Chemical and Biochemical Studies of 2-Propynylpyrrolidine Derivatives. Restricted-Rotation Analogues of *N*-Methyl-*N*-(1-methyl-4-pyrrolidino-2-butynyl)acetamide (BM-5)

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A series of optically pure 2-[substituted-3-aminopropynyl]pyrrolidine derivatives, which are restricted-rotation analogues of the muscarinic agent *N*-methyl-*N*-(1-methyl-4-pyrrolidino-2-butynyl)acetamide (BM-5, compound 1), have been prepared from *d*- and *l*-proline. The compounds when tested in a series of in vitro muscarinic assays [[^{3}H]CD (cortex), [^{3}H]QNB (cortex), [^{3}H]PZ (cortex), [^{3}H]QNB (heart), [^{3}H]QNB + GppNHp (heart)] were found to have weaker muscarinic properties than compound 1. The decrease in affinity was attributed to the increased size of the molecule resulting from the addition of a methylene group to form the pyrrolidine ring. The use of optically active compounds provided a more detailed examination of the complex pharmacological effects of the flexible muscarinic agent 1. The *R* enantiomers in the acetamide derivatives **12b**, **12d**, and **12f** had a 5–10-fold greater affinity for the muscarinic receptor than the corresponding *S* enantiomers. A 5-fold difference or less found in the (*R*)- and (*S*)-carbamate derivatives **9**, **15**, and **16** suggested close overlap of the two enantiomers in the receptor binding domain. The affinity differences found in the enantiomeric acetamido derivatives when compared to those of the carbamate analogues may be the result of limited rotation of the acetamido group.

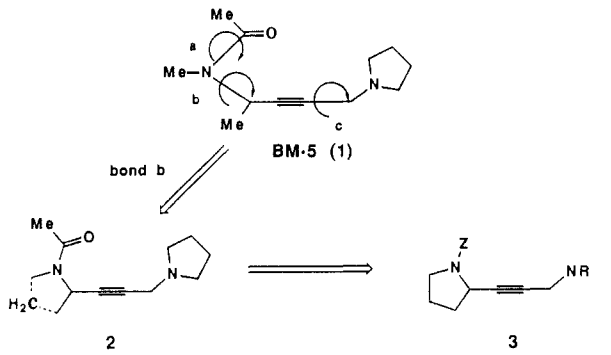
Introduction

Senile dementia of the Alzheimer's Type (SDAT) is a neurodegenerative disease which results in the progressive impairment of memory and dementia. Autopsied brain tissue from SDAT patients has shown a consistent and significant decrease in cholinergic markers, such as choline acetyltransferase and acetylcholine esterase, most notably in the nucleus basalis of Meynert, cerebral cortex, and hippocampus.[1] It is suggested that the degeneration occurs presynaptically while the postsynaptic muscarinic receptors remain intact.[2] These observations in part form the basis for the cholinergic hypothesis of age-related memory loss.[3] A series of drug-discovery projects have been initiated in our geriatric program to discover selective cholinergic agents to ameliorate the cognitive symptoms of this degenerative disease.

Cholinergic neurons are found not only in the brain but also throughout the periphery (cardiac tissue, smooth muscle, secretory glands). The predominant acetylcholine-sensitive receptors found in these tissues and in different areas of the brain are not identical.[4] Pharmacologically, the assignment of predominant receptor subtypes to a specific tissue has been accomplished with the use of a series of radiolabeled anticholinergic agents which display differential binding characteristics, such as pirenzepine,[5] 11-[[2-[(diethylamino)methyl]-1-piperidinyl]-acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one (AFDX-116),[6] and 4-(diphenylacetoxy)-*N*-methylpiperidine methiodide (4-DAMP).[7] Molecular biology has confirmed the diversity of muscarinic receptors by the discovery of five distinct human genes for muscarinic receptors which have been cloned and expressed in various cell lines.[8] Four of these receptor subtypes are regionally expressed in different densities in human brain. Biochemical assays with transfected cells[9] demonstrate the differential effects of the selective anticholinergic agents and therefore support the observed differences in the tissue experiments.[4] In theory the search for a pharmacologically useful compound for the treatment of SDAT should begin with the synthesis of an agent which may have some preference for one (or more) of these subtypes in order to stimulate the appropriate receptors while not affecting the unrelated muscarinic receptors.

