isomers), 4.35 (q, 1H, C*H*CH_3), 2.67 (AB q, 1H, CH_2C=), 2.45 and 2.19 (2m, 4H, N(C*H*$_2$$CH_2$)$_2$), 2.30 (AB q, 1H, CH_2C=), 2.18 (s, 3H, NMe), 2.06 and 1.90 (2s, 3H, NCOMe, two amide isomers), 1.60 (m, 4H, N(CH_2C*H*$_2$)$_2$), 1.22 (d, 3H, CHC*H*$_3$). Anal. ($C_{16}H_{25}N_3O_6$) C, H, N.

Preparation of (*E*)- and(*Z*)-2,8-Dimethyl-1-oxa-8-azaspiro[4.5]decan-3-one Oxime (6a). The starting ketone **2a** (9.09 g or 0.049 mol) was dissolved in absolute ethanol (750 mL). The hydroxylamine hydrochloride (4.1 g, mmol) and pyridine (0.72mL, 9.8 mmol) were added, and the reaction mixture was stirred under N_2 for 16 h. The reaction was complete by silica gel TLC. The ethanol was stripped on a Rotovapor, and the residue was partitioned between chloroform and saturated aqueous sodium carbonate. The aqueous layer was extracted four more times with chloroform, and the combined organic layers were dried with sodium sulfate and stripped. Purification by preparative LC using a silica Prep-pak (2–20% methanol/chloroform gradient, with ammoniation) gave 2.96 g of the *E*- or anti isomer and 1.73 g of the *Z*- or syn isomer, and 2.34 g mixed. Total yield 72%. The *E*- and *Z*-mixture was converted to the fumarate salt (ethyl acetate–ether), mp 170–172 °C. Anal. ($C_{14}H_{22}N_2O_6$) C, H, N.

The *Z*-isomer: mp 122.5–124 °C (toluene/petroleum ether); ^{13}C NMR ($CDCl_3$) 165.01 (C=N), 78.24 (quaternay C), 71.03 (CH), 52.42 and 52.04 (CH_2N), 45.84 (NCH_3), 40.39 (*C*H_2C=N, syn), 37.10 and 34.06 (*C*H_2CCH_2), 18.48 (CH_3); ^{1}H NMR ($CDCl_3$) 4.75 [q, 1H, C*H*CH_3 syn; overlaps HOD after exchange], 2.7–2.3 (bm, overlapped with a sharp singlet, 9H, CH_2NCH_2, CH_2C=N, NCH_3), 1.95–1.5 (4H, bm, CH_2CCH_2), 1.45 (d, 3H, CH_3); IR (KBr) 3220 cm^{-1}; CIMS (M + H)$^+$ *m/z* 199. Anal. ($C_{10}H_{18}N_2O_2$) C, H, N.

The *E*-isomer: mp 159–161 °C (toluene/petroleum ether); ^{13}C NMR ($CDCl_3$) 164.58 (C=N), 78.588 (quat. C), 72.14 (CH), 52.57 and 52.30 (NCH_2), 45.90 (NCH_3), 37.82 (*C*H_2C=N, *E*-isomer), 37.45 and 34.5 (CH_2), 19.41 (CH_3); ^{1}H NMR ($CDCl_3$) 4.48 (q, 1H, *E*-isomer, C*H*CH_3), 2.8–2.3 (complex m, 9H), 1.95–1.6 (complex m, 4H, CH_2CCH_2), 1.4 (d, 3H, CH_3); IR (KBr) 3220 cm^{-1}; CIMS (M + H) *m/z* 199. Anal. ($C_{10}H_{18}N_2O_2$) C, H, N.

1-(Ethoxycarbonyl)-4-[1-ethoxy-1-[(trimethylsilyl)oxy]-methylene]piperidine (16). Under a nitrogen atmosphere, a solution of 11.38 g (0.0496 mol) of ethyl 1-(ethoxycarbonyl)-piperidine-4-carboxylate in 25 mL of tetrahydrofuran was added dropwise over a 20 min period to a solution of 25 mL of 2.0 M lithium diisopropylamide in 50 mL of tetrahydrofuran, while stirring at −78 °C. The resulting solution was allowed

to warm gradually to 0 °C and then recooled to −78 °C; to this was added a solution of trimethylsilyl chloride (7.5 mL, 0.0591 mol) in 15 mL of tetrahydrofuran. The resulting solution was warmed slowly to ambient temperature, concentrated *in vacuo*, and then bulb-to-bulb distilled at 90−100 °C (0.1 Torr) to give 13.11 g of a 10:1 mixture of ketene acetal **16** (71%) and starting ester. **16**: ^{1}H NMR ($CDCl_3$) 0.03 (s, 9H, $Si(CH_3)_3$), 1.05 (t, 3H, J = 7.4 Hz, OCH_2CH_3), 1.09 (t, 3H, J = 7.4 Hz, OCH_2CH_3), 1.98 (m, 4H, $N(CH_2CH_2)_2$), 3.19 (m, 4H, $N(CH_2CH_2)_2$), 3.61 (q, 2H, J = 7.4 Hz, OCH_2CH_3), 3.94 (q, 2H, J = 7.4 Hz, OCH_2CH_3).

