Resolved Pyrrolidine, Piperidine, and Perhydroazepine Analogues of the Muscarinic Agent *N*-Methyl-*N*-(1-methyl-4-pyrrolidino-2-butynyl)acetamide

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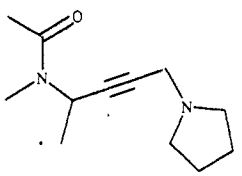
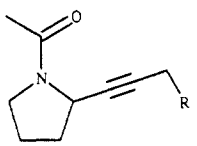
A series of conformationally restricted analogues of the partial muscarinic agonist *N*-methyl-*N*-(1-methyl-4-pyrrolidino-2-butynyl)acetamide (BM 5; **1**) was synthesized. Three of the racemic derivatives were resolved into the enantiomers. The compounds were investigated for muscarinic and antimuscarinic activity in the isolated guinea pig ileum. They were found to be fairly potent muscarinic antagonists or weak partial agonists. The new compounds were either equally or less potent than **1** in inhibiting (-)-[^{3}H]-*N*-methylscopolamine binding in homogenates of the rat cerebral cortex. Thus, structural modifications to **1** in which the amide moiety and the methyl group in the butynyl chain have been joined to form a six- or seven-membered ring preserve affinity but abolish efficacy. The *R* enantiomers were found to have 14-79 times higher affinity to ileal muscarinic receptors than the respective antipodes. The enantiomeric affinity ratios were nearly identical in both preparations studied. As suggested by molecular mechanics calculations, the difference in affinity between the five-membered and the six- and seven-membered ring analogues may be rationalized in conformational terms.

Introduction

Muscarinic agonists[1] with a selective effect on the central nervous system might be of clinical use for the treatment of certain psychiatric disorders associated with impaired central cholinergic transmission, e.g. presenile dementia (Alzheimer's disease).[2] In this context, the muscarinic agent *N*-methyl-*N*-(1-methyl-4-pyrrolidino-2-butynyl)-acetamide (BM 5; **1**)[3] has attracted some interest. By



1: BM 5



2: R = N

3: R = NMe_2

4: R = NMe_3^+

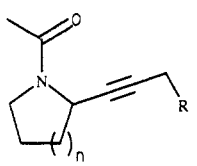
virtue of its partial agonism, **1** shows tissue selectivity both in vitro and in vivo.[4] Compound **1** has been claimed to act both as a presynaptic antagonist and as a postsynaptic agonist at muscarinic receptors.[5] More recently, **1** has been characterized as a partial agonist at adenylyl cyclase-coupled (m2 and m4) receptors and an antagonist at phosphoinositide turnover-coupled (m1 and m3) receptors.[6,7]

In order to study the effects of restricting the mobility of **1** and thereby limiting the number of accessible conformations, a series of cyclic analogues of **1** has been prepared and evaluated pharmacologically. The racemic five-membered ring analogues **2-4**, formally formed by joining the *N*-methyl substituent of the acetamide moiety and the methyl substituent in the butynyl chain of **1** with a methylene group, were found to be antagonists of moderate potency or weak partial agonists.[8] In the present report we describe the synthesis of some conformationally restricted analogues of **1**, in which the *N*-methyl substituent and the methyl group of the butynyl chain are joined to form a six- or a seven-membered ring (**5-10**). The most potent analogues in the five (**2**),[8] six (**5**), and seven membered ring (**8**) series were resolved into the enantiomers.

Table I. Yields and GC Purities of the Methoxylated Compounds **13**, **14**, and **21-23**

no.	method[a]	scale, mmol	% yield[b]	% GC purity[c]	R_f[d] (TLC)
13	B	35	87[e]	96	0.38 (A)
14	B	25	99	≥99	0.38 (A)
21	B	140	97	95	0.24 (B)
22	B	110	94	92[f]	0.37 (B)
23	B	100	95	98	0.37 (B)

[a] Letters refer to the methods of preparation as described in the Experimental Section. [b] After evaporation of the volatiles at 0.04 mmHg, 45 °C. [c] Capillary GC: DB-5 (oven 150 °C/injector 200 °C). [d] A, SiO_2 (2.5% MeOH-ether); B, SiO_2 [ether-*n*-hexane (2:8)]. [e] After chromatography on Al_2O_3 with ether as eluent. [f] The sample contained about 6% of an unidentified impurity.



Compd.	n	R
5	2	N
6	2	NMe_2
7	2	NMe_3^+
8	3	N
9	3	NMe_2
10	3	NMe_3^+

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[∥] Deceased, October 14, 1989.

(1) For recent reviews on muscarinic agents, see: (a) Ringdahl, B. In *The Muscarinic Receptors*; Brown, J.H., Ed.; Humana Press Inc.: Clifton, NJ, 1989; pp 151-218. (b) Krogsgaard-Larssen, P.; Jensen, B.; Falch, E. Jörgensen, F. S. *Drugs Future*, **1989**, *14*, 541-561. (c) Baker, R.; Saunders, J. *Annu. Rep. Med. Chem.* **1989**, *24*, 31-40.
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(5) (a) Nordström, Ö.; Alberts, P.; Westlind, A.; Undén, A.; Bartfai, T. *Mol. Pharmacol.* **1983**, *24*, 1-5. (b) Casamenti, F.; Cosi, C.; Pepeu, G. *Eur. J. Pharmacol.* **1986**, *122*, 288-290.

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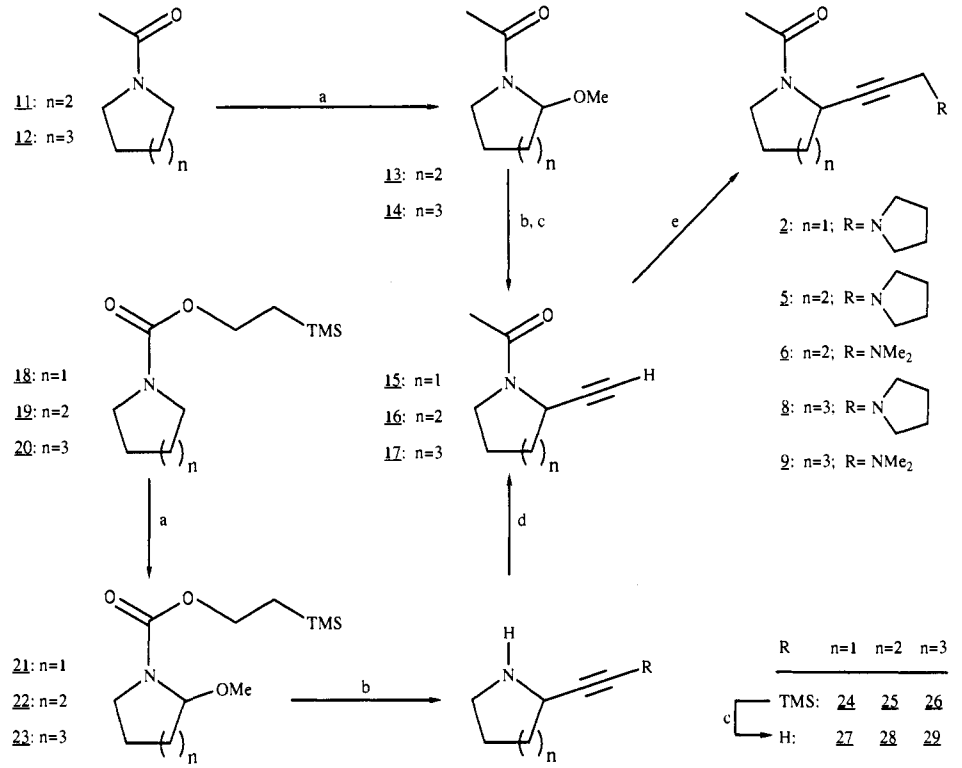
Scheme I[a]

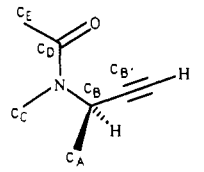


[a] Reagents: (a) $-2e^-$, $-2H^+$; n-Bu_4NBF_4, MeOH, 10 °C; (b) Bis(trimethylsilyl)acetylene, $AlCl_3$, CH_2Cl_2, −30 to 0 °C; (c) KF, MeOH, 66 °C; (d) AcCl, Et_3N, ether, 4 °C; (e) pyrrolidine or dimethylamine, $(HCHO)_n$, HOAc, CuCl, dioxane, 40 °C.