Several known cholinergic agonists and partial agonists can be used as models or leads for a chemical synthesis project. One of these, *N*-methyl-*N*-(1-methyl-4-pyrrolidino-2-butynyl)acetamide (1, BM-5) has been reported[10] to be a presynaptic antagonist, which should

Scheme I



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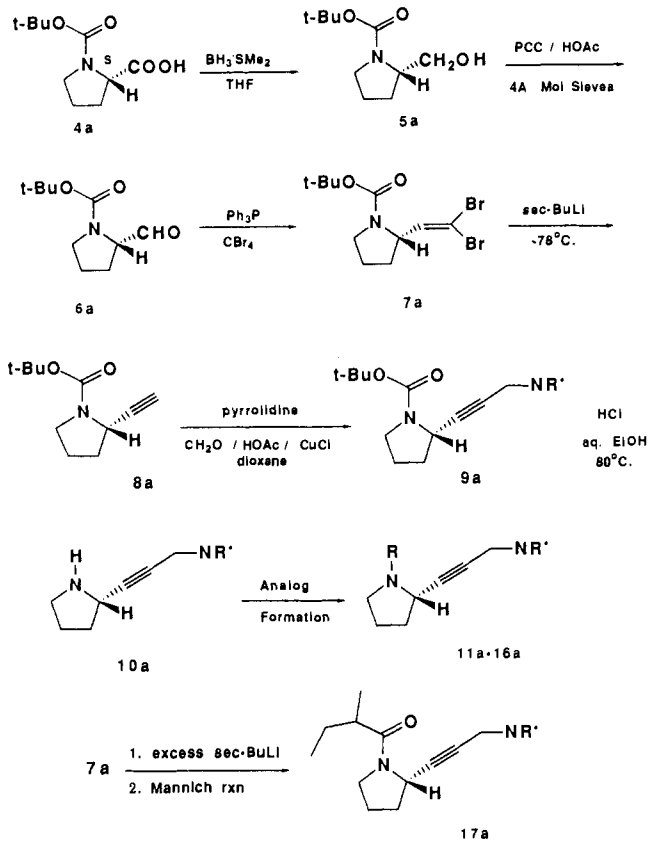
Scheme II

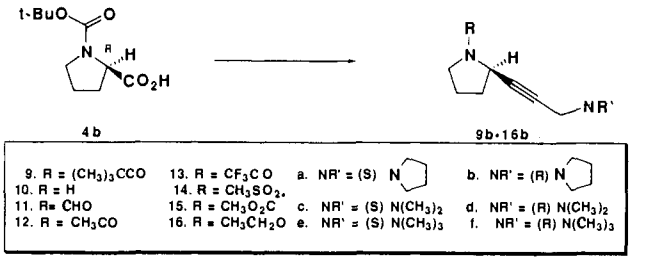


disinhibit the release of endogenous acetylcholine, and a postsynaptic partial agonist, which should mimic some of the effects of acetylcholine. Studies of compound **1** have explored both the structure–activity relationships[11] and the stereochemical requirements for biological activity.[12] Chemically, compound **1** is a fairly flexible molecule (Scheme I, bonds a, b, c) and, as a result, can assume a number of different conformations. An investigation of the different possible conformations of compound **1** has been reported;[13] however, the target molecules were racemic. The present research describes the asymmetric synthesis and the biochemical activity of a series of derivatives of **1** in which one degree of freedom (bond b) has been restricted. Connection of the *N*-methyl group to the butynyl methyl group in compound **1** by the addition of a methylene fragment generates an acetylated 2-alkynylpyrrolidine derivative **2**. The generalization of compound **2** produces structure **3** which represents the compounds described in this report.

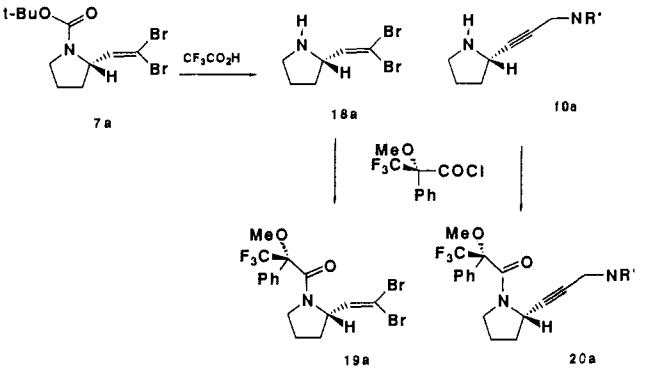
Chemistry

Since the target structure **2** contains an asymmetric center and the muscarinic receptor is asymmetric, the probability for the discovery of a selective agent may be increased by preparing optically active compounds instead of racemic mixtures.[14] The enantioselective synthesis of

Scheme III



Scheme IV



the target structures became straightforward when a pair of optically pure and commercially available starting materials was identified. The chiral center in target molecule **2** can be related to the chiral center of the amino acid proline. Starting from the *t*-Boc-protected form of (*R*)- and (*S*)-proline (**4**), each enantiomer of **2** (compounds **12a** and **12b**) was synthesized in seven steps (Schemes II and III).