Ethyl 1-(Ethoxycarbonyl)-4-[[1-(tributylstannyl)ethoxy]methyl]piperidine-4-carboxylate (17). A solution of 1.85 g (4.88 mmol) of [1-(methoxymethoxy)ethyl]tributylstannane (**21**) in 10 mL of dichloromethane was added dropwise over 10 min to a solution of titanium tetrachloride (4.4 mL of a 1.0 M solution in dichloromethane) in 50 mL of dichloromethane cooled to −78 °C under a nitrogen atmosphere. Ketene acetal **16** (1.64 g, 4.85 mmol) was added, and the reaction mixture was warmed to ambient temperature over 1 h and then stirred for an additional 4 h. After the reaction was quenched with 50 mL of saturated aqueous ammonium chloride, the reaction mixture was diluted with 100 mL of water, the layers were separated, and the aqueous layer was extracted with 100 mL of dichloromethane. The combined organic layer was dried over anhydrous magnesium sulfate, concentrated *in vacuo*, and flash chromatographed through neutral silica gel using an 85:15 mixture of hexane and ethyl acetate as the eluant, giving 817 mg (29%) of ester **17** as a light yellow oil: ^{1}H NMR ($CDCl_3$) 0.91 (m, 18H), 1.39 (cp, 20 H), 2.07 (m, 2H, $N(CH_2HCH)_2$), 2.99 (m, 2H, $N(HCHCH_2)_2$), 3.20 and 3.53 (AB, 2H, J = 8.5 Hz, CCH_2O), 3.68 (q, 1H, J = 6.9 Hz, $OCHCH_3$), 3.89 (m, 2H, $N(HNHCH_2)_2$), 4.11 (q, 2H, J = 6.9 Hz, OCH_2CH_3), 4.16 (dq, 2H, J = 1.7, 6.9 Hz, OCH_2CH_3); IR (neat) 2957, 2927, 1728, 1704 cm^{-1}; MS (CI) $(M + 1)^+$ = 578.

1-(Ethoxycarbonyl)-4-[[1-(tributylstannyl)ethoxy]methyl]piperidine-4-carbonyl Chloride (18). A solution of 3.10 g (5.38 mmol) of ester **17** in 50 mL of petroleum ether was added to 50 mL of Claisen's alkali (prepared from 35 g of potassium hydroxide, 25 mL of water and 100 mL of methanol), and the reaction mixture was stirred at ambient temperature for 48 h. The reaction mixture was poured into 250 mL of ice-cold 2 N aqueous hydrochloric acid and extracted with chloroform (3 × 100 mL). The combined organic layer was dried over anhydrous magnesium sulfate, concentrated *in vacuo*, and filtered through a short pad of neutral silica gel using ethyl acetate as the eluant to provide 2.75 g of the acid as a colorless oil. The acid was then dissolved in 60 mL of dichloromethane and cooled in an ice/acetone bath to −5 °C under a nitrogen atmosphere. Dimethylformamide (0.1 mL) was added, followed by the dropwise addition of 8.0 mL of a 2.0 M solution of oxalyl chloride in dichloromethane over 15 min. The resulting solution was warmed gradually to ambient temperature over 1 h, then stirred at ambient temperature for 1 h, concentrated *in vacuo*, and flash chromatographed through neutral silica gel using a 2:1 mixture of hexane and ethyl acetate as the eluant to give 2.15 g (70% overall) of acid chloride **18** as a colorless oil: ^{1}H NMR ($CDCl_3$) 0.89 (m, 15H), 1.37 (cp, 20 H), 2.20 (m, 2H, $N(CH_2HCH)_2$), 3.08 (m, 2H, $N(HCHCH_2)_2$), 3.32 and 3.69 (AB, 2H, J = 8.5 Hz, CCH_2O), 3.72 (q, 1H, J = 7.4 Hz, $OCHCH_3$), 3.88 (m, 2H, $N(HNHCH_2)_2$), 4.11 (q, 2H, J = 6.9 Hz, OCH_2CH_3); IR (neat) 2957, 2927, 1793, 1705 cm^{-1}.