The compounds were investigated for muscarinic and antimuscarinic activity on the isolated guinea pig ileum and for ability to inhibit the binding of the muscarinic antagonist (−)-[^{3}H]-*N*-methylscopolamine ([^{3}H]NMS) to homogenates of the rat cerebral cortex. They were found to be muscarinic antagonists or weak partial agonists in the preparations used. The most potent analogues (**5** and **8**) had an affinity to guinea pig ileal muscarinic receptors which exceed that of **1**. The *R* enantiomers of **2**, **5**, and **8** had higher affinity to ileal and cortical muscarinic receptors than the respective antipodes.

Chemistry

Synthesis. The synthetic sequence affording the racemic amides **16** and **17** (Scheme I), which involves a Lewis acid promoted amidoalkylation reaction as the key synthetic step, is based on a method previously used for the preparation of 1-acetyl-2-ethynylpyrrolidine:[8] anodic oxidation[9] of *N*-acetylpiperidine (**11**) and *N*-acetylperhydroazepine (**12**) in MeOH afforded α-methoxylated amides **13**[10] and **14**[10] (Table I; method B). Treatment of **13** and **14** with bis(trimethylsilyl)acetylene[11] (BTMSA) and anhydrous $AlCl_3$ in CH_2Cl_2 at −30 °C (method C) followed by desilylation with KF in refluxing MeOH[12] afforded the α-acetylenic amides **16** and **17**.[13]



(R)-30

Figure 1. Definition of τ_h: $\tau(C_B'-C_B-N-C_D)$.

The synthesis of the resolved key intermediates **15**–**17** (Scheme I) has been briefly described previously.[14] Detailed experimental procedures are now given in the Experimental Section. Carbamates **18**–**20** were prepared from the corresponding cyclic amines (method A). Anodic oxidation of **18**–**20** afforded the methoxylated carbamates **21**–**23** (Table I; method B).[15] Carbamates **21**–**23** were

(6) (a) Baumgold, J.; Drobnick, A. *Mol. Pharmacol.* **1989**, *36*, 465–470. (b) Bradbury, B. J.; Baumgold, J.; Jacobson, K. A. *J.* Med. *Chem.* **1990**, *33*, 741–748.

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(9) For reviews, see, e.g.: (a) Shono, T. *Tetrahedron* **1984**, *40*, 811–840. (b) Wistrand, L.-G. *Janssen Chim. Acta* **1986**, *4*, 34–39.

(10) Mitzlaff, M.; Warning, K.; Jensen, H.; *J. Liebigs Ann. Chem.* **1978**, 1713–1733.

(11) Walton, D. R. M.; Waugh, F. *J. Organometal. Chem.* **1972**, *37*, 45–56.

(12) Kraihanzel, C. S.; Poist, J. E. *J. Organometal. Chem.* **1967**, *8*, 239–243.

(13) Elimination of MeOH from the methoxylated amides to form the corresponding enamides was found to compete with the coupling reaction and lower the yield of the α-acetylenic amides. This side reaction seems to be more favored in the six-membered ring than in the seven- and the five-membered ring homologues, as indicated by the yields of coupled product (44% vs 68% and 70%, compare ref 8). Changed reaction conditions (the order of addition, reaction temperature, and/or equivalent ratio) did not improve the yield of (±)-**16**.

(14) Lundkvist, J. R. M.; Wistrand, L.-G.; Hacksell, U. *Tetrahedron Lett.* **1990**, *5*, 719–722.

(15) The yield and purity of **21**–**23** were slightly lower than that of **13**–**14** (Table I) because the bigger electrodes used in the oxidation of **18**–**20** absorbed some of the starting material from the electrolysis solution due to the porous nature of the graphite used. This fraction of unconsumed material was released when the electrodes were immersed in MeOH (500 mL) to extract the product after the reaction.

Table II. Yields and Physical Data of Racemic 5-10, 16-20, and 24-26 and the Enantiomers of 2, 5, 8, 15-17, and 24-29

compd	method[a]	% yield	mp/bp, °C	recryst solvent[b]	$[\alpha]^{22}_D$,[c] deg	R_f[d] (TLC)	formula
(*R*)-**2**-oxalate	G	87	65-75[e]	-	+94.9	0.43 (A)	$C_{15}H_{22}N_2O_5 \cdot {}^3/_4H_2O$
(*S*)-**2**-oxalate	G	83	65-75[e]	-	-93.8		$C_{15}H_{22}N_2O_5 \cdot {}^3/_4H_2O$
(*R*)-**5**-oxalate	G	85	114-115	A	+115	0.44 (B)	$C_{16}H_{24}N_2O_5 \cdot 0.5MeOH$
(*S*)-**5**-oxalate	G	81	113-114	A	-118		$C_{16}H_{24}N_2O_5$
(±)-**5**-oxalate	G	82	117-118	A	-		$C_{16}H_{24}N_2O_5 \cdot 0.5MeOH$
(±)-**6**-oxalate	G	86	128-129	A	-	0.27 (C)	$C_{14}H_{22}N_2O_5$
(±)-**7**	H	94	160-161	A	-		$C_{13}H_{23}IN_2O$
(*R*)-**8**-citrate	G	85	87-92[e]	-	+76.6	0.40 (D)	$C_{21}H_{32}N_2O_8 \cdot 1.0H_2O$
(*S*)-**8**-citrate	G	89	96-101[e]	-	-76.6		$C_{21}H_{32}N_2O_8 \cdot 0.3H_2O$
(±)-**8**-oxalate	G	65	121-122	A	-		$C_{17}H_{26}N_2O_5$
(±)-**9**-oxalate	G	85	83-84	A	-	0.36 (E)	$C_{15}H_{24}N_2O_5$[f]
(±)-**10**	H	78	200-201	A	-	-	$C_{14}H_{25}IN_2O$
(*R*)-**15**	F	76	43-47	B	+146	0.39 (F)	$C_8H_{11}NO$
(*S*)-**15**	F	70	43-47	B	-143		$C_8H_{11}NO$
(*R*)-**16**	F	75	51-52	B	+180	0.36 (G)	$C_9H_{13}NO$
(*S*)-**16**	F	87[g]	51-53	B	-177		$C_9H_{13}NO$
(±)-**16**	C	44	78-79 (0.4)[h]	-	-		$C_9H_{13}NO$
(*R*)-**17**	F	89	101-102	B	+197	0.45 (H)	$C_{10}H_{15}NO$
(*S*)-**17**	F	79	101-102	B	-194		$C_{10}H_{15}NO$
(±)-**17**	C	68	71-73	B	-		$C_{10}H_{15}NO$
18	A	74	78-79 (0.2)[h]	-	-	0.53 (1)	$C_{10}H_{21}NO_2Si$
19	A	75	64-65 (0.03)[h]	-	-	0.76 (1)	$C_{11}H_{23}NO_2Si$
20	A	71	80-82 (0.03)[h]	-	-	0.75 (I)	$C_{12}H_{25}NO_2Si$
(*R*)-**24**-oxalate	D	74[i]	160-161	A	+33.8	0.45 (J)	$C_{11}H_{19}NO_4Si$
(*S*)-**24**-oxalate	D	65[i]	159-161	A	-34.4		$C_{11}H_{19}NO_4Si$
(±)-**24**-oxalate	C	92	146-147 (144-145)[j]	A	-		$C_{11}H_{19}NO_4Si$
(*R*)-**25**-oxalate	D	9[i]	137-138	A	+20.4	0.63 (J)	$C_{12}H_{21}NO_4Si$
(*S*)-**25**-oxalate	D	6[i]	135-137	A	-18.7		$C_{12}H_{21}NO_4Si$
(±)-**25**-oxalate	C	88	178-179 (174-175)[j]	A	-		$C_{12}H_{21}NO_4Si$
(*R*)-**26**-oxalate	D	52[i]	145-146	A	+18.3	0.74 (J)	$C_{13}H_{23}NO_4Si$
(*S*)-**26**-oxalate	D	58[i]	145-146	A	-18.7		$C_{13}H_{23}NO_4Si$
(±)-**26**-oxalate	C	86	152-153 (148-149)[j]	A	-		$C_{13}H_{23}NO_4Si$
(*R*)-**27**-oxalate	E	74	139-140	A	+27.7	0.27 (J)	$C_8H_{11}NO_4$
(*S*)-**27**-oxalate	E	84	139-140	A	-28.6		$C_8H_{11}NO_4$
(*R*)-**28**-oxalate	E	88	116-117	A	+16.0	0.38 (J)	$C_9H_{13}NO_4$
(*S*)-**28**-oxalate	E	86	118-119	A	-16.5		$C_9H_{13}NO_4$
(*R*)-**29**-oxalate	E	92	156-157	A	+12.8	0.58 (J)	$C_{10}H_{15}NO_4$
(*S*)-**29**-oxalate	E	88	156-157	A	-12.7		$C_{10}H_{15}NO_4$