Reduction of the acid group in **4** with borane–methyl sulfide complex afforded alcohol **5**. Oxidation of the alcohol function in **5** with pyridinium chlorochromate[15] gave aldehyde **6** which, without isolation, was treated with triphenylphosphine and carbon tetrabromide[16] to afford dibromide **7**. Reaction of compound **7** with slightly less than 2 equiv of *sec*-butyllithium at −78 °C gave acetylene **8**. Treatment of acetylene **8** with a secondary amine (pyrrolidine, **a**, **b**; dimethylamine, **c**, **d**) under Mannich reaction conditions gave the corresponding aminocarbamate **9**. Removal of the *t*-Boc protecting group in **9** was best accomplished with aqueous ethanolic hydrogen chloride. The resulting diamine **10** was isolated and purified as its dihydrochloride salt. The diamine was then used to prepare the acyl, sulfonyl, and carbamoyl derivatives **11**–**16** (Table I).

The only problematic step in the synthesis was the reaction of *sec*-butyllilthium with compound **7**. If an excess of the organolithium was used, reaction at the carbamate group was observed producing amide **17** as a byproduct. Traces of amide **17** were removed during the crystallization of the dihydrochloride salt of **10**.

In the synthetic route there are two steps where racemization may occur, namely, the formation of aldehyde **6** and the formation of acetylene **8** with the use of an organolithium. The optical purity of the synthetic intermediates was assayed chemically with Mosher's reagent[17]

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Table I. Chemical Data for Restricted-Rotation Analogues of Compound 1

compound	(absolute config) $[\alpha]_D^{26}$ [a]	NR' [b]	R	mp, °C	emp fmla	mol wt	anal.
9a	(S) -100	Pyr	$CO_2C(CH_3)_3$		$C_{16}H_{26}N_2O_2$	278.39	C,H,N
9b	(R) +100	Pyr	$CO_2C(CH_3)_3$		$C_{16}H_{26}N_2O_2$	278.39	C,H,N
9c	(S) -109	NMe_2	$CO_2C(CH_3)_3$		$C_{14}H_{24}N_2O_2$	252.36	C,H,N
9d	(R) +108	NMe_2	$CO_2C(CH_3)_3$		$C_{14}H_{24}N_2O_2$	252.36	C,H,N
10a·2HCl	(S) -20	Pyr	H	196-197	$C_{11}H_{18}N_2 \cdot 2HCl$	251.2	C,H,N
10b·2HCl	(R) +21	Pyr	H	196-197	$C_{11}H_{18}N_2 \cdot 2HCl$	251.2	C,H,N
10c·2HCl	(S) -23	NMe_2	H	190-191	$C_9H_{16}N_2 \cdot 2HCl$	225.16	C,H,N
10d·2HCl	(R) +22	NMe_2	H	190-191	$C_9H_{16}N_2 \cdot 2HCl$	225.16	C,H,N
11a	(S) -126	Pyr	CHO		$C_{12}H_{18}N_2O \cdot 0.2H_2O$	206.29	C,H,N
11b	(R) 131	Pyr	CHO		$C_{12}H_{18}N_2O \cdot 0.2H_2O$	206.29	C,H,N
12a	(S) -117	Pyr	$COCH_3$		$C_{13}H_{20}N_2O$	220.32	C,H,N
12a·HCl	(S) -103	Pyr	$COCH_3$		$C_{13}H_{20}N_2O \cdot HCl \cdot 0.8H_2O$	256.78	C,H,N
12b	(R) +13	Pyr	$COCH_3$		$C_{13}H_{20}N_2O$	220.31	C,H,N
12b·HCl	(R) +85	Pyr	$COCH_3$		$C_{13}H_{20}N_2O \cdot HCl \cdot 1.2H_2O$	256.77	C,H,N
12c	(S) -124	NMe_2	$COCH_3$		$C_{11}H_{18}N_2O \cdot H_2O$	194.28	C,H,N
12d	(R) +141	NMe_2	$COCH_3$		$C_{11}H_{18}N_2O \cdot H_2O$	194.28	C,H,N
12e	(S) -90	NMe_3	$COCH_3$		$C_{12}H_{21}IN_2O$	336.22	C,H,N
12f	(R) +90	NMe_3	$COCH_3$		$C_{12}H_{21}IN_2O$	336.22	C,H,N
13a	(S) +124	Pyr	$COCF_3$		$C_{13}H_{17}F_3NO_2$	274.29	C,H,N
13a·HCl	(S) +103	Pyr	$COCF_3$	156-157	$C_{13}H_{17}F_3NO_2 \cdot HCl$	310.75	C,H,N
13b	(R) -124	Pyr	$COCF_3$		$C_{13}H_{17}F_3NO_2$	274.29	C,H,N
13b·HCl	(R) -102	Pyr	$COCF_3$	156-157	$C_{13}H_{17}F_3NO_2 \cdot HCl$	310.75	C,H,N
14a	(S) -85	Pyr	SO_2CH_3	46-47	$C_{12}H_{20}N_2O_2S$	256.36	C,H,N
14a·HCl	(S) -81	Pyr	SO_2CH_3	198-199	$C_{12}H_{20}N_2OS \cdot HCl$	292.83	C,H,N
14b	(R) +81	Pyr	SO_2CH_3	46-47	$C_{12}H_{20}N_2O_2S \cdot 0.2H_2O$	256.36	C,H,N
14b·HCl	(R) +78	Pyr	SO_2CH_3	198-199	$C_{12}H_{20}N_2OS \cdot HCl$	292.82	C,H,N
15a	(S) -131	Pyr	CO_2CH_3		$C_{13}H_{20}N_2O_2 \cdot 0.25H_2O$	236.32	C,H,N
15b	(R) +128	Pyr	CO_2CH_3		$C_{13}H_{20}N_2O_2 \cdot 0.25H_2O$	236.32	C,H,N
16a	(S) +126	Pyr	$CO_2CH_2CH_3$		$C_{14}H_{22}N_2O_2 \cdot 0.25H_2O$	250.34	C,H,N
16b	(R) +125	Pyr	$CO_2CH_2CH_3$		$C_{14}H_{22}N_2O_2 \cdot 0.25H_2O$	250.34	C,H,N

[a] Optical rotations were carried out in methylene chloride for free bases and in methanol for hydrochloride salts and are in degrees. [b] Pyr = pyrrolidinyl.

at these two stages (Scheme IV, compounds **7** and **10**). The *t*-Boc group in compound **7** was hydrolyzed to produce amine **18**. Both diamine **10** and amine **18** were treated with Mosher's reagent and the optical purity of the adducts, compounds **19** and **20**, was determined by NMR. However, the NMR spectra of Mosher derivatives required some intrepretation. Rotation around an amide bond can be slow on the NMR time scale and at room temperature two sets of absorptions appeared in the spectrum of the adducts. This effect has been observed for some derivatives of compound **1**[8] and was also noted in the spectra of the acetamido derivatives **12** and **13**. The NMR spectra of the Mosher derivatives were taken in DMSO heated to 140 °C to allow free rotation about the amide bonds.[18] If the two sets of absorptions were due to a diastereomeric mixture, heating of the samples should have had no effect. In each case, the multiplicity of peaks coalesced into single sets of absorptions and optical purity was estimated to be greater than 90%.[19] In addition the optical rotation of each Mosher derivative, intermediate, and target compound was determined (Table I).

Pharmacology

Displacement of [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) in muscarinic receptor binding assays has been used extensively as a rapid and efficient screen for test compounds that interact at cholinergic receptors.[20] Little information regarding the potential intrinsic activity or pharmacological selectivity of the compound for muscarinic receptor subtypes is obtained in this assay. Meaningful estimates of intrinsic activity and pharmacological selectivity can nevertheless be determined from the apparent equilibrium dissociation constants (K_i) for test compounds. This information can be obtained with a series of assays that employ tritiated agonists as well as antagonists to label receptor populations in several different tissues.

A method for using the relative affinities (K_i values) of test compounds to displace [^{3}H]-*N*-methylscopolamine ([^{3}H]NMS, antagonist) and [^{3}H]oxotremorine-M ([^{3}H]-OXO-M, agonist) as an index of their efficacy at cortical muscarinic receptors (NMS/OXO-M ratio) has been described.[21] Alternatively, [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB, antagonist) can be used to label both the high- and low-affinity states[22] and [^{3}H]-*cis*-[methyl(trimethylammonio)methyl]-1,3-dioxolane ([^{3}H]CD, agonist) can be used to label the high-affinity agonist state of the muscarinic receptor in competition assays.[23] The experimental conditions were chosen to maximize the difference between agonists and antagonists, and the ratio of K_i values for the two assays provides a useful index of agonist efficacy. Muscarinic antagonists display similar affinity in both binding assays (K_i ratio is ~1.0). Full muscarinic agonists displace [^{3}H]CD from the muscarinic receptor with high affinity relative to their displacement of [^{3}H]QNB. Thus, full agonists such as carbachol exhibit large ratio (K_i ratios ≈ 500–2000). Compounds that display partial muscarinic

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agonist activity such as oxotremorine in functional assays have intermediate ratios (K_i ratio ≈ 50–500). The ratio of K_i values determined in the two assays correlates well with the ability of agonists to stimulate cortical phosphatidylinositol turnover.[20]

The results for the restricted-rotation analogues **9** and **11–16** and several standards, including compound **1**, are presented (Table II). All of the restricted-rotation analogues, compounds **9** and **11–17**, showed weaker affinity in the five binding assays when compared to compound **1**.

The reported pharmacological profile of compound **1** as a mixed agonist–antagonist implies some type of muscarinic subtype specificity.[10] To assess possible pharmacological subtype specificity of compound **1** and the prepared restricted-rotation analogues **9** and **11–16**, three additional receptor assays were performed. To evaluate pharmacological selectivity of these analogues, displacement of [^{3}H]pirenzepine ([^{3}H]PZ) from rat cortical receptors was used in conjunction with displacement of [^{3}H]QNB from rat cardiac muscarinic receptors. Pirenzepine (PZ) is an antagonist that selectively labels a subpopulation of muscarinic receptors (M_1) in central and peripheral tissues relative to the classical antagonist QNB. Approximately 80% of rat cortical receptors labeled by [^{3}H]QNB bind PZ with high affinity (M_1) while the remaining receptors bind PZ with low affinity (M_2).[5b,c] Using a low concentration of [^{3}H]PZ, the M_1 subpopulation of muscarinic receptors can be preferentially labeled. Rat cardiac receptors are essentially of the M_2 subtype with low affinity for PZ.[24] With this nearly homogenous population, the M_2 subtype of receptors can be separately labeled with rat cardiac tissue and [^{3}H]QNB.

Guanine nucleotides shift M_2 muscarinic receptor affinity for agonists to a single low-affinity state.[25] Displacement of [^{3}H]QNB from rat cardiac M_2 was performed in the absence and presence of 10 μM guanyl-5′-yl imidodiphosphate (Gpp(NH)p) to assess binding of compounds to all affinity states and to the low-affinity state, respectively, of the muscarinic receptors.[26]

Muscarinic receptor (M_1) selectivity was determined from the apparent K_i ratio of K_i(QNB + Gpp(NH)p) and K_i(PZ), and potential M_2 agonist activity was determined from the apparent K_i ratio of K_i(QNB + Gpp(NH)p) and K_i(QNB)—without Gpp(NH)p. The results are presented in Table II. All of the restricted-rotation analogues, compounds **9**, and **11–17**, showed weaker affinity in both the cardiac [^{3}H]QNB and cortical [^{3}H]PZ binding assays when compared to compound **1**.

Discussion

The pharmacological profile attributed to compound **1**, as a presynaptic (M_2) antagonist and a postsynaptic (M_1) agonist, provides an excellent conceptual match for an agent which may have therapeutic utility in the treatment of Alzheimer's disease. The molecule is quite flexible and rotation about three bonds (bonds a, b, c) provides a number of potential conformations (Scheme I). The putative dual role of compound **1** as an antagonist and agonist may be the result of the molecule interacting with muscarinic receptors in two or more different conformations.

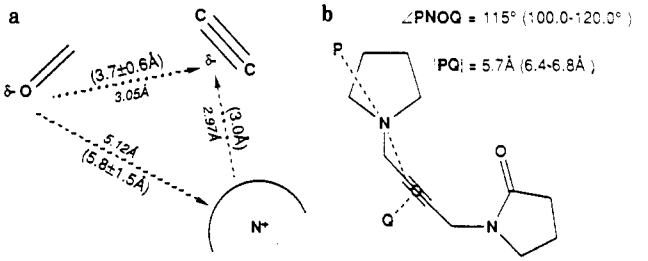

Figure 1. (a) Calculated muscarinic pharmacophore distances of oxotremorine which were used (calculated by Kier[27]); (b) calculated muscarinic agonist recognition site which was used (calculated by Schulman[28]).

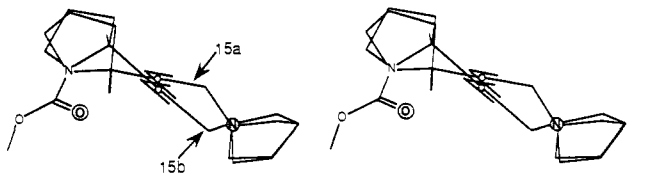

Figure 2. Stereoview of the overlap between the pharmacophoric regions of **15a** and **15b**.

In one conformation the compound may act as an antagonist and in the other as an agonist. To test this hypothesis, the synthesis of a series of analogues was undertaken to lock (bond b) the molecule into a specific conformation. The hypothesis was to see if the pharmacological effects of the parent molecule could be separated.

In the five biochemical assays [[^{3}H]CD (cortex), [^{3}H]-QNB (cortex), [^{3}H]PZ (cortex), [^{3}H]QNB (heart) [^{3}H]QNB + Gpp(NH)p (heart)], analogues **9** and **11–16** exhibited affinities weaker than that of compound **1**, with the largest decrease in affinity observed in the [^{3}H]CD binding assay. Two possible explanations for the decrease in affinity can be rationalized. One is that the locked conformation of compound **1** is not important for its pharmacological effects and the other is that the increased steric effects of the added methylene group to form the pyrrolidinone ring decreased the affinity of the analogues for the receptors.

A computer model[27] of oxotremorine was constructed and the conformation of this agonist was adjusted to fit a muscarinic pharmacophore calculated by using extended Hückel theory[28] (Figure 1a) and to fit the model for muscarinic agonist recognition[29] (Figure 1b). When a model of **12b** was superimposed onto oxotremorine, good overlap of the pharmacophoric groups was observed. These findings imply (but do not prove) that the decrease in affinity was likely the result of additional steric effects of the methylene group.

Since the [^{3}H]CD binding affinities are more affected than the [^{3}H]QNB binding affinities, analogues **9** and **11–16** are expected to have more overall antagonistlike and less agonistlike characteristics than compound **1**. This

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Table II. Biochemical Data for Restricted-Rotation Analogues of Compound 1

compound	absolute config	[^{3}H]QNB K_i, μM, rat cortex[a]	[^{3}H]CD K_i, μM, rat cortex[a]	agonist ratio [^{3}H]QNB/[^{3}H]CD[b]	[^{3}H]PZ K_i, μM, rat cortex[c]	[^{3}H]QNB K_i, μM rat heart[c]	[^{3}H]QNB + Gpp(NH)p K_i, μM, rat heart	selectivity ratios: M_2 agonist: [^{3}H]QNB + Gpp(NH)p/[^{3}H]QNB	selectivity ratios: M_1 agonist: [^{3}H]QNB + Gpp(NH)p/[^{3}H]PZ	selectivity ratios: M_1 agonist: [^{3}H]QNB/[^{3}H]PZ[d]
1		0.08 ± 0.02	0.004 ± 0.001	20.0	0.04 (0.05, 0.03)	0.07 (0.09, 0.05)	0.18 (0.20, 0.16)	2.6	4.5	1.8
carbachol		4.99 ± 0.32	0.004 ± 0.001	1247	5.8 (7.2, 4.4)	2.34 (2.86, 1.82)	160 (192, 128)	68	28	0.4
oxotremorine		0.06 ± 0.01	0.0005 ± 0.0001	120	0.14 (0.17, 0.11)	0.004 (0.006, 0.002)	0.14 (0.12, 0.16)	35	1.0	0.03
9a	(*S*)	0.98 ± 0.62	0.33 ± 0.04	3.0	0.07	0.13	0.90	6.9	13	1.9
9b	(*R*)	0.66 ± 0.08	0.22 ± 0.02	3.0	0.42	1.32	0.96	1.5	4.7	3.1
9c	(*S*)	11.4 (6.8, 15.9)	3.3 (3.5, 3.1)	3.5	9.04	13.4	14.1	1.1	1.6	1.5
9d	(*R*)	3.2 (1.1, 5.3)	0.25 (0.1, 0.4)	12.8	5.6	5.16	19.6	3.8	3.5	0.9
10a·2HCl	(*S*)	2.8 ± 0.33	5.67 ± 0.79	0.5	2.22	1.55	2.08	1.3	0.9	0.7
10b·2HCl	(*R*)	3.22 ± 0.25	1.28 ± 0.38	2.5	6.5	12.9	19.6	1.5	3.0	2.0
10c·2HCl	(*S*)	8.5	37.35	0.2	13.2 (15.6, 10.8)	20.0 (13.0, 27.0)	19.1 (16.0, 22.2)	1.0	1.4	1.5
10d·2HCl	(*R*)	18.7	37.08	0.5	25.2 (30.4, 20.1)	28.5 (19.9, 37.1)	29.3 (25.7, 32.9)	1.0	1.2	1.1
11a	(*S*)	4.59	4.88	1.0	1.23	7.62	77.0	10	62	6.2
11b	(*R*)	2.39	3.35	0.7	1.18	3.26	3.12	1.0	2.5	2.7
12a·HCl	(*S*)	4.96 ± 0.12	2.55 ± 0.21	1.9	2.06	15.5	22.6	1.5	11	7.5
12b·HCl	(*R*)	0.43 ± 0.02	0.45 ± 0.12	1.0	0.17	0.61	1.63	2.7	9.6	3.6
12c	(*S*)	48.9 ± 7.16	15.5 ± 6.24	3.2	40.0 (39.7, 40.2)	51.7 (40.4, 62.9)	175 (154, 196)	3.4	4.4	1.3
12d	(*R*)	13.8 ± 2.11	1.65 ± 0.59	8.4	5.25 (8.97, 1.53)	11.8 (5.85, 17.8)	42.4 (41.1, 43.6)	3.6	8.1	2.2
12e	(*S*)	20.9 (24.3, 17.5)	4.15 (4.2, 4.3)	5.0	13.9 (19.4, 8.6)	16.4 (21.4, 11.4)	47.1	2.9	3.4	1.2
12f	(*R*)	2.23 ± 0.14	0.11 ± 0.05	20.3	1.63 (1.78, 1.48)	2.07	5.47	2.6	3.4	1.3
13a·HCl	(*S*)	3.45 ± 2.25	2.22 ± 0.48	1.6	3.10	6.40	8.86	1.4	2.9	2.1
13b·HCl	(*R*)	0.36 ± 0.06	0.44 ± 0.08	0.8	0.14	1.23	1.07	0.9	7.6	8.8
14a·HCl	(*S*)	3.02 ± 0.35	3.92 ± 1.21	0.8	6.05	9.19	15.1	1.6	2.5	1.5
14b·HCl	(*R*)	1.92 ± 0.20	1.98 ± 0.72	1.0	4.11	5.91	13.4	2.3	3.3	1.4
15a	(*S*)	0.62 ± 0.03	1.44 ± 0.24	0.4	1.48	4.42 (7.03, 1.81)	5.75 (8.11, 3.39)	1.3	3.9	3.0
15b	(*R*)	0.81 ± 0.01	0.53 ± 0.10	1.5	1.59	2.94 (7.37, 2.75)	5.06 (7.37, 2.75)	1.7	3.2	1.8
16a	(*S*)	0.31 ± 0.03	0.61 ± 0.13	0.5	0.10	0.85	1.0	1.2	10	8.5
16b	(*R*)	1.16 ± 0.11	0.51 ± 0.16	2.3	0.19	0.94	1.04	1.1	5.5	4.9

[a] A mean of two determinations or the standard deviation from the mean for three or more determinations. [b] The [^{3}H]QNB data is from rat cortex. [c] From one determination or the mean of two determinations. [d] The [^{3}H]QNB data is from rat heart.

affect can be expressed by the [^{3}H]QNB/[^{3}H]CD ratio. Except for the ratio of quaternary ammonium salt **12f** (20.3), the ratios of analogues **9** and **11–16** (0.2–12.8) were all less than the ratio of compound **1** (20.3). The guanine nucleotide-induced shift of the [^{3}H]QNB binding data using heart tissue is a second indicator of a compound's agonist activity. The guanine nucleotide induced shift represents the M_2 agonist activity of the compound and confirms the [^{3}H]QNB/[^{3}H]CD ratio which is a measure of overall agonist activity. A minimal shift in affinity of compound **1** (2.5) and analogues **9** and **11–16** (0.2–6.9) was observed, supporting the negligible M_2 agonist activity of these compounds. The M_1 selectivity, expressed in the [^{3}H]QNB/[^{3}H]PZ ratios, for compound **1** and analogues **9** and **11–16** was greater for each analogue than for oxotremorine and carbachol, but the slight improvement in selectivity occured at the expense of agonist activity ([^{3}H]QNB/[^{3}H]CD ratio). On the basis of these data, compound **1** and analogues **9** and **11–16** are overall antagonists ([^{3}H]QNB/[^{3}H]CD ratio) and should be expected to produce little if any M_1 and M_2 agonist activity.

Within the series of analogues **9** and **11–16**, some observations about the binding properties of the compounds can be made. In reported pharmacological data of oxotremorine and analogues of compound **1**, the *R* enantiomers have been found to be more potent than the *S* isomers.[12] A 5–10-fold difference in affinities between the *R* and *S* enantiomers was observed among the acetamide (**12**) and trifluoroacetamido analogues (**13**) (Table II). In the sulfonamide analogues (**14**) and carbamoyl analogues (**9**, **15**, and **16**), the difference between *R* and *S* enantiomers was less than 5-fold. The compounds can be put into two categories: an enantiomeric difference of 5–10 (compounds **12** and **13**) and an enantiomeric difference of less than 5 (analogues **9**, **14–16**). A similar grouping of the compounds can be made on the basis of their NMR spectra. The acetamide and trifluoroacetamide analogues produce complex NMR spectra which are the result of amide bond resonances (bond a).[11] Coalescence of the multiple peaks was achieved only on heating the acetamide samples to 120 °C. These results imply restricted movement of the pharmacophoric carbonyl oxygen atom. The NMR spectra of the sulfonamide analogues **14** and the methyl and ethyl carbamates **15** and **16**, respectively, were less complex, suggesting freer movement of the pharmacophoric oxygen atom. The NMR spectra of the *t*-Boc derivatives **9**, a bulkier carbamate, had some multiplicity; however, coalescence was achieved by heating the sample to 60 °C.

The similarity in the affinities of the (*R*)- and (*S*)-carbamates **9**, **15**, and **16** suggests a close overlap of the two enantiomers in the receptor binding domain. Figure 1 depicts one possible overlap of he *R* and *S* enantiomers. In the acetamido derivatives the *R* enantiomer may be able to conform into the preferred fit, whereas, the *S* enantiomer with some barrier to rotation is less able to adapt to the receptor pocket. An alternate explanation for the decrease in selectivity of the carbamates is that there are two oxygen atoms in the carbamate functionality capable of receptor binding interactions and only one oxygen atom in the acetamido analogues. The decrease in the binding energy of the (*S*)-carbamate enantiomer using the sp^2 oxygen atom may be compensated for by interactions with the sp^3 oxygen atom. In the acetamido derivatives this alternate mode is not available. The latter explanation is possible but unlikely since differences in the binding interactions would produce significantly different steric environments for the enantiomers which would alter affinity for the receptor.

In summary, a series of optically pure, restricted-rotation analogues of compound **1** have been prepared from (*R*)- and (*S*)-proline. The availability of the optically active compounds and a series of biochemical assays provided detailed examination of the complex pharmacological effects of the flexible muscarinic agent *N*-methyl-*N*-(1-methyl-4-pyrrolidino-2-butynyl)acetamide (**1**). The decrease in the enantioselectivity of the carbamate derivatives suggests close overlap of the two enantiomers in the receptor binding domain. The affinity differences found in the enantiomeric acetamido derivatives may be a function of limited rotation of the acetyl group.

The compounds, when tested in a battery of in vitro muscarinic assays, were found to have weaker muscarinic properties than compound **1**.

Experimental Section

All melting points were determined with a Mel-Temp apparatus and are uncorrected. All ^{1}H NMR spectra were measured in $CDCl_3$ or DMSO-d_6 with either a General Electric QE 300 or GN 500 spectrometer. The coupling constants are recorded in hertz (Hz) and the chemical shifts are reported in parts per million (δ, ppm) downfield from tetramethylsilane (TMS), which was used as an internal standard. IR spectra were recorded on a Nicolet 20 SXB FT-IR spectrophotometer. Low-resolution mass spectra (MS-LR) were recorded on a Finnigan MAT-90 mass spectrometer, and high-resolution mass spectra (MS-HR) were recorded on either a Finnigan MAT-90 for electron impacct (EI) or a VG ZAB-SE mass spectrometer for (FAB). Specific rotations, $[\alpha]^{26}_D$, were recorded on a Perkin-Elmer 241 polarimeter with the sample concentration in grams per milliliter of solution. Thin-layer chromatography was conducted with silica gel (Analtech) or aluminum oxide (Analtech) plates. For flash column chromatography, Kieselgel 60 (230–400 mesh ASTM, E. M. Science) was used, and for gravity column chromatography, alumina deactivated with 4.5% H_2O was used. Celite (Johns Mansville) was used as a filter aid and Magnesol (FMC Corp.), a hydrous magnesium silicate, was used for plug filtrations. Anhydrous sodium sulfate was used for the drying of organic solvents.

(*S*)-2-(Hydroxymethyl)-1-pyrrolidinecarboxylic Acid 1,1-Dimethylethyl Ester (5a). Borane–methyl sulfide complex (30 mL, 0.3 mol) was added dropwise to a solution of 50 g (23 mmol) of **4a** in 500 mL of dry THF which was cooled to 0 °C. When gas evolution ceased, the ice bath was removed and the solution was refluxed for 1 h. The solution was cooled and slowly 400 mL of methanol was added. The resulting solution was concentrated in vacuo. The residue was redissolved twice in 200 mL of MeOH and in 200 mL of toluene and reconcentrated in vacuo to give 46 g of **5a** as an oil. Upon standing, the oil crystallized to give **5a** as colorless crystals: mp 55–56 °C; IR (neat) 1662 cm^{-1} (C=O); NMR ($CDCl_3$) δ 1.47 (s, 9, 3 CH_3), 1.82 (m, 2, CH_2), 2.0 (m, 2, CH_2), 3.33 (m, 1, CH), 3.45 (m, 1, CH), 3.58 (m, 2, CH_2), 3.96 (br s, 1, CH), 4.8 (br s, 1, OH); MS-LR M^+ m/z 202; $[\alpha]^{26}_D$ = −46° (c = 0.85, CH_2Cl_2). Anal. ($C_{10}H_{19}NO_3$) C, H, N.

(*R*)-2-(Hydroxymethyl)-1-pyrrolidinecarboxylic acid 1,1-dimethylethyl ester (5b): colorless crystals; mp 50–53 °C; $[\alpha]^{26}_D$ = +47° (c = 1.16, CH_2Cl_2). Anal. ($C_{10}H_{19}NO_3$) C, H, N.

(*S*)-2-(2,2-Dibromoