Acknowledgment. This work was supported in part by US PHS grant NS 23523.

Registry No. 2, 525-66-6; 3, 95685-31-7; 3 free base, 1202-55-7; 4, 118868-54-5; 5, 20904-18-1; 6, 118868-55-6; 7, 118868-56-7; 7 free base, 75384-45-1; 8, 118868-57-8; 8 free base, 28204-46-8; 9, 118868-58-9; 10, 26243-11-8; 11, 118868-59-0; 11 free base, 118868-73-8; (±)-12, 118868-60-3; 13, 118868-61-4; 13 free base, 87272-77-3; 14, 26243-10-7; 15, 118868-62-5; 15 free base, 118868-74-9; 16, 118868-63-6; 16 free base, 118868-75-0; 17, 118868-64-7; 17 free base, 118868-76-1; 18, 118868-65-8; 18 free base, 117263-75-9; 19, 118868-66-9; 19 free base, 118868-77-2; 20, 118868-67-0; 20 free base, 50882-68-3; 21, 63722-04-3; 22, 118868-68-1; 22 free base, 118890-25-8; 23, 118868-70-5; 23 free base, 118868-69-2; 24, 118890-24-7; 24 free base, 118868-78-3; 1-bromo-4-phenylbutane, 13633-25-5; dimethylamine hydrochloride, 506-59-2; 1-bromo-3-(2-methoxyphenoxy)propanamine, 118868-71-6; *N*-(2-(1-naphthyloxy)ethyl)phthalimide, 118868-72-7; 1-acetylnaphthalene, 941-98-0; 1-(3-bromopropoxy)naphthalene, 3351-50-6; 1-(4-bromobutoxy)naphthalene, 87723-21-5; 1-(2-bromoethoxy)naphthalene, 13247-79-5; *N*-propyl-1-propanamine, 142-84-7; *N*-butyl-1-butanamine, 111-92-2; *N*-methyl-1-propanamine, 627-35-0; *N*-methylbenzenemethanamine, 103-67-3; *N*-ethyl-1-butanamine, 13360-63-9.

Conformationally Restricted Analogues of the Muscarinic Agent *N*-Methyl-*N*-(1-methyl-4-pyrrolidino-2-butynyl)acetamide

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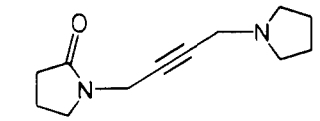
Conformationally restricted analogues of the selective partial muscarinic agonist *N*-methyl-*N*-(1-methyl-4-pyrrolidino-2-butynyl)acetamide (BM 5; **2**) were synthesized. The compounds were tested for muscarinic and antimuscarinic activity in the isolated guinea pig ileum and in intact mice. They were found to be moderately potent muscarinic antagonists or weak partial agonists. The new compounds were less potent than **2** in inhibiting (-)-[^{3}H]-*N*-methylscopolamine binding in the rate cerebral cortex. Thus, structural modifications of **2** in which part of the amide moiety has been connected with the methyl group in the butynyl chain to form a five-membered ring decrease affinity and in most cases abolish efficacy.

A large series of oxotremorine (**1**)[1] analogues has been synthesized during the past 25 years.[2] Structural modifications of **1** include (a) reduction or prolongation of the intermediate butynyl chain, (b) introduction of substituents (mainly methyl groups) in the lactam ring, in the intermediate chain and in the pyrrolidine moiety, (c) variations of the amino moiety, including quaternization, (d) exchange of the pyrrolidone moiety for succinimide, phthalimide, and a large number of other ring systems, and (e) opening of the pyrrolidone ring. One of the more interesting derivatives is *N*-methyl-*N*-(1-methyl-4-pyrrolidino-2-butynyl)acetamide (BM 5; **2**).[3] In contrast to **1**, which stimulates various muscarinic responses in a relatively uniform manner, **2** acts as an antagonist at some muscarinic sites (including certain presynaptic sites in the brain) while being an agonist at most others.[4,5] Thus, it has been suggested that **2** might have potential for the therapy of Alzheimer-type dementia and related disorders in which central cholinergic transmission is deficient.[6]



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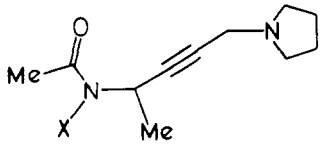

2 : X = Me

3 : X = MeCO

In the present paper we describe the synthesis and pharmacological evaluation of some conformationally restricted analogues of **2** in which, formally, the acetyl or the *N*-methyl substituent of the acetamide moiety has been connected with the methyl substituent in the butynyl chain. We have also included derivatives of the recently reported[7] acyclic imide **3** in this study.

The new compounds (**4**–**12**) were compared to **2** in their 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 also investigated for tremorogenic and tremorolytic activity in mice and for muscarinic and antimuscarinic activity on the isolated guinea pig ileum (Table II). None of the compounds had a pharmacological profile similar to that of **2**, which behaves like a partial agonist in the ileum and is a potent tremorolytic agent. However, **9** and **12** were partial agonists of weak potency and **7** and **10** were moderately potent tremorolytic agents.

† University of Uppsala.
‡ University of California.

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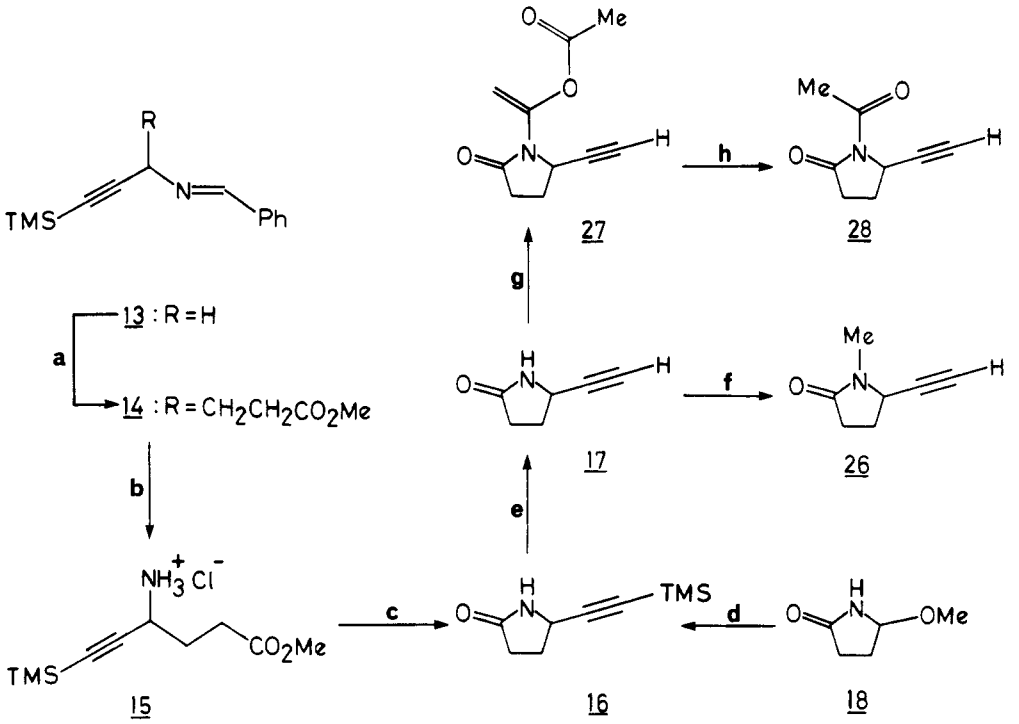
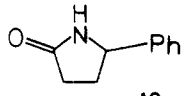
Scheme I[a]