[a] Letters refer to methods of preparation as described in the Experimental Section. [b] A, MeOH-ether; B, $CHCl_3$-n-hexane. [c] *c* 1.4, MeOH. [d] A, free base on Al_2O_3 (3% MeOH-ether); B, free base on Al_2O_3 ($CHCl_3$); C, free base on Al_2O_3 (5% EtOAc-$CHCl_3$); D, free base on Al_2O_3 (1% MeOH-ether); E, free base on Al_2O_3 (2% MeOH-ether); F, SiO_2 (3% MeOH-ether); G, Al_2O_3 [ether-light petroleum (7:3)]; H, Al_2O_3 [ether-light petroleum (8:2)]; I, SiO_2 [ether-light petroleum (1:1)]; J, free base on SiO_2 [ether-NH_3 (concentrated) (19:1)]. [e] Recrystallization was not possible. The crude salt was dried at 0.05 mmHg and 35 °C for several hours. [f] Satisfactory elemental analysis was obtained for the base ($C_{13}H_{22}N_2O$). [g] After chromatography on Al_2O_3 with either/light petroleum (1:1) as eluant. [h] Boiling point (mmHg). [i] Yield of diastereomeric salt from the resolution. [j] In parentheses are melting points of the crude oxalates.

coupled with BTMSA in the presence of anhydrous $AlCl_3$ (method C) to produce racemic **24-26**.[16] These racemates were resolved by fractional recrystallization of diastereomeric salts (method D) formed with tartaric acid, di-*O*-4-toluoyltartaric acid and mandelic acid, respectively. The resolved amines **24-26** were desilylated (method E) and acetylated (method F) to give the enantiomers of **15-17**.

The absolute configuration of (+)-(*R*)-**15** has been unambiguously determined by chemical correlation with (+)-(*R*)-proline,[18] and the absolute configuration of the enantiomers of **16** and **17** was deduced by comparison of their circular dichroism (CD) spectra with that of **15**;[14] the dextrorotary enantiomers of **16** and **17** also have the *R* configuration.

Racemic **5**, **6**, **8**, and **9**, and the enantiomers of **2**, **5**, and **8** were obtained by cuprous-catalyzed Mannich reactions[19] of the acetylenic precursors with pyrrolidine or dimethylamine (method G). Methylation of the dimethylamino functon with iodomethane in acetone gave the quaternary ammonium derivatives **7** and **10** (method H). Physical data of the new acetylenic derivatives are presented in Table II.

Molecular Mechanics Calculations. In order to investigate if the conformational preference of **2**, **5**, and **8** differ from that of **1**, we studied the conformational distribution of model compounds by molecular mechanics calculations using the MMX-89.0 force field.[20] For convenience, calculations were done on model compounds lacking the pyrrolidinomethyl moiety [(*R*)-**15-17** and (*R*)-**30**; Figure 1]; it is known that, e.g. in oxotremorine,

(16) (a) No carbamate-containing product was observed (IR ν 1700 cm^{-1}) when workup was performed at a fixed pH of about 8 (excess saturated sodium-potassium tartrate solution), conditions during which a carbamate should be stable.[17] This suggests that the carbamate group is cleaved off during the reaction. (b) Normally, 2.0-2.5 equiv of $AlCl_3$ were used. Only starting material was detected (GC) after 15 h at 22 °C in an attempted reaction with only 1.0 equiv of $AlCl_3$. This suggests that 1 equiv of $AlCl_3$ is complexed either to BTMSA or to the carbamate and that additional Lewis acid would be necessary to catalyze or promote the reaction.

(17) Greene, T. W. *Protective Groups in Organic Synthesis*, John Wiley: New York, 1981; pp 323-324.

(18) Trybulski, E. J.; Mangano, R.; Kramss, R.; Brabander, H.; Rusinko, A. Poster presented at the Fourth International Symposium on Subtypes of Muscarinic Receptors, Wiesbaden, West Germany, July 20-22, 1989.

(19) For a review, see Tramontini, M. *Synthesis* **1973**, 703-775.

(20) MMX is a molecular mechanics program that is an enhanced version of Allinger's MMP2 program. The MMX program is part of the molecular modeling package PCMODEL (Serena Software, P.O. Box 3076, Bloomington, IN 47402-3076).

the conformational preferences of the lactam and the pyrrolidine ring are affected very little by the other ring moiety.[21] Therefore, we assumed that the geometries and energies obtained for conformations of the model compounds would be similar to those of **1**, **2**, **5**, and **8**.

Twelve starting geometries for each of the **E** and **Z** rotational isomers of (*R*)-**30** were constructed by an incremental change of the dihedral angle $\tau_h = \tau(C_B\text{–}C_B\text{–}N\text{–}C_D)$ (Figure 1) in steps of 30°, so that τ_h = 0°, 30°, 60°, 90°, 120°...330°. Energy minimization of the 24 starting geometries resulted in five conformations with relative steric energies below 3 kcal/mol (see the supplementary material). The two conformations with relative steric energies below 1 kcal/mol adopt τ_h values (defined in Figure 1) of 137° and 132°, respectively.

Starting geometries for energy minimizations of the ring-containing model compounds were constructed by taking into account the flexibility of the ring system and the possibility of *E* and *Z* geometries about the amide bond. In total, we constructed 4, 16, and 20 starting geometries of (*R*)-**15**, (*R*)-**16**, and (*R*)-**17**, respectively. The calculations resulted in 4, 4 and 14 low-energy conformations of (*R*)-**15**, (*R*)-**16**. and (*R*)-**17**, resectively (see the supplementary material). As a consequence of the ring size, the τ_h values in these conformations vary considerably. The low-energy conformations of pyrrolidine derivative (*R*)-**15** adopt τ_h values (see Figure 1) from 53° to 79°. In conformations of piperidine derivative (*R*)-**16** with relative steric energies below 0.5 kcal/mol, τ_h varies from 88° to 107°, and conformations of (*R*)-**17** with relative steric energies below 1.8 kcal/mol adopt τ_h values from 84° to 138°. It is noteworthy that the latter value is similar to that of the global energy minimum of (*R*)-**30**.