8-(Ethoxycarbonyl)-3-methyl-2-oxa-8-azaspiro[4.5]decan-4-one (19). Under an argon atmosphere, a solution of 245 mg (0.43 mmol) of acid chloride **18** and 80 mg of palladium tetrakis(triphenylphosphine) in 5 mL of tetrahydrofuran was refluxed for 6 h. Upon cooling to ambient temperature, the reaction mixture was diluted with 100 mL of ether, washed with 100 mL of water, dried over anhydrous magnesium sulfate, and concentrated to an orange oil. Flash chromatography through neutral silica gel gave 90 mg (86%) of spirofuranone **19** as a light yellow oil: ^{1}H NMR ($CDCl_3$) 1.30 (t, 3H, J = 6.9 Hz, OCH_2CH_3), 1.36 (d, 3H, J = 6.9 Hz, $CHCH_3$), 1.63 (m, 4H, $N(CH_2CH_2)_2$, 2.57 (m, 2H, $N(HCHCH_2)_2$, 3.85 and 4.23 (AB, 2H, J = 10.3 Hz, CCH_2O), 3.89 (m, 2H, $N(HCHCH_2)_2$, 3.93 (q, 1H, J = 6.9 Hz, $CHCH_3$), 4.16 (q, 2H, J = 6.9 Hz, OCH_2CH_3); IR (neat) 1754, 1696 cm^{-1}; MS (CI) $(M + 1)^+$ = 242.

3,8-Dimethyl-2-oxa-8-azaspiro[4.5]decan-4-one (20). Under a nitrogen atmosphere, a solution of 740 mg (3.06 mmol) of spirofuranone **19** in 10 mL of tetrahydrofuran was added dropwise over 10 min to a solution of 5.0 mL of Vitride (3.4 M in toluene) in 25 mL of tetrahydrofuran. The resulting solution was refluxed for 4 h and then cooled in an ice bath. 2-Propanol was added dropwise to destroy excess hydride, followed by water until a white precipitate formed. The clear solution was decanted, and the precipitate was washed with ether. The combined organic solution was concentrated *in vacuo*, and the resulting oil was dissolved in 5 mL of dichloromethane and then added to a solution of 2.0 mL of 2.0 M oxalyl chloride and 1.0 mL of dimethyl sulfoxide in 20 mL of dichloromethane, stirring at −78 °C under a nitrogen atmosphere. After stirring for 20 min, 1.0 mL of diisopropylethylamine was added and the reaction mixture was warmed to 0 °C over 1 h. The reaction mixture was quenched with 50 mL of saturated aqueous sodium bicarbonate, diluted with 100 mL of water and 100 mL of dichloromethane, and separated. The organic layer was dried over anhydrous magnesium sulfate and concentrated *in vacuo* to a yellow oil. Formation of the maleate salt in ethyl acetate gave 357 mg (39% overall) of furanone **20**, maleate salt: mp 134−136 °C (EtOAc); ^{1}H NMR (DMSO-d_6) 1.10 (d, 3H, J = 6.9 Hz, $CHCH_3$), 1.80 (m, 3H, $HCHCH_2NCH_2CH_2$), 2.79 (s, 3H, NCH_3), 3.41 [m, 5H, $N(CH_2CH_2)_2$ + $N(CH_2HCH)$], 3.87 and 4.25 (AB, 2H, CCH_2O), 4.07 (q, 1H, J = 6.9 Hz, $CHCH_3$), 6.05 (s, 2H, CH=CH); IR (KBr) 1745, 1585 cm^{-1}; MS (CI) $(M + 1)^+$ 184. Anal. ($C_{14}H_{21}NO_6$) C, H, N.

[1-(Methoxymethoxy)ethyl]tributylstannane (21). Under a nitrogen atmosphere, 25 mL of 2.0 M lithium diisopropylamide (in heptane/tetrahydrofuran/ethylbenzene) was added to 100 mL of ice-cold tetrahydrofuran, followed by 13.4 mL (50 mmol) of tributyltin hydride. The resulting solution was stirred for 15 min and then cooled to −78 °C, and a solution of 2.80 mL (50 mmol) of acetaldehyde in 10 mL of tetrahydrofuran was added over 2 min. After the mixture was stirred for an additional 5 min, the reaction was quenched with 25 mL of saturated aqueous ammonium chloride and 25 mL of water. The reaction mixture was diluted with 300 mL of water and extracted with 400 mL of petroleum ether. The organic layer was dried over sodium sulfate and concentrated *in vacuo* to a colorless oil, which was dissolved in 225 mL of dichloromethane containing 12.5 mL of *N,N*-dimethylaniline. This solution was cooled in an ice bath, under a nitrogen atmosphere, and 5.70 mL (75 mmol) of chloromethyl methyl ether was added. After stirring for 1 h, the reaction mixture was poured into 400 mL of petroleum ether and extracted with 0.05 N aqueous hydrochloric acid, 100 mL of water, and 100 mL of saturated aqueous sodium bicarbonate. After drying over sodium sulfate, the organic layer was concentrated *in vacuo*, and the residual oil was flash chromatographed through neutral silica gel using a mixture of 95:5 hexane/ethyl acetate as the eluant, giving 12.02 g (63%) of organostannane **21** as a colorless oil: ^{1}H NMR ($CDCl_3$) 0.91 (m, 15H), 1.42 (cp, 15H), 3.34 (s, 3H, OCH_3), 4.08 (q, 1H, J = 8.0 Hz, $OCHCH_3$), 4.53 and 4.66 (AB, 2H, J = 6.9 Hz, OCH_2O).