[a] Reagents: (a) i, *n*-BuLi, THF, −75 °C; ii, $CH_2CHCOOMe$, −75 °C; iii, NH_4Cl (aq); (b) 1 M HCl, Et_2O; (c) Et_3N, THF; (d) BTMSA, $AlCl_3$, CH_2Cl_2, −5 °C; (e) KF, MeOH; (f) i, KH, THF; ii, MeI; (g) AcCl, Et_3N, 3 °C; (h) 1 M HCl, Et_2O.

Chemistry

The key intermediate 5-ethynyl-2-pyrrolidone (**17**) was synthesized by two alternative routes. In the first method (Scheme I), the lithium salt of *N*-benzylidene derivative **13** was reacted with methyl acrylate to form **14**.[8] Selective removal of the *N*-benzylidene function formed ammonium ester **15**.[9] Treatment of **15** with triethylamine in tetrahydrofuran gave the desired lactam **16**. This reaction sequence could preferably be performed without isolation of the intermediates. In such instances, we also isolated small amounts of 5-phenyl-2-pyrrolidone (**19**). Most likely,



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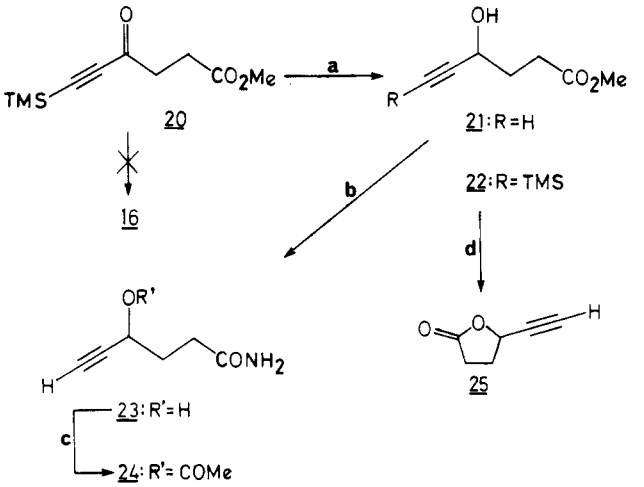
this product originates from Michael attack of the benzylic carbanion on methyl acrylate (the *N*-benzylidene anion is an ambident nucleophile). Desilylation of **16**, giving **17**, was readily accomplished with potassium fluoride in methanol.[10] Alternatively, and in a similar overall yield, **16** was prepared by an aluminum chloride catalyzed reaction of 5-methoxy-2-pyrrolidone (**18**) with bis(trimethylsilyl)acetylene (Scheme I). Methoxy lactam **18** is readily obtained by electrochemical decarboxylation/methoxylation of 2-pyrrolidone-5-carboxylic acid.[11]

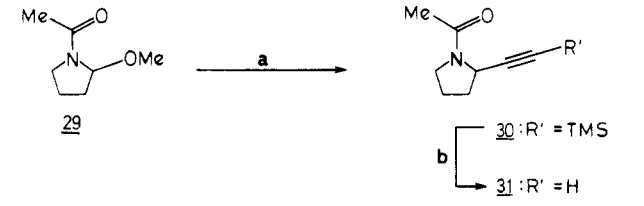
Several other attempts to synthesize **17** were unsuccessful; for example, attempted formation of the lactam ring by reductive amination of keto ester **20** resulted in formation of the corresponding alcohol (**21**) and base-catalyzed cyclization of amide acetate **24** produced lactone **25** as the sole product (Scheme II).

Scheme II[a]



[a] Reagents: (a) $NaBH_4$, MeOH, −30 °C; (b) NH_3(l), MeOH, −15 °C; (c) AcCl, 4-DMAP, pyridine, 0 °C; (d) Δ.

Scheme III[a]



[a] Reagents: (a) BTMSA, $AlCl_3$, CH_2Cl_2, −5 °C; (b) KF, MeOH.

N-Methylation of **17** using potassium hydride and iodomethane gave **26**. The N-acetylated derivative **28** was produced by treatment of **17** with a large excess of acetyl chloride and triethylamine in ether[7] followed by acid-catalyzed hydrolysis of the resulting enol ester **27** (Scheme I).

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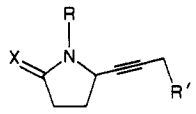
Table I. Physical Data of the Compounds Tested



compd	R	X	R′	prepn method[a]	yield, %	recrystn solvent[b]	mp, °C	formula
4	CH_3	O	NC_4H_8	A	86	A′	91–92	$C_{12}H_{18}N_2O\cdot C_2H_2O_4$
5	CH_3	O	$N(CH_3)_2$	A	82	A′	118–119	$C_{10}H_{16}N_2O\cdot C_2H_2O_4$
6	CH_3	O	$N(CH_3)_3^+$	B	49[c]	B′	211–212	$C_{11}H_{19}IN_2O$
7	CH_3CO	O	NC_4H_8	A	81	A′	140–142	$C_{13}H_{18}N_2O_2\cdot C_2H_2O_4$
8	CH_3CO	O	$N(CH_3)_2$	A	46	C′	71–73	$C_{11}H_{16}N_2O_2\cdot C_2H_2O_4$
9	CH_3CO	O	$N(CH_3)_3^+$	B	52[c]	B′	171–172	$C_{12}H_{19}IN_2O_2$
10	CH_3CO	H_2	NC_4H_8	A	81	C′	101–103	$C_{13}H_{20}N_2O\cdot C_2H_2O_4$
11	CH_3CO	H_2	$N(CH_3)_2$	A	82	A′	128–129	$C_{11}H_{18}N_2O\cdot 1.5C_2H_2O_4$
12	CH_3CO	H_2	$N(CH_3)_3^+$	B	55[c]	A′	198–200	$C_{12}H_{21}IN_2O$

[a] See the Experimental Section. [b] A′, MeOH/Et_2O; B′, MeOH/acetone; C′, no recrystallization. [c] Recrystallized yield.