Stereoscopic views of the minimum energy (MMX) conformations of (*R*)-**15**–(*R*)-**17** and (*R*)-**30** are shown in Figure 2.

NMR Spectroscopy. Due to the relatively high energy barrier to rotation about the amide bond, (*R*)-**15**–(*R*)-**17** and (*R*)-**30** exist as equilibrium mixtures of the *E* and *Z* rotational isomers. The *E*–*Z* interconversion is sufficiently slow on the NMR time scale to allow for observation of the two isomers.[22] The isomeric preference of each model compound was determined according to the method of LaPlanche and Rogers[23] by integration of ^{1}H NMR spectroscopic signals due to each rotational isomer: in $CDCl_3$ solutions of **16**, **17**, and **30** (at 23 °C), the *Z* rotamer predominates [83% in (*R*)-**30**; 72% in (*R*)-**16**; 68% in (*R*)-**17**]. In contrast, the *E* rotamer of (*R*)-**15** appeared to be slightly favored (55%) in the same solvent. The rotameric preference of test compounds **1**, **2**, **5**, and **8** (free base in $CDCl_3$ at 23 °C) paralleled that of the model compounds (^{1}H and ^{13}C NMR). The molecular mechanics calculations indicate that the *Z* rotamer prevails in (*R*)-**15**, (*R*)-**16**, and (*R*)-**30** and that the *E* rotamer of (*R*)-**17** should be slightly energetically favored. It should be noted, however, that the differences between experimental and theoretical results are small since neither indicate a pronounced preference for a particular rotational isomer.

Pharmacology

Racemic **5**–**10** and the enantiomers of **2**, **5**, and **8** were tested for muscarinic and antimuscarinic activity in the isolated guinea pig ileum. The results are presented in Table III. For comparison, relevant data for **1**–**4**, carbachol, and atropine are included as well. In Table III, K_D values are the equilibrium dissociation constants of the drug–receptor complex. They were obtained from the ability of the test compounds to antagonize carbachol-induced contractions in the ileum.[24] In contrast to **1**, none of the tertiary amines were spasmogenic on the guinea pig ileum. Instead, they acted as antagonists to carbachol. The antagonism appeared to be competitive in all cases since the slopes of the Schild plots[25] were not significantly different from 1. The pyrrolidinyl-substituted derivatives

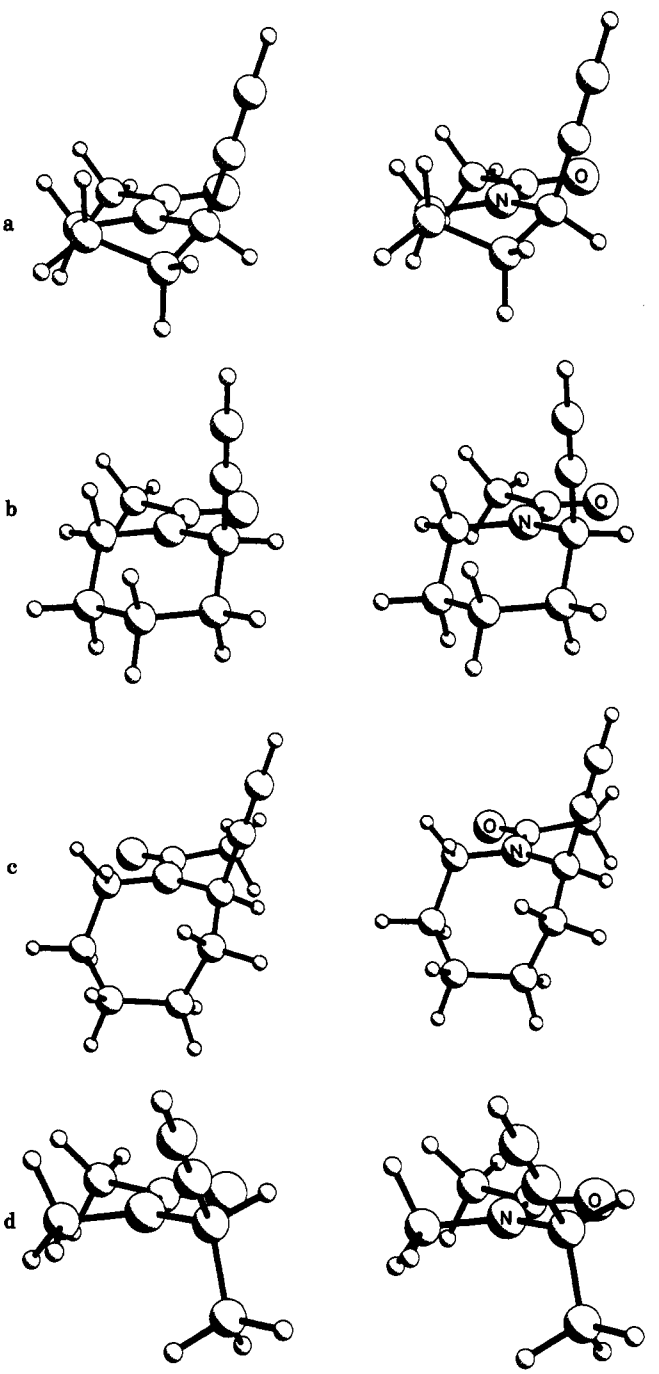

Figure 2. Stereoscopic representations of minimum energy (MMX) conformations of model compounds (*R*)-**15** (a), (*R*)-**16** (b), (*R*)-**17** (c), and (*R*)-**30** (d).

(21) See Nilsson, B. M.; Ringdahl, B.; Hacksell, U. *J. Med. Chem.* **1990**, *33*, 580–584 and references cited therein.
(22) For a review on NMR studies of amides, see: Stewart, W. E.; Siddall, T. H., III *Chem. Rev.* **1970**, *70*, 517–551.
(23) LaPlanche, L. A.; Rogers, M. T. *J. Am. Chem. Soc.* **1963**, *85*, 3728–3730.
(24) Furchgott, R. F. *Adv. Drug. Res.* **1966**, *3*, 21–55.
(25) Arunlakshana, O.; Schild, H. O. *Br. J. Pharmacol.* **1959**, *14*, 48–58.