Dihydro-5′-methylspiro[1-azabicyclo[2.2.2]octane-3,5′-(4′H)-furan]-3′-one Hydrochloride (22). Sodium hydride (60% dispersion in mineral oil, 2.0 g, 51 mmol) was washed with hexane under a nitrogen atmosphere, suspended in DMF (20 mL), and cooled in an ice bath. Ethyl lactate (6.0 g) was added dropwise to keep the foaming under control, and the mixture was stirred at room temperature for 1.5 h. 3-(Carbethoxymethylene)quinuclidine[17] (2.0 g, 10.2 mmol) was added dropwise, and the solution was stirred overnight. The reaction mixture was acidified with a saturated solution of HCl in ethanol, and the mixture was diluted with ether (50 mL) followed by hexane (100 mL). The top liquid layer was decanted, and the bottom layer was diluted with ether (100 mL), precipitating a yellow solid. The material was suspended in $CHCl_3$−MeOH, 3:1 and filtered through Celite, and the solvent was evaporated. The residue, **23**, was heated at reflux

in 1 N HCl (20 mL) for 4 h, the reaction mixture was cooled, basified with 25% NaOH, and extracted with two portions of $CHCl_3$, the combined extracts were dried ($MgSO_4$), and the solvent was evaporated. The product was purified by flash chromatography through NH_3-deactivated silica gel, eluting with $CHCl_3$–MeOH, 20:1 then 10:1, affording 0.927 g (46% yield) of the title compound as a pale yellow oil. The HCl salt had mp (2-PrOH) 200 °C (dec) (lit.[8] mp 188–190 °C dec). Anal. ($C_{11}H_{16}NO_2Cl·2.97H_2O$) C, H, N.

Conformational Analysis. Using the Sybyl molecular modeling software program,[5] molecules were constructed by BUILD command. MAXIMIN2 was used to configurationally minimize the structures. RING SEARCH was then used for searching the global minimum. Overlap of two molecules was done by FIT. The 2-methyl group in the 5-membered ring was assigned as the *S*-configuration. The ether oxygen of **2a** or the 1-oxygen of **3** was found to be at the equatorial position after minimization.

Biological Assay. To measure binding affinity at muscarinic receptors, rat brain crude membrane preparation is incubated with a radiolabeled agonist [^{3}H]oxotremorine-M, (Oxo-M) or antagonist [^{3}H]-*N*-methylscopolamine (NMS) and various concentrations of the test compound at 30 °C for 60 min. Membranes are collected by vaccuum filtration, and the receptor bound radioactivity is determined by liquid scintillation spectroscopy.[13] The affinities (K_i) of the test compound are determined from the competition binding curves using a nonlinear iterative curve fitting computer software program developed by Lundon.

Affinity for M_1 receptors was measured in rat hippocampal membranes using 10 nM [^{3}H]pirenzepine.[14a] The assay buffer, 50 mM sodium phosphate, pH 7.4, contained 0.3 mM $MgCl_2$ and 0.1 mM GppNp. Nonspecific binding for this and the M2 assay below was measured in the presence of 1 μM atropine. Bound radioactivity was collected by vacuum filtration and liquid scintillation. Binding parameters were estimated as above.

Intrinsic activity at M_1 receptors was estimated by measuring stimulation of phosphatidyl inositol (PI) hydrolysis rates in rat hippocampal tissue by a modification of the method described by Crews et al.[14b,c] Cross-chopped slices (350 μm) of tissue were pre-incubated with [^{3}H]myo-inositol for 1 h in a Krebs–Hensleit buffer and then exposed to agonists (10^{-7}–10^{-3} M) for 2 h. Following chloroform/methanol extraction (C/M), inositol phosphates were isolated from the aqueous phase by ion exchange chromatography. Rates of inositol phosphate formation were corrected for variations in incorporation of isotope into phosphatidyl inositol by counting the C/M fraction. Results are expressed as a percent of hydrolysis rates in the presence of carbachol.

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