Table II. Muscarinic and Antimuscarinic Effects and Receptor Binding Affinities of Some Oxotremorine Analogues[a]

	guinea pig ileum				intact mice:	rat cerebral cortex:
compd	*N*	EC_{50},[b] μM	E_{max}[c]	K_D,[d] μM	tremorolytic dose,[e] μmol/kg	[^{3}H]NMS displacement, K_i,[f] μM
2	4	0.19 ± 0.03[g]	0.83 ± 0.003[g]	0.24 ± 0.07[g]	0.6[h]	0.064 ± 0.007
3	7		0	0.28 ± 0.04	3.2 ± 1.0	
4	5		0	45.3 ± 3.8[i]	78.3 ± 16.5	18.3 ± 1.6
5	4		0	496 ± 26	>200	204 ± 17
6	3	>500	<0.6		ND[k]	145 ± 17
7	6		0	1.43 ± 0.2[i]	3.5 ± 0.6	0.78 ± 0.09
8	3		0	95.6 ± 36.2	160 ± 37	53.2 ± 5.0
9	4	13.4 ± 1.3	0.78 ± 0.06		ND[k]	6.4 ± 0.6
10	5		0	1.4 ± 0.2[i]	6.3 ± 0.6	0.67 ± 0.01
11	4		0	125 ± 19	119 ± 24	25.4 ± 3.3
12	4	7.5 ± 0.4	0.80 ± 0.08		ND[k]	6.1 ± 0.4
carbachol	6	0.10	1.00	15.3 ± 3.1	ND[k]	
atropine	4			0.0009 ± 0.0001	0.57 ± 0.06	

[a] Values are means plus or minus standard errors. [b] The concentration of an agonist or a partial agonist that elicits 50% of its own maximum contractile response. [c] The maximum contractile respone relative to that elicited by carbachol. [d] Dissociation constant of the drug–receptor complex. [e] Dose required to double the dose of oxotremorine inducing a predetermined (grade 2) tremor intensity. [f] Based on three separate determinations, each performed in triplicate. [g] Values are from ref 36. [h] Value is from ref 4. [i] Value is from ref 7. [j] The slopes of the Schild plot were 1.07 ± 0.15, 0.98 ± 0.10, and 0.90 ± 0.13 for compounds **4**, **7**, and **10**, respectively. [k] Not determined.

The *N*-acetylpyrrolidine derivative **31** was produced in good yield by treatment of **29** with anhydrous aluminum chloride and bis(trimethylsilyl)acetylene in methylene chloride[12] followed by desilylation using potassium fluoride in methanol (Scheme III).

The desired tertiary amines were obtained by cuprous chloride catalyzed Mannich reactions[13] of the appropriate acetylenic precursor with pyrrolidine or dimethylamine (method A). Methylation of the dimethylamino function with iodomethane in acetone gave the quaternary ammonium derivatives (method B).

Pharmacology

Compounds **4–12** were tested for muscarinic and antimuscarinic activity in the isolated guinea pig ileum and in intact mice. The results are presented in Table II. For comparison, relevant data for **2**, **3**, carbachol, and atropine are also included. In Table II, K_D is the equilibrium dissociation constant of the drug–receptor complex and is a measure of affinity.[14] K_D values of the antagonists were obtained from their ability to antagonize carbachol-induced contractions in the ileum (spasmolytic activity).

None of the tertiary amines showed visible muscarinic effects in vivo or spasmogenic activity on the guinea pig ileum. Instead, **4**, **7**, **8**, **10**, and **11** antagonized oxotremorine-induced tremors and therefore showed central antimuscarinic activity. All the tertiary amines were antagonists to carbachol on the ileum (Figure 1). This antagonism was competitive for **4**, **7**, and **10** as evidenced by the slopes of their Schild plots,[15] which were not significantly different from one (Table II). Because of the low affinities of **5**, **8**, and **11**, these compounds could not be tested over a sufficiently wide concentration range to allow for a meaningful Schild analysis. Spasmolytic as well as

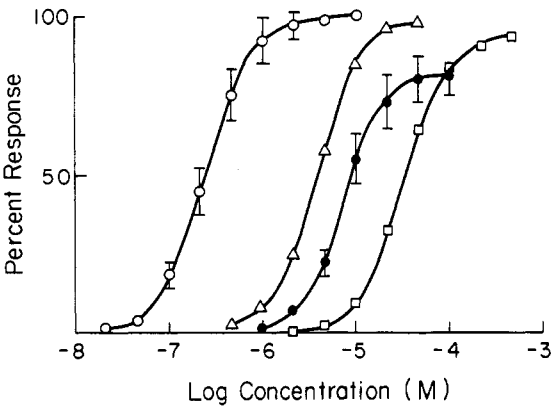


Figure 1. Concentration–response curves in the isolated guinea pig ileum of **12** (●), carbachol alone (○) and carbachol in the presence of 20 μM (△) and 200 μM (□) of **10**. Responses are expressed relative to the maximum response elicited by carbachol. Vertical bars show standard errors.

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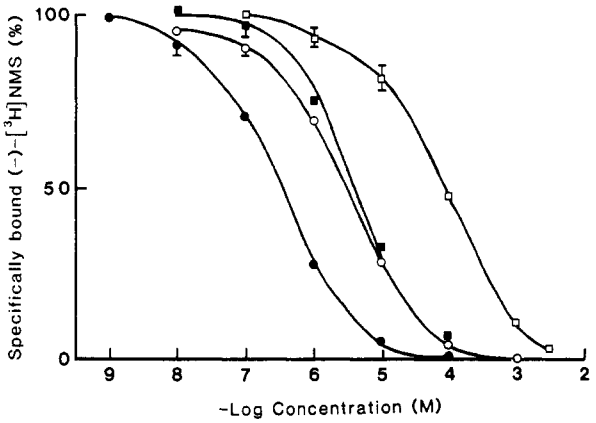

Figure 2. Competitive inhibition of (-)-[^{3}H]NMS binding in rat cerebral cortex by **2** (●), **4** (□), **7** (■), and **10** (○). Values are means ● standard errors from three experiments, each performed in triplicate. The concentration of (-)-[^{3}H]NMS used was 0.3 nM. The theoretical curves are the least-squares fit to the data.

tremorolytic activity were much higher for the pyrrolidino (**4**, **7**, and **10**) than for the dimethylamino derivatives (**5**, **8**, and **11**) (Table II). The most potent antagonists (**7** and **10**) were those having *N*-acetyl substituents.

The trimethylammonium derivatives **9** and **12** behaved as relatively weak partial agonists on the ileum (Figure 1) whereas **6** was virtually inactive.

The compounds inhibited the specific binding of (-)-[^{3}H]NMS in the rat cerebral cortex in an apparently competitive manner (Figure 2). The binding data were analyzed with a one-site binding equation to give K_i values (Table II).

Discussion

Compounds **4–6** are semirigid derivatives of the partial muscarinic agent **2** in which the acetyl methyl and the methyl group of the butynyl chain have been connected to form a five-membered ring. The resulting geometry, in which the two methylene groups are close to eclipsed, corresponds to an unfavorable conformation of **2** [as suggested by preliminary molecular mechanics (MMX) calculations]. In contrast to **2** and its dimethylamino and trimethylammonium analogues,[3,16] **4–6** are unable to stimulate muscarinic receptors. They also are poor antagonists having at least 100-fold lower affinity than **2** for central and peripheral muscarinic receptors. These observations indicate that the carbonyl group of **4–6** occupies a relative spatial position which does not allow for an optimal receptor interaction.

In compounds **10–12**, the five-membered ring is formed by connection of the *N*-methyl of **2** with the butynyl methyl via a methylene group. The resulting *N*-acetylpyrrolidine derivatives show considerably higher spasmolytic and tremorolytic potencies than the *N*-methylpyrrolidone derivatives **4–6**. However, they are still less potent than **2**. Pyrrolidine derivative **10**, which is the most potent antagonist among **10–12**, is about 10 times less potent than **2** and also has 10-fold lower affinity for cortical muscarinic receptors. Whereas **10** and **11** behave as antagonists, trimethylammonium derivative **12** is a relatively weak partial agonist on the ileum (Figure 1).

Imides **7–9** are semirigid analogues of the acyclic imide **3**, which is a fairly potent antagonist.[7] Alternatively, **7–9** can be considered as hybrids of **4–6** and **10–12**. The pharmacological potencies and receptor binding affinities of **7–9** parallel those of **10–12**. Thus, the pyrrolidone carbonyl does not appear to enhance or diminish affinity or efficacy whereas the *N*-acetyl carbonyl seems to be much better positioned for binding to muscarinic receptors. It has been shown previously that introduction of larger substituents, such as ethyl groups, in the butynyl chain and in the *N*-methyl group of **2** and related compounds yields antagonists, i.e. decreases efficacy.[3,17] Thus, the added methylene group in **7–12** (as compared to **2**) may constitute a steric hindrance for an effective activation of muscarinic receptors. Alternatively, the relative rigidity of **4–12** may reduce their efficacy since conformational changes of both agonist and receptor may be required for the induction of a response.[18]

The effects of variations in the amino moiety on pharmacological potency and receptor binding affinity were similar regardless of substitution pattern in the amide moiety; the pyrrolidino derivatives were the most potent antagonists both in vivo and in vitro. The trimethylammonium derivatives were more efficacious than the corresponding tertiary amines. These results are consistent with those from other studies of oxotremorine-related muscarinic/antimuscarinic agents.[2]

Experimental Section

Chemistry. General Comments. Melting points (uncorrected) were determined in open glass capillaries on a Thomas-Hover apparatus. IR spectra were recorded on a Perkin-Elmer 298 infrared spectrophotometer. ^{1}H 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. Mass spectra were obtained on a LKB 9000 spectrometer using a direct insertion probe. All spectra were in accordance with the assigned structures. Flash chromatography was carried out as described by Still et al.[19] Thin-layer chromatography (TLC) 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. Solvents used for eluting TLC plates were the following: A, Et_2O/*n*-hexane (1:1); B, Et_2O/*n*-hexane (8:2); C, $CHCl_3$; D, Et_2O/MeOH (99:1); E, Et_2O/MeOH (9:1); F, Et_2O. TLC spots were visualized with UV light or by spraying with aqueous potassium permanganate. 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 value. Commercial reagent grade chemicals were used throughout. THF was distilled from sodium benzophenone ketal under N_2 immediately before use. CH_2Cl_2 was dried over P_2O_5, distilled under N_2, and stored with molecular sieves. Et_3N was dried over KOH pellets. All other solvents were of reagent grade quality and were used as received.

5-[(Trimethylsilyl)ethynyl]-2-pyrrolidone (16). Preferentially, the following reaction seequence was carried out without isolation of intermediates.

Crude methyl 4-(benzylideneamino)-6-(trimethylsilyl)-5-hexynoate (**14**) was prepared from *N*-benzylidene-3-(trimethylsilyl)propargylamine (**13**)[8,20] (10.0 g, 46.4 mmol), *n*-BuLi in hexane (1.55 M; 30 mL, 47 mmol), and methyl acrylate (3.8 mL, 42 mmol) in dry THF (330 mL) at -75 °C according to a literature method.[8] The reaction mixture was quenched by pouring it into a stirred and saturated NH_4Cl solution (500 mL). The mixture was partitioned between water and Et_2O. The organic layer was washed repeatedly with brine, dried (Na_2SO_4), and filtered. Evaporation of the volatiles afforded 14 g of a thick, dark-red oil consisting mainly of **14** and recovered starting material in a 3:1 ratio ac-

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cording to GC and ^1H NMR analysis. Crude **14** could be purified by rapid bulb-to-bulb distillation but considerable polymerization took place during the distillation: bp 150–155 °C (0.1 mmHg) [lit.[8] bp 140–144 °C (0.2 mmHg)]; IR (neat) 2160, 1735, 1642, 840 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.20 (s, 9 H), 2.00–2.64 (m, 4 H), 3.64 (s, 3 H), 4.45–4.61 (m, 1 H), 7.20–7.80 (m, 5 H), 8.53 (d, J = 1.8 Hz, 1 H).

A solution of crude **14** (18.6 g, approximately 62 mmol) in Et_2O (100 mL) was added to a mixture of 1 M aqueous HCl (185 mL, 185 mmol) and Et_2O (150 mL) at room temperature. The mixture was stirred vigorously for 10 min. The layers were separated, and the organic layer was extracted with 1 M aqueous HCl (150 mL). The combined aqueous layers were filtered through glass wool and then rapidly concentrated and dried [first by coevaporation with Et_2O and C_6H_6, and then at reduced pressure (0.05 mmHg) at 35 °C] to give 8.28 g of a crystalline mass consisting of **methyl 4-amino-6-(trimethylsilyl)-5-hexynoate hydrochloride** [**15**; R_f 0.58 (Al_2O_3; D); IR (KBr) 2160, 1735 cm^{-1}; ^1H NMR (CD_3OD) δ 0.20 (s, 9 H), 1.77–2.42 (m, 2 H), 2.53–2.70 (m, 2 H), 3.70 (s, 3 H), 4.25 (dd, J_1 = 9.1 Hz, J_2 = 5.3 Hz, 1 H)] and **4-amino-6-(trimethylsilyl)-5-hexynoic acid** [IR (KBr) 1720 cm^{-1}; ^1H NMR (CD_3OD) δ 0.19 (s, 9 H), 1.74–2.34 (m, 2 H), 2.49–2.66 (m, 2 H), 4.23 (dd, J_1 = 8.8 Hz, J_2 = 5.6 Hz, 1 H)].

A solution of the above mixture and dry Et_3N (23 mL, 164 mmol) in dry THF (100 mL) was heated to reflux overnight under N_2. Et_2O was added and precipitated Et_3N·HCl was filtered off. The resulting solution was concentrated and the residue was purified by flash chromatography using Et_2O/MeOH (1.5–3%) as eluant. The yield of pure **16** was 2.28 g (19% from **13**): mp 90–90.5 °C (Et_2O/n-hexane); R_f 0.45 (Al_2O_3, D); IR (KBr)[21] 3200 (br), 2170, 1710, 1655 cm^{-1}; mass spectrum (40 eV), m/z (relative intensity) 181 (48, M^+); ^1H NMR ($CDCl_3$) δ 0.15 (s, 9 H), 2.05–2.65 (m, 4 H), 4.28–4.43 (m, 1 H), 6.30 (br s, 1 H). Anal. ($C_9H_{15}NOSi$) C, H, N.

An isolated byproduct was identified as **5-phenyl-2-pyrrolidone (19)**: yield 0.30 g (3% from **13**); mp 106–107 °C (n-hexane/Et_2O) (lit.[22] mp 110 °C).

Alternative Preparation of 5-[(Trimethylsilyl)-ethynyl]-2-pyrrolidone (16). Anhydrous $AlCl_3$ (2.4 g, 18 mmol) was added in portions to a vigorously stirred solution of bis(trimethylsilyl)acetylene[23] (1.8 g, 11 mmol) and 5-methoxy-2-pyrrolidone (**18**)[11] (0.78 g, 7.0 mmol) in dry CH_2Cl_2 (14 mL) kept under N_2 at -5 °C. The reaction mixture was stirred at -10 °C overnight and then poured on ice (30 g). The aqueous layer was extracted with CH_2Cl_2, and the combined organic layers were washed with aqueous 0.5 M potassium–sodium tartrate and with saturated aqueous $NaHCO_3$, dried (Na_2SO_4), filtered, and concentrated. Flash chromatography using Et_2O as eluant gave crude **16**, which was further purified by recrystallization to give 0.23 g (18%) of pure **16**.

5-Ethynyl-2-pyrrolidone (17).[24] A solution of **16** (2.6 g, 14 mmol) and KF (2.5 g, 43 mmol) in MeOH (100 mL) was heated to reflux under N_2 for 1 h. The mixture was concentrated and the residue was triturated with CH_2Cl_2. Evaporation of the solvent gave 1.6 g (99%) of **17**: mp 101–103 °C (Et_2O/MeOH); R_f 0.24 (Al_2O_3, D); IR (KBr) 3225, 2117, 1690 cm^{-1}; mass spectrum (70 eV), m/z (relative intensity) 109 (44, M^+); ^1H NMR ($CDCl_3$) δ 2.05–2.72 (m, 4 H), 2.39 (d, J = 2.2 Hz, 1 H), 4.30–4.46 (m, 1 H), 6.20 (br s, 1 H).

Methyl 4-Oxo-6-(trimethylsilyl)-5-hexynoate (20).[25,26] This compound was prepared in 0.12-mol scale according to a literature method[25] from bis(trimethylsilyl)acetylene,[23] 3-(methoxycarbonyl)propionyl chloride,[26] and anhydrous $AlCl_3$: yield, 81% after purification by flash chromatography using Et_2O/light petroleum (6:4) as eluent; bp 71–73 °C (0.05 mmHg) [lit.[26] bp 80–85 °C (0.1 mmHg)].

Methyl 4-Hydroxy-5-hexynoate (21). $NaBH_4$ (0.085 g, 2.3 mmol) was added in one portion to a solution of **20** (1.5 g, 7.1 mmol) in dry MeOH (30 mL) kept at -30 °C under N_2. The solution was allowed to reach room temperature after 3 h, and the stirring was continued for another 37 h. A saturated aqueous NH_4Cl solution (2 mL) was added and the MeOH was evaporated. The residue was partitioned between Et_2O and H_2O. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated to give 0.93 g (93%) of pure **21** as an oil: bp 74–76 °C (0.3 mmHg); R_f 0.31 (SiO_2, A); IR (neat liquid) 3400 (br), 2117, 1737 cm^{-1}; mass spectrum (70 eV), m/z (relative intensity) 128 (30, $M^+ - 14$); ^1H NMR ($CDCl_3$) δ 1.90–2.14 (m, 2 H), 2.47–2.64 (m, 2 H), 2.47 (d, J = 2.0 Hz, 1 H), 2.73 (br d, J = 6.0 Hz, 1 H), 3.68 (s, 3 H), 4.47 (ddt, J_1 = 2.0 Hz, J_2 = 6.0 Hz, J_3 = 5.9 Hz, 1 H). Anal. ($C_7H_{10}O_3$) C, H.

When the reaction above was quenched more rapidly, the main product was **methyl 4-hydroxy-6-(trimethylsilyl)-5-hexynoate (22):** bp 140-142 °C (0.8 mmHg); R_f 0.48 (SiO_2, A); IR (neat liquid) 3450 (br), 2175, 1743 cm^{-1}; mass spectrum (70 eV), m/z (relative intensity) 199 (13, M – CH_3); ^1H NMR ($CDCl_3$) δ 0.15 (s, 9 H), 1.89-2.13 (m, 2 H), 2.30 (br s, 1 H), 2.46-2.64 (m, 2 H), 3.68 (s, 3 H), 4.46 (t, J = 6.2 Hz, 1 H). Anal. ($C_{10}H_{18}SiO_3$) C, H.

4-Hydroxy-5-hexynamide (23). Liquid NH_3 (50 mL) was added to a stirred solution of a 1:3 mixture of **21** and **22** (4.00 g, 21.8 mmol) in dry MeOH (150 mL) kept at -15 °C. Stirring was continued for 48 h and the temperature was allowed to slowly rise to room temperature. Concentration of the reaction mixture and flash chromatography of the residue using Et_2O/MeOH (10:1) as eluant afforded 2.57 g (93%) of **23** as an oil: R_f 0.41 (SiO_2, E); IR (neat liquid) 3300 (v br), 2112, 1665 cm^{-1}; mass spectrum (70 eV), m/z (relative intensity) 110 (4, $M^+ - 17$), 59 (55), 44 (75); ^1H NMR [$(CD_3)_2CO$] δ 1.80–2.03 (m, 2 H), 2.35–2.52 (m, 2 H), 2.82 (d, J = 2.1 Hz, 1 H), 4.38 (ddt, J_1 = 2.1 Hz, J_2 = 6.0 Hz, J_3 = 6.0 Hz, 1 H), 4.79 (d, J = 6.0 Hz, 1 H), 6.25-6.85 (br d, 2 H). Anal. ($C_6H_9NO_2$) C, H, N.

4-Acetoxy-5-hexynamide (24). Acetyl chloride (0.14 mL, 1.9 mmol) was added dropwise to mixture of **23** (0.10 g, 0.79 mmol), 4-(dimethylamino)pyridine (0.01 g, 0.08 mmol), and dry pyridine (1.5 mL, 19 mmol) kept at 0 °C under N_2. After 4 h the reaction mixture was concentrated and the residue was purified by flash chromatography using Et_2O/acetone (10:1) followed by Et_2O/acetone (3:1) as eluants. The yield of **24** was 0.14 g (99%): mp 80-81 °C (MeOH/Et_2O); R_f 0.56 (SiO_2, E); IR (KBr) 2120, 1735, 1650 cm^{-1}; mass spectrum (60 eV), m/z (relative intensity) 126 (17, $M^+ - CH_3CO$), 59 (18), 44 (46), 43 (100); ^1H NMR ($CDCl_3$) δ 1.99–2.25 (m, 2 H), 2.09 (s, 3 H), 2.33-2.44 (m, 2 H), 2.51 (d, J = 2.1 Hz, 1 H), 5.43 (dt, J_1 = 5.8 Hz, J_2 = 2.1 Hz, 1 H), 5.92 (br d, 2 H). Anal. ($C_8H_{11}NO_3$) C, H, N.

5-Ethynyl-γ-butyrolactone (25). Compound **21** (0.91 g, 6.4 mmol) was heated overnight at 180 °C (oil bath temperature) in a flask equipped with a condenser and kept under N_2. The residue was triturated with Et_2O. The Et_2O extract was concentrated and purified by flash chromatography using Et_2O/light petroleum (1:1) as eluant. Distillation afforded 0.41 g (58%) of **25** as an oil: bp 64–65 °C (0.9 mmHg); R_f 0.38 (Al_2O_3, A); IR (neat liquid) 2125, 1780 cm^{-1}; mass spectrum (70 eV), m/z (relative intensity) 110 (7, M^+); ^1H NMR ($CDCl_3$) δ 1.95-2.92 (m, 4 H), 2.63 (d, J = 2.0 Hz, 1 H), 5.01-5.17 (m, 1 H). Anal. ($C_6H_6O_2$) C, H.

5-Ethynyl-1-methyl-2-pyrrolidone (26). Compound **16** (2.5 g, 14 mmol) was added to a stirred suspension of KH (0.60 g, 15 mmol) in dry THF (100 mL) under N_2. After 10 min the solution was chilled to 5 °C and MeI (1.0 mL, 16 mmol) was added. The mixture was stirred for 1 h and MeOH (0.6 mL) was added. The volatiles were evaporated, and the residue was partitioned between H_2O and Et_2O. The organic layer was dried (Na_2SO_4), filtered, and concentrated. The residue (partially desilylated product) was treated with KF (1.0 g, 17 mmol) in boiling MeOH (60 mL) for 1 h. The solvent was evaporated and the residue was triturated with CH_2Cl_2. The combined organic extracts were filtered, concentrated, and purified by chromatography on Al_2O_3 using Et_2O/MeOH (0–2%) as eluant to give 1.27 g (75%) of **26** as an oil: bp 44–46 °C (0.04 mmHg), which solidified upon cooling; mp 35-36 °C (n-hexane/Et_2O); R_f 0.44 (Al_2O_3, D); IR (neat liquid) 3225, 2110, 1690 cm^{-1}; mass spectrum (70 eV), m/z (relative

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intensity) 123 (56, M^+), 122 (60); ^1H NMR ($CDCl_3$) δ 1.85–2.50 (m, 4 H), 2.41 (d, J = 2.1 Hz, 1 H), 2.83 (s, 3 H), 4.17–4.33 (m, 1 H). Anal. (C_7H_9NO) C, H, N.

1-(1-Acetoxyethenyl)-5-ethynyl-2-pyrrolidone (27). Acetyl chloride (6.2 mL, 87 mmol) was added slowly (15 min) from a dry syringe to a vigorously stirred mixture of **17** (0.95 g, 8.7 mmol), dry Et_3N (6.1 mL, 46 mmol), and dry Et_2O (95 mL) kept at 3 °C under N_2. The stirring was continued overnight and the reaction mixture was allowed to reach room temperature. The solution was filtered, and the volatiles were evaporated. The residue was partitioned between CH_2Cl_2 and water, and the organic layer was washed with a saturated aqueous $NaHCO_3$ solution until pH 7–8 and with brine. The organic layer was dried (Na_2SO_4), filtered, and concentrated. Flash chromatography using Et_2O/light petroleum (7:3) as eluant gave 1.4 g (84%) of **27** as an oil and 0.06 g (5%) of **1-acetyl-5-ethynyl-2-pyrrolidone (28).**

27: bp 120-122 °C (0.9 mmHg); R_f 0.50 (SiO_2; F); IR (neat liquid) 3270, 2110, 1770, 1720, 1660 cm^{-1}; mass spectrum (22 eV), m/z (relative intensity) 193 (5, M^+), 151 (80), 123 (100), 95 (36), 43 (97, CH_3CO); ^1H NMR ($CDCl_3$) δ 1.80-2.75 (m, 4 H), 2.12 (s, 3 H), 2.42 (d, J = 2.3 Hz, 1 H), 4.44-4.59 (m, 1 H), 4.70 (d, J = 2.9 Hz, 1 H), 4.98 (d, J = 2.9 Hz, 1 H). Anal. ($C_{10}H_{11}NO_3$) C, H, N.