Table III. Muscarinic and Antimuscarinic Effects and Receptor Binding Affinities of Racemic 1-10 and the Enantiomers of 1, 2, 5, and 8[a]

	guinea pig ileum					rat cerebral cortex:
compd	N[b]	EC_{50}, μM	E_{max}[c]	K_D,[d] μM	slope of Schild plot	[^{3}H]NMS displacement; K_i,[e] μM
(*R*)-1	7	0.094 ± 0.009[f]	0.83 ± 0.03[f]	0.20 ± 0.03[f]		0.064 ± 0.007[g]
(*S*)-1	6			3.23 ± 0.35[f]		
(*R*)-2	4		0	1.16 ± 0.10	1.05 ± 0.10	0.41 ± 0.02
(*S*)-2	4		0	16.01 ± 3.41	0.80 ± 0.15	5.16 ± 0.17
(±)-2	5		0	1.4 ± 0.28	0.90 ± 0.13[g]	0.67 ± 0.01[g]
(±)-3	4		0	125 ± 19[g]		25.4 ± 3.3[g]
(±)-4	4	7.5 ± 0.4[g]	0.80 ± 0.08[g]	49.5 ± 7.6		6.1 ± 0.4[g]
(*R*)-5	5			0.080 ± 0.007	0.86 ± 0.05	0.016 ± 0.001
(*S*)-5	4			6.32 ± 0.95	0.99 ± 0.25	1.482 ± 0.020
(±)-5	6		0	0.097 ± 0.016	0.88 ± 0.16	0.044 ± 0.002
(±)-6	4		0	8.3 ± 0.6	0.90 ± 0.05	2.26 ± 0.06
(±)-7	4	2.8 ± 0.4	0.75 ± 0.02			0.75 ± 0.01
(*R*)-8	4		0	0.161 ± 0.034	1.27 ± 0.17	0.042 ± 0.004
(*S*)-8	4		0	7.85 ± 0.93	1.17 ± 0.15	2.070 ± 0.331
(±)-8	5		0	0.11 ± 0.02	1.17 ± 0.23	0.082 ± 0.01
(±)-9	4		0	7.0 ± 1.0	1.10 ± 0.19	2.90 ± 0.20
(±)-10	5			3.55 ± 0.13	1.03 ± 0.04	1.23 ± 0.03
carbachol	6	0.1	1.00			
atropine	4			0.0009 ± 0.0001[h]		

[a] Values are means plus or minus standard errors. [b] Number if ileal preparations used. [c] The maximum contractile response relative to that elicited by carbachol. [d] Dissociation constant of the drug–receptor complex. [e] Based on three separate determinations, each performed in triplicate. [f] Values are from ref 37. [g] Values are from ref 8. [h] Value is from ref 42.

(*R*)-5 and (*R*)-8 were the most potent, both having an affinity on the ileum greater than that of (*R*)-1. The six- and seven-membered ring analogues having the same tertiary amine moiety displayed similar affinity to ileal muscarinic receptors. The trimethylammonium derivative **7** behaved as a partial agonist on the ileum whereas **10** was a weak antagonist.

The compounds inhibited the specific binding of (−)-[^{3}H]NMS in the rat cerebral cortex in an apparently competitive manner. The radioligand data were analyzed and satisfactorily fit by a one site binding equation to give K_i values shown in Table III. The rank order of potency of the muscarinic agents in the receptor binding assay also paralleled their affinity for the ileal muscarinic receptor.

Discussion

The new compounds are conformationally restricted analogues of the partial muscarinic agent **1** in which, formally, the *N*-methyl substituent of the acetamide moiety and the methyl group of the butynyl chain have been joined in a five-, six-, or seven-membered ring. The analogues were found to be muscarinic antagonists or partial muscarinic agonists on the guinea pig ileum. For the most potent compounds (**5** and **8**), the affinity for ileal and cortical muscarinic receptors was similar to that observed for **1**. Thus, ring expansion from the five-membered (**2**–**4**) to the six- and seven-membered ring derivatives (**5**–**10**) increased the affinity by about 10 times. On the other hand, the efficacy did not increase since all the tertiary amines were antagonists. The larger steric bulk of the seven-membered ring as compared to that of the six-membered ring did not appear to interfere with the drug–receptor interaction, since the affinity constants of (*R*)-5 and (*R*)-8 are similar.

The effects of variations of the amino moiety on the pharmacological potency and receptor binding affinity were similar regardless of the ring size of the amide moiety; the pyrrolidino derivatives (**5** and **8**) were the most potent antagonists. The trimethylammonium derivative **7** was more efficacious than the corresponding tertiary amines. These results agree with previous observations in other series of oxotremorine analogues.[1a] Surprisingly, the other trimethylammonium derivative (**10**) was not spasmogenic on the ileum. Apparently, it lacked intrinsic activity.

Compounds **2**, **5**, and **8** exhibited the same enantioselectivity as **1**,[26] the (*R*) enantiomers being the more potent receptor ligands. Compound (*R*)-**2** was found to have a 5-fold lower affinity for ileal muscarinic receptors compared to (*R*)-**1** (see Table III). On the other hand, (*R*)-**5** and (*R*)-**8** had higher affinity than (*R*)-**1** in the same preparation. The eudismic ratio[27] of **2** was nearly identical with that established for **1** (**14** vs **16**, see Table III), whereas the corresponding ratios of **5** and **8** were significantly higher (79 and 49, respectively). For each enantiomeric pair the eudismic ratio in the ileal preparation almost paralleled that in the rat cortical preparation.

In an attempt to rationalize the differences in affinity among **1**, **2**, **5**, and **8**, we compared low energy (MMX) conformations of model compounds (*R*)-**15**–(*R*)-**17** (*Z* rotamers) with the conformation of lowest energy of (*R*)-**30** (Figure 3).[28] In computer-aided fittings, the amide moiety and the ring carbons adjacent to the nitrogen of (*R*)-**15**–(*R*)-**17** were superimposed on the corresponding structural elements of (*R*)-**30** (Figure 3). An almost perfect fit was obtained when (*R*)-**30** (a model for (*R*)-**1**) was compared with a low-energy conformation (ΔE_s = 0.8 kcal/mol) of (*R*)-**17** [a model for (*R*)-**8**]. The comparisons with (*R*)-**16** [a model for (*R*)-**5**] and (*R*)-**15** [a model for (*R*)-**2**] produced less good and poor fits, respectively (Figure 3). The quality of the above fits may also be expressed in differences in τ_h values: in low-energy conformations of (*R*)-**15**–(*R*)-**17**, this particular torsional angle differs more than 58°, 30°, and 1°, respectively, from the τ_h value of the lowest energy conformation of (*R*)-**30**. The difference in conformational preferences of model compounds (*R*)-**15**–(*R*)-**17** may account for the difference in affinity among (*R*)-**2**, (*R*)-**5**, and (*R*)-**8**. Thus, (*R*)-**5** and (*R*)-**8**, but not (*R*)-**2**, appear to readily adopt conformations that may bind efficiently to the receptor.

In contrast to (*R*)-**1**, which behaves as a partial agonist in the ileum assay, the conformationally restricted ana-

(26) Dahlbom, R.; Jenden, D. J.; Resul, B.; Ringdahl, B. *Br. J. Pharmacol.* **1982**, *76*, 299–304.
(27) Ariens, E. J.; Lehman, P. A.; De Miranda, R. J. F. *Prog. Drug Res.* **1976**, *20*, 101–142.
(28) It has been suggested that the biological activity of **1** should reside in the *Z* rotational isomer.[3]

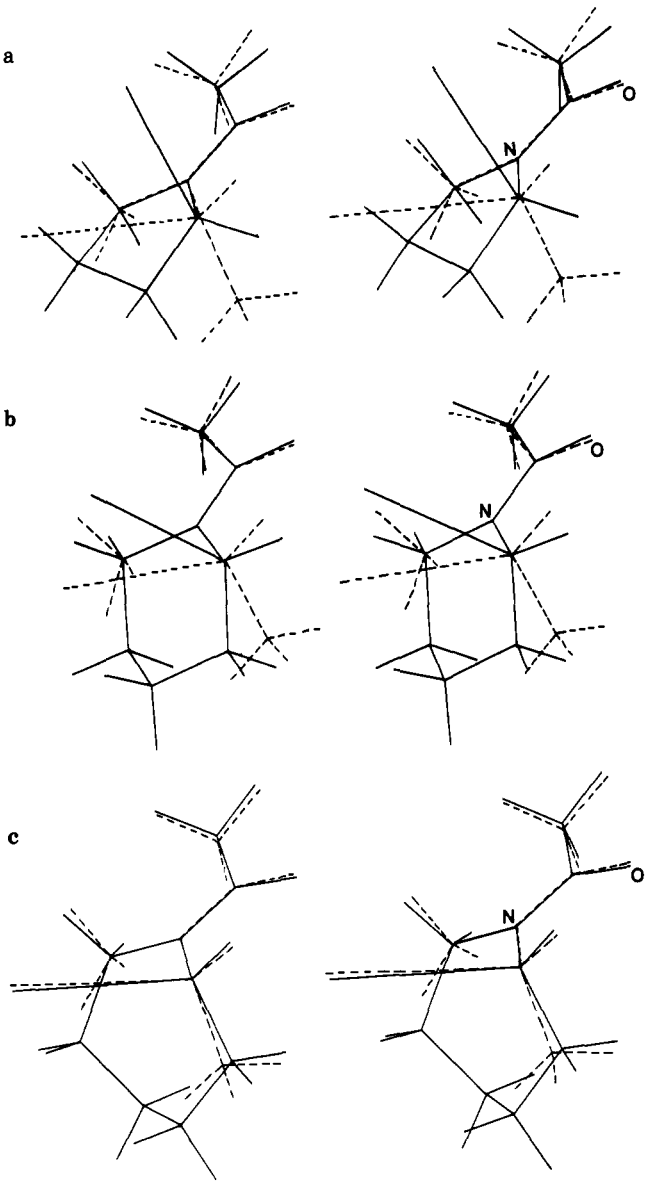

Figure 3. Computer-generated (MIMIC)[43] stereoparis of the best fit of (*R*)-**30** (dashed lines) in its minimum-energy conformation with (a) that of (*R*)-**15**, (b) that of (*R*)-**16** and (c) a low-energy conformation (ΔE_s = 0.8 kcal/mol) of (*R*)-**17** (solid lines).

logues (*R*)-**2**, (*R*)-**5**, and (*R*)-**8** appear to lack intrinsic activity or efficacy. This reduction in efficacy may be related to the steric bulk of the five-, and six- and seven-membered rings. Alternatively, the restricted mobility of (*R*)-**2**, (*R*)-**5**, and (*R*)-**8** might decrease their ability to undergo a conformational change which may be a prerequisite for activation of the receptor.[29]

Experimental Section

Chemistry. General Comments. Melting points (uncorrected) were determined in open glass capillaries using a Thomas-Hoover apparatus. ^1H and ^{13}C NMR spectra were recorded on a JEOL FX 90Q spectrometer at 90.0 and 22.5 MHz, respectively, and were referenced to internal tetramethylsilane. All spectra were in accordance with the assigned structures. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter. Capillary GC analysis of the compounds were performed on a Carlo-Erba 6000 Vega instrument equipped with DB-5 fused silica column (30 m, i.d. = 0.32 mm) or a DB-1701 fused silica column (30 m, i.d. = 0.25 mm). Helium (80 kPa) was used as the carrier gas. The elemental analyses were performed by Mikro Kemi AB, Uppsala, Sweden or Analytische Laboratorien, Gummersbach, West Germany and were within 0.4% of the calculated values. Flash chromatography was carried out as described by Still et al.[30] Thin-layer chromatography was carried out on aluminum sheets precoated with silica gel 60 F_{254} (0.2 mm) or aluminum oxide 60 F_{254} neutral (type E) (E. Merck). TLC spots were visualized with UV light or by spraying with aqueous H_2SO_4 and $KMnO_4$. All reactions except the electrochemical oxidation were performed in an atmosphere of dry nitrogen. 1-Acetylpiperidine and 1-acetylperhydroazepine were prepared according to a standard Schotten–Baumann procedure.

Electrolysis Apparatus (0.10–0.14 mol Scale). The solution to be electrolyzed was placed in an open, undivided, water-jacketed cell (500-mL volume, 7-cm i.d.) provided with a magnetic stirrer and a thermometer. Circulating, cold, tap water kept the temperature at +10 °C during the electrolysis. Two rectangular graphite electrodes[31] were immersed 120 mm into the solution and kept approximately 10 mm apart. The electrodes were connected to a direct-current power supply (0–18 V) in series with a mA meter (0–1000 mA).

Below follow representative examples of methods A–H:

Method A. 1-[[2-(Trimethylsilyl)ethoxy]carbonyl]-pyrrolidine (18). A solution of 2-(trimethylsilyl)ethanol (19.7 mL, 0.138 mol) in dry toluene (40 mL) was added during 1 h to a stirred solution of phosgene in toluene (1.93 M, 215 mL, 0.42 mol) (**CAUTION, TOXIC**[32]) at −35 °C. When the addition was complete, the dry ice bath was removed. The solution was purged with dry N_2 for 3 h and then concentrated by rotary evaporation on a water bath at 20 °C until most of the solvent was removed [the bath temperature should not exceed 20 °C, since the chloroformate is volatile (bp 42–43 °C/4 mmHg)]. The crude chloroformate was dissolved in dry ether (100 mL) and immediately added, during 30 min, to a mechanically stirred solution of pyrrolidine (11.6 mL, 0.138 mol) dry Et_3N (29 mL, 0.21 mol), and ether (400 mL) kept at −5 °C. The mixture was stirred overnight at 22 °C and finally refluxed for 2 h. Suction filtration of the mixture and concentration and distillation of the filtrate afforded **18** as an oil.

Method B.[9b] **2-Methoxy-1-[[2-(trimethylsilyl)ethoxy]-carbonyl]perhydroazepine (23).** A solution of 1-[[2-(trimethylsilyl)ethoxy]carbonyl]perhydroazepine (**23**; 24.3 g, 0.100 mol) in MeOH (400 mL) containing *n*-Bu_4NBF_4 (6.6 g, 0.020 mol) as a supporting electrolyte was oxidized at a constant current of about 400 mA. The progress of the oxidation was followed by GC analysis of small aliquots from the electrolysis solution. Column: DB-5 (oven 150 °C/injector 200 °C).[33] After passing 2.1–2.4 *F*/mol of electricity through the cell, only traces of starting material remained (1% according to GC). The solution was suction filtered and the electrodes were immersed in a stirred MeOH bath (500 mL) to dissolve some additional product. The combined methanol solutions were concentrated by rotary evaporation at 30 °C. The oily residue was treated with ether (400 mL) and left at 4 °C overnight. The salt was filtered off by suction and washed twice with ether.[34] The combined filtrates were dried twice (Na_2SO_4), filtered, and concentrated by rotary evaporation at 30 °C. The resulting oil was triturated with light petroleum (400 mL) to precipitate some additional salt (0.5–1 g). Evaporation of volatiles gave **23** as an oil.

Method C. (±)-2-[2-(Trimethylsilyl)ethynyl]pyrrolidine [(±)-24]. A solution of bis(trimethylsilyl)acetylene[11] (14.3 g, 0.0840 mol) in dry CH_2Cl_2 (6 mL) was added dropwise to a mechanically stirred suspension of anhydrous $AlCl_3$ (19.6 g, 0.147 mol) in dry CH_2Cl_2 (50 mL) at 0 °C. When the addition was complete, the dark mixture was cooled on a dry ice bath to −30 °C. *n*-Decane (4.98 g, 0.035 mol) was added as internal GC reference and a

(29) Burgen, A. S. V. *Fed. Proc. Am. Soc. Exp. Biol.* **1981**, *40*, 2723–2728.

(30) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.

(31) AUC-graphite (Union Carbide Europe S.A., Uddevalla, Sweden): electrode size, 9 × 50 × 170 and 9 × 40 × 170 mm, respectively.

(32) *Hazards in the Laboratory*; Bretherick, L., Ed.; The Royal Society of Chemistry: London, 1981; p 440.

(33) The α-methoxylated products decompose in the injector at temperatures above 200 °C.

(34) The recovered *n*-Bu_4NBF_4 was reused several times.

solution of 2-methoxy-1-[[2-(trimethylsilyl)ethoxy]carbonyl]-pyrrolidine (**21**; 17.2 g, 0.070 mol) in dry CH_2Cl_2 (28 mL) was added dropwise to the mixture above during 40 min. The mixture was stirred overnight while slowly warming to 0 °C.

The reaction mixture was poured onto a stirred mixture of crushed ice (200 g) and sodium–potassium tartrate (50 g). Ether was added (150 mL) and stirring was continued until the ice had melted. The mixture was carefully alkalinized to pH 8–9[35] with a saturated aqueous Na_2CO_3 solution (200–250 mL) and extracted with ether (3 × 200 mL). The organic phase was washed with saturated brine (100 mL), dried (K_2CO_3), and filtered. The amine was precipitated by adding a solution of oxalic acid (6.9 g, 0.077 mol) in ether (100 mL) to the clear filtrate from above under stirring. The resulting oxalate was filtered off by suction, washed with small portions of ether, and dried to constant weight. The salt thus obtained was ≥95% pure (GC, ^{1}H NMR). An analytical sample was obtained by recrystallization.

The workup procedure for acetamides (±)-**16** and (±)-**17**, which involved a desilylation step, is described below. The reaction mixture was poured slowly onto crushed ice and the mixture was extraction with CH_2Cl_2 (3 × 20 mL). The combined organic layers were washed with aqueous 0.5 M potassium–sodium tartrate, saturated aqueous sodium carbonate, and brine. Drying ($MgSO_4$), filtration, and concentration of the organic layer afforded a dark-brown oil, which was treated with KF (2.0 g, 0.034 mol) in refluxing MeOH (30 mL) for 45 min. The solvent was evaporated and the residue was triturated with CH_2Cl_2 (2 × 20 mL). Repeated flash chromatography of the crude product with ether–light petroleum (7:3; **16**) or $CHCl_3$–EtOAc (95:5; **17**) as eluent afforded 0.81 g of (±)-**16** (44% from 0.012 mol of **11**) and 1.48 g of (±)-**17** (68% from 0.013 mol of **12**).

Method D. Resolution of Racemates by Fractional Crystallization of Diastereomeric Salts. (*R*)- and (*S*)-2-[2-(Trimethylsilyl)ethynyl]pyrrolidine [(*R*)- and (*S*)-24]. The oxalate of (±)-**24** (39.9 g, 0.155 mol) was treated with an excess of saturated aqueous $KHCO_3$ and then extracted with ether (4 × 200 mL). The combined ether layers were dried (K_2CO_3), filtered, and concentrated by rotary evaporation at 20 °C to afford 25.0 g (0.149 mol) of (±)-**24**. The base was added to a boiling solution of (±)-L-tartaric acid (22.4 g, 0.149 mol) and 95% EtOH (350 mL). The solution was kept at 22 °C for 2 days and the crystals were filtered off. One recrystallization from 95% EtOH (150 mL) afforded 17.4 g (74%) of crystalls (needles): mp 130–131 °C; $[\alpha]_D^{22}$ = +39.2° (*c* 1.3, MeOH); optical purity of the base, 99.0% ee. This salt (8.8 g, 0.028 mol) was treated with an excess of saturated aqueous $KHCO_3$ and was extracted with ether (4 × 50 mL). The combined ether layers were dried (K_2CO_3), filtered, and treated with a solution of oxalic acid (2.8 g, 0.031 mol) in ether. The oxalate was filtered off, washed with small portions of ether, dried, and recrystallized from MeOH–ether to yield 6.3 g (88%) of (*R*)-**24**·oxalate.

The combined mother liquors from the resolution above were concentrated by rotary evaporation. The residue was treated with an excess of saturated aqueous $KHCO_3$ and was extracted with ether (4 × 150 mL). The combined ether layers were dried (K_2CO_3), filtered, and concentrated by rotary evaporation at 20 °C. The residual oil was treated with (–)-D-tartaric acid (13.0 g, 0.0866 mol) and 95% EtOH (170 mL) as described above. Two recrystallizations afforded 15.5 g (65%) of crystals (needles): mp 130–131 °C; $[\alpha]_D^{22}$ = –38.9° (*c* 1.4, MeOH); optical purity of the base 99.0% ee. The salt (9.9 g, 0.031 mol) was converted into the oxalate yield 7.18 g (90%) of (*S*)-**24**·oxalate after recrystallization.

(*R*)- and (*S*)-2-[2-(Trimethylsilyl)ethynyl]piperidine Oxalate [(*R*)- and (*S*)-25·Oxalate]. The base of (±)-**25** (19.7 g, 0.109 mol) was added to a warm solution of (–)-di-*O*-4-toluoyl-L-tartaric acid (42.0 g, 0.109 mol) in 95% EtOH (80 mL). Ether (300 mL) was added slowly to the gently refluxing solution. The solution was allowed to cool at 22 °C and was replaced in the refrigerator overnight. The precipitate was filtered off and recrystallized three times from 95% EtOH and ether to afford 2.8 g (9%) of crystals (needles); mp 143–145 °C; $[\alpha]_D^{22}$ = –89.7° (*c* 1.4, MeOH); optical purity of the base, 95.0% ee. The salt (2.6 g, 4.7 mmol), which contained about 5% of the corresponding desilylated amine (*R*)-**28** (GC), was converted into the oxalate to yield 0.70 g (56%) of (*R*)-**25**·oxalate after three recrystallizations from MeOH–ether.

Partially resolved (*S*)-**25** (16% ee), obtained from the combined mother liquors from above, was converted into the oxalate. Repeated recrystallizations from MeOH-ether afforded an oxalate which contained only 0.1% of **28**·oxalate (GC). This salt was converted into the base (2.15 g, 11.9 mmol) which was treated with (+)-di-*O*-4-toluoyl-D-tartaric acid (4.58 g, 11.9 mmol), 95% EtOH (2 mL), and ether (50 mL) as described above. Four recrystallizations afforded 0.20 g (6% yield) of needles: mp 143–145 °C; $[\alpha]_D^{22}$ = +88.7° (*c* 1.4, MeOH); optical purity of the base, 94.0% ee. Part of this salt (0.11 g, 0.19 mmol) was converted into the oxalate to yield 0.044 g (83%) of (*S*)-**25**·oxalate after recrystallization.

(*R*)- and (*S*)-2-[2-(Trimethylsilyl)ethynyl]perhydroazepine [(*R*)- and (*S*)-26]. The base of (±)-**26** (22.2 g, 0.114 mol) was added to warm solution of (–)-D-mandelic acid (17.3 g, 0.114 mol) in 95% EtOH (20 mL) with stirring. Ether (100 mL) was added slowly to the gently refluxing solution. The solution was allowed to cool at 22 °C and was put in a refrigerator for 2 days. The crystals were filtered off and recrystallized from 95% EtOH and ether to give 10.2 g (52%) of needles: mp 117–118 °C; $[\alpha]_D^{22}$ = –29.8° (*c* 1.4 MeOH); optical purity of the base, 99.8% ee. The mandelate (5.51 g, 15.9 mmol) was converted into the oxalate to yield 4.10 g (91%) of *R*)-**26**·oxalate after one recrystallization from MeOH–ether.

Partially resolved (*S*)-**26** (13.4 g, 0.0686 mol), generated from the combined mother liquors from above, was treated with (+)-L-mandelic acid (10.4 g, 0.0686 mol), 95% EtOH (10 mL), and ether (60 mL) as described for (*R*)-**26**. Two recrystallizations afforded 11.4 g (58%) of needles: mp 117–118 °C; $[\alpha]_D^{22}$ = +31.7° (*c* 1.4, MeOH); optical purity of the base, 99.8% ee. The mandelate (5.51 g, 15.9 mmol) was converted into the oxalate to yield, after recrystallization, 3.59 g (79%) of (*S*)-**26**·oxalate.

General Procedure for Determination of the Percent Enantiomeric Excess.[36] The enantiomeric excess of the resolved enantiomers of **24–26** was determined indirectly, as follows. The appropriate diastereomeric salt (0.10 mmol) was mixed with CH_2Cl_2 (0.5 mL) and freshly prepared aqueous 0.5 M $NaHCO_3$ (1.0 mL) at 20 °C. After 5 min, a solution of (*R*)-2-methoxy-2-phenylacetyl chloride (0.12 mmol) [freshly prepared from (–)-(*R*)-2-methoxy-2-phenylacetic acid (0.020 g) by stirring with thionyl chloride (2 mL) for 2 h at 20 °C and then evaporation of the volatiles] in CH_2Cl_2 (0.5 mL) was added with vigorous stirring. After stirring for 1 h at 20 °C, the mixture was extracted with ether. The organic layer was analyzed by GC [column, DB-1701; temperature 230 °C (oven)/300 °C (injector)]; level of detection, >99.8% de].

The enantiomeric excess of the resolved enantiomers of **27–29** was determined by treating the respective oxalate (0.10 mmol) identically. No difference in optical purity was found between the enantiomers of **24–26** and the desilylated analogues **27–29**.

Method E.[12] **(*R*)-2-Ethynylpyrrolidine Oxalate [(*R*)-27·Oxalate].** A mixture of (*R*)-**24**·oxalate (4.37 g, 17.0 mmol), KF (2.0 g, 34 mmol), and MeOH (80 mL) was heated to reflux during 1.5 h. The cooled solution was stirred with oxalic acid (3.06 g, 34.0 mmol) for 5 min and was concentrated. The solid residue was dissolved in saturated aqueous K_2CO_3 and the solution (pH 9–10) was extracted with ether (10 × 50 mL). The combined ether layers were dried (K_2CO_3), filtered, treated with a solution of oxalic acid (1.8 g, 20 mmol) in ether (50 mL), and concentrated in a stream of dry N_2 to about 200 mL. The crude oxalate was filtered off by suction and recrystallized to afford (*R*)-**27**·oxalate.

Method F. (*R*)-1-Acetyl-2-ethynylpyrrolidine [(*R*)-15]. (*R*)-**27**·oxalate (0.72 g, 3.9 mmol) was treated with an excess of saturated aqueous K_2CO_3 and the mixture was extracted with ether (6 × 50 mL). The combined ether layers were dried (K_2CO_3), filtered, and cooled on an ice–water bath to 4 °C. Dry Et_3N (0.58 mL, 4.1 mmol) and a solution of acetyl chloride (0.28 mL, 3.9 mmol) in ether (10 mL) were added to the stirred ether solution. The cooling bath was removed and the stirring was continued for

(35) The pH was kept at or below 9 to minimize desilylation.

(36) Helmchen, G.; Strubert, W. *Chromatographia* **1974**, *12*, 713–715.

30 min. The mixture was filtered by suction and the filtrate was concentrated to afford (*R*)-**15** as an oil, which solidified in the freezer. Recrystallization afforded an analytical sample.

Method G. (±)-1-Acetyl-2-(3-pyrrolidino-1-propynyl)-piperidine [(±)-5].[19] A mixture of pyrrolidine (0.14 mL, 1.7 mmol), paraformaldehyde (0.051 g, 1.7 mmol), HOAc (0.092 mL, 1.7 mmol), CuCl (0.03 g), (±)-**16** (0.23 g, 1.49 mmol), and dry dioxane (4 mL) was stirred at 40 °C (oil-bath temperature) overnight and then concentrated by rotary evaporation. The residue was dissolved in CH_2Cl_2 (5 mL), chilled on an ice bath, and extracted with cold 1 M aqueous HCl (3 × 10 mL). The combined aqueous phases were chilled on an ice bath, carefully made alkaline with saturated aqueous Na_2CO_3 to pH 8–9 (foaming), and then extracted with CH_2Cl_2 (3 × 20 mL). The combined organic layers were dried (K_2CO_3), filtered and concentrated. The residue was chromatographed on Al_2O_3 using 1–2% MeOH in ether as eluent to afford (±)-**5** as an oil. The base was isolated as the oxalate. Recrystallization afforded an analytical sample.

Method H. (±)-1-Acetyl-2-[3-(dimethylamino)-1-propynyl]piperidine Methiodide [(±)-7]. Iodomethane (0.18 mL, 2.9 mmol) was added to (±)-**6** (0.16 g, 0.72 mmol) in dry acetone (2 mL). The mixture was stirred for 1 h at 20 °C and was then concentrated by rotary evaporation. The crystalline residue was washed repeatedly with dry ether and dried in a stream of dry N_2 to afford 0.24 g (94%) of (±)-**7**. An analytical sample was obtained by recrystallization.

Pharmacology. Guinea Pig Ileum. A standard guinea pig ileum preparation was set up in Tyrode solution (pH 7.4) at 37 °C as described previously.[37] The Tyrode solution contained hexamethonium (0.3 mM). Contractions were recorded isotonically at 1 g of tension. Concentration–response curves were constructed by the cumulative dose-response technique by stepwise increase of the concentration of agonist by a factor of 2.15.

Spasmogenic activity (EC_{50} values) was estimated by interpolation at the 50% response level of each compound. Dissociation constants (K_D) of antagonists were estimated with carbachol as the agonist.[25,37] The antagonist was allowed to equilibrate with the tissue for 15 min before the addition of carbachol. At least four different concentrations of each antagonist were used.

Muscarinic Receptor Binding Assay. Cerebral cortex from male Sprague–Dawley rats (200–300 g of body weight) was homogenized in 50 volumes of 50 mM sodium–potassium phosphate buffer (pH 7.4). The homogenate was centrifuged at 30000*g* for 10 min and resuspended in phosphate buffer to a concentration of 10 mg of original wet tissue weight/mL of buffer.

The binding of (–)-[^{3}H]NMS (80 Ci/mmol) was measured by the filtration assay of Yamamura and Snyder.[38] Homogenate of cortex (0.1 mL) was incubated with nonlabeled ligand and (–)-[^{3}H]NMS (0.3 nM) in a total volume of 2 mL of 50 mM phosphate buffer. Incubations lasted for 30 min at 30 °C. Binding in the presence of 10 μM atropine was defined as nonspecific. IC_{50} values (concentration that causes half-maximal inhibition of specific (–)-[^{3}H]NMS binding) of nonlabeled ligands were obtained by fitting a one-site competitive inhibition equation to the lig-and/(–)-[^{3}H]NMS competition data by nonlinear regression.[39] The IC_{50} values were corrected for receptor occupancy by (–)-[^{3}H]NMS as described by Cheng and Prusoff[40] to give K_i values (concentration of nonlabeled ligand that causes half-maximal receptor occupancy in the absence of (–)-[^{3}H]NMS).

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Supplementary Material Available: ^{13}C and ^{1}H NMR spectral data for compounds **5–10** and **16–29**, list of geometries and relative steric energies of low energy (MMX) conformations of (*R*)-**15–17** and (*R*)-**30** (8 pages). Ordering information is given on any current masthead page.

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