1-Acetyl-5-ethynyl-2-pyrrolidone (28). A mixture of **27** (0.83 g, 4.3 mmol), Et_2O (20 mL), and 1 M aqueous HCl (3 mL, 3 mmol) was stirred vigorously at room temperature for 90 min. To complete the hydrolysis, 5 M aqueous HCl (1 mL) was added and stirring was continued for another 40 min. Solid $NaHCO_3$ (2 g) was added. The aqueous phase was washed with Et_2O, and the combined organic extracts were dried twice (Na_2SO_4). Evaporation of the volatiles afforded 0.65 g (quantitative yield) of pure **28**: bp 86–88 °C (0.6 mmHg); R_f 0.48 (SiO_2, B); IR (neat liquid) 3260, 2110, 1740, 1700 cm^{-1}; mass spectrum (70 eV), m/z (relative intensity) 151 (6, M^+), 43 (100, CH_3CO); ^1H NMR ($CDCl_3$) δ 2.11-3.05 (m, 8 H), 4.92-5.03 (m, 1 H). Anal. ($C_8H_9NO_2$) C, H, N.

1-Acetyl-2-[(trimethylsilyl)ethynyl]pyrrolidine (30). Compound **30** was prepared from 1-acetyl-2-methoxypyrrolidine (**29**)[27] (5.0 g, 35 mmol) by the same procedure as described for the preparation of **16**. The crude product was purified by flash chromatography using Et_2O/light petroleum (6:1), Et_2O, and Et_2O/MeOH (2%) as eluants to give 4.93 g (68%) of pure **30**. Impure fractions (1.61 g) were desilylated and purified as described below to give 0.43 g of **31**.

30: bp 81-82 °C (0.1 mmHg); R_f 0.82 (SiO_2, E); IR (neat liquid) 2165, 1655 cm^{-1}; mass spectrum (50 eV), m/z (relative intensity) 209 (67, M^+), 194 (65), 166 (39), 73 (100), 43 (83, CH_3CO); ^1H NMR ($CDCl_3$) δ 0.10, 0.12 (s's, 9 H), 1.70-2.25 (m, 7 H), 3.20–3.70 (m, 2 H), 4.35-4.76 (m, 1 H).

1-Acetyl-2-ethynylpyrrolidine (31). A solution of **30** (3.2 g, 16 mmol) and KF (3.0 g, 52 mmol) in MeOH (150 mL) was heated to reflux for 1 h. The volatiles were evaporated, and the residue was triturated with CH_2Cl_2. Evaporation of the solvent and distillation afforded 1.8 g (83%) of **31**: bp 92-94 °C (1.0 mmHg); R_f 0.61 (SiO_2, E); IR (neat liquid) 3220, 2100, 1640 cm^{-1}; mass spectrum (50 eV), m/z (relative intensity) 137 (28, M^+), 94 (61), 67 (99), 43 (100, CH_3CO); ^1H NMR ($CDCl_3$) δ 1.60-2.40 (m, 8 H), 3.00-3.70 (m, 2 H), 4.25-4.75 (m, 1 H). Anal. ($C_8H_{11}NO$) C, H, N.

Below follow representative examples of methods A and B.

1-Methyl-5-(3-pyrrolidino-1-propynyl)-2-pyrrolidone (4). Method A. A mixture of pyrrolidine (0.26 mL, 3.2 mmol), paraformaldehyde (0.095 g, 3.2 mmol), HOAc (2 mL), CuCl (0.05 g), **24** (0.33 g, 2.6 mmol), and dry dioxane (15 mL) was stirred at 40 °C for 24 h under N_2. The mixture was concentrated and the residue was dissolved in Et_2O (50 mL) and extracted with 2.5 M aqueous HCl (15 mL). The aqueous layer was alkalinized with solid Na_2CO_3 to pH ~10[28] and then extracted with Et_2O (5 × 30 mL). The combined organic layers were dried (Na_2SO_4), filtered, and concentrated. The residue was purified by chromatography on Al_2O_3 using Et_2O/MeOH (2%) as eluant to afford 0.47 g (86%) of pure **4** as a colorless oil. The base was converted into the oxalate: mass spectrum (40 eV), m/z (relative intensity) 206 (57, M^+); ^1H NMR (CD_3OD) δ 2.00–2.70 (m, 8 H), 2.86 (s, 3 H), 3.25–3.60 (m, 4 H), 4.17 (d, J = 1.8 Hz, 2 H), 4.35–4.60 (m, 1 H).

1-Methyl-5-[3-(dimethylamino)-1-propynyl]-2-pyrrolidone Methiodide (6). Method B. Iodomethane (0.35 mL, 5.6 mmol) was added to a solution of 1-methyl-5-[3-(dimethylamino)-1-propynyl]-2-pyrrolidone (**5**) (0.20 g, 1.1 mmol) in dry acetone (8.0 mL) under N_2. The mixture was stirred for 1 h at room temperature, and the volatiles were evaporated. The crystalline residue was washed repeatedly with dry Et_2O and dried in a stream of N_2 to yield, after recrystallization, pure **6**: IR (KBr) 1690 cm^{-1}; mass spectrum (15 eV), m/z (relative intensity) 180 (18, M^+ – MeI), 142 (92, MeI); ^1H NMR (D_2O) δ 2.13–2.88 (m, 4 H), 2.97 (s, 3 H), 3.31 (s, 9 H), 4.38–4.48 (d, 2 H), 4.58–4.80 (m, 1 H).

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.[29] 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 increasing stepwise 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.[15,29] The antagonist was allowed to equilibrate with the tissue for 15 min before the addition of carbachol. At least three different concentrations of each antagonist were used.

Tremorolytic Activity in Mice. Male Swiss–Webster mice (24–32 g body weight) were used. The test compounds were administered ip at a dose of 200 μmol/kg to groups of six or more mice, while six control animals remained untreated. Twenty minutes after drug administration, the ED_{50} value of oxotremorine, injected iv, was estimated by the up-and-down method[30] using intermittent spontaneous (grade 2) tremor[31] as the end point. Compounds that showed no significant antagonism of oxotremorine under these conditions were not tested further. Compounds that significantly blocked oxotremorine at a dose of 200 μmol/kg were tested at two additional doses. For the latter compounds, the ED_{50} value of oxotremorine was plotted against the dose of antagonist (including zero) used for premedication. The dose of antagonist which doubled the ED_{50} value of oxotremorine was estimated by linear regression analysis.[4] Under these conditions, atropine and **2** had tremorolytic doses of 0.9 and 0.6 μmol/kg, respectively.

Muscarinic Receptor Binding Assay. Cerebral cortex from male Sprague–Dawley rats (200–300 g 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 (–)-[^3H]NMS (80 Ci/mmol) was measured by the filtration assay of Yamamura and Snyder.[32] Homogenate of cortex (0.1 mL) was incubated with nonlabeled ligand and (–)-[^3H]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. The binding parameters were determined from the experimental data by nonlinear least-squares regression analysis. IC_{50} values (concentration that causes half-maximal inhibition of specific (–)-[^3H]NMS binding) of nonlabeled ligands were obtained by fitting a one-site competetive inhibition equation to the ligand/(–)-[^3H]NMS competition data.[33] The IC_{50} values were corrected for receptor occupancy by (–)-[^3H]NMS as described by Cheng and Prusnoff[34] to give K_i values (concentration of nonlabeled ligand that causes half-maximal

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receptor occupancy in the absence of (–)-[^{3}H]NMS). The dissociation constant of (–)-[^{3}H]NMS (0.070 nM) was determined independently by Scatchard analysis of seven-point (–)-[^{3}H]NMS binding isotherms using a centrifugation assay.[35]

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Registry No. **4**, 118800-02-5; **4**·$C_2H_2O_4$, 118800-20-7; **5**, 118800-21-8; **5**·$C_2H_2O_4$, 118800-22-9; **6**, 118800-03-6; **7**, 118800-04-7; **7**·$C_2H_2O_4$, 118800-23-0; **8**, 118800-05-8; **8**·$C_2H_2O_4$, 118800-24-1; **9**, 118800-06-9; **10**, 118800-07-0; **10**·$C_2H_2O_4$, 118800-25-2; **11**, 118800-08-1; **11**·$C_2H_2O_4$, 118800-26-3; **12**, 118800-09-2; **13**, 57790-32-6; **14**, 57735-00-9; **15**, 111886-97-6; **16**, 111886-98-7; **17**, 105457-63-4; **18**, 63853-74-7; **19**, 22050-10-8; **20**, 88761-59-5; **21**, 118800-10-5; **22**, 118800-11-6; **23**, 118800-12-7; **24**, 118800-13-8; **25**, 118800-14-9; **26**, 118800-15-0; **27**, 118800-16-1; **28**, 118800-17-2; **29**, 63050-21-5; **30**, 118800-18-3; **31**, 118800-19-4; methyl acrylate, 96-33-3; 4-amino-6-(trimethylsilyl)-5-hexynoic acid, 111886-96-5; bis(trimethylsilyl)acetylene, 14630-40-1; 3-(methoxycarbonyl)-propionyl chloride, 1490-25-1; pyrrolidine, 123-75-1; dimethylamine, 124-40-3.

Supplementary Material Available: ^{13}C NMR chemical data for compounds **4–12**, **15–17**, and **21–28** and ^{1}H NMR chemical data for compounds **5** and **7–12** (3 pages). Ordering information is given on any current masthead page.

Identification of Structural Requirements for Analogues of Atrial Natriuretic Peptide (ANP)† To Discriminate between ANP Receptor‡ Subtypes

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The structure–activity relationships for affinity and selective binding of atrial natriuretic peptide (ANP) and analogues to guanylate cyclase coupled (CC) and non-cyclase coupled (NC) receptors in rabbit lung membranes are described. We have designed a series of peptides to try to identify the minimal sequence involved in specific recognition of each receptor subtype. The affinity of the peptides was determined from competitive binding experiments. Several peptides derived from the rat ANP sequence, e.g., des-[Phe^{106},Gly^{107},Ala^{115},Gln^{116}]ANP-(103–125)NH_2 (**4**), des-[Cys^{105,121}]ANP-(104–126) (**5**), and [Acm-Cys^{105}]ANP-(105–114)NH_2 (**9**) have high affinity and selectivity for the noncoupled site. Peptide **4** was the most selective ligand with an affinity superior to that of ANP-(103–126). This compound does not displace the radiolabeled ligand from the guanylate cyclase coupled receptor at the highest concentration tested (100 nM). The structure–activity relationship for affinity and selectivity is discussed. Comparison of the peptide sequences suggests that the structural feature responsible for recognition of the NC site resides in a single sequence of seven contiguous amino acids from the cyclic core of the hormone. The corresponding heptapeptide retains affinity to the guanylate cyclase uncoupled binding site and is proposed to encompass the minimal sequence for specific recognition of the non-guanylate cyclase coupled ANP receptor.

Recently, several reports have pointed to the existence of two distinct types of endogeneous binding proteins specific for the peptides of the atrial natriuretic peptide (ANP) family.[1,3,5,25] One subtype is coupled to guanylate cyclase and the other is not.[2,6–12] Although the circulating 28-mer, ANP (99–126), and closely related analogues bind to both types of specific binding proteins with similar subnanomolar affinities, so far, only the class of binding protein coupled to guanylate cyclase (CC, guanylate cyclase coupled) has been firmly established as a receptor correlated with the physiological responses of ANP.[6,21] The role of the non-guanylate cyclase coupled protein is still a matter for debate.[7] Affinity cross-linking studies have

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† Abbreviations are according to the IUPAC-IUB Commission on Biochemical Nomenclature (*Pure Appl. Chem.* **1974**, *40*, 317). The sequence of the human atrial natriuretic peptide (ANP) is as follows: S^{99}-L-R-R-S-S-C^{105}-F-G-G-R-M^{110}-D-R-I^{113}-G-A-Q-S-G-L-G-C^{121}-N-S-F-R-Y^{126}. The rat sequence has an Ile residue instead of the Met residue at position 110 and is abbreviated rat ANP-(99–126). An alternative nomenclature for ANP is published in *New Engl. J. Med.* **1987**, *316*, 1278.

‡ Receptor is taken in a broad sense. As described in the text, only one of the ANP binding proteins has been shown to be associated with a second messenger system producing physiological responses. The second binding protein is actually a binding site and its physiological significance is under study.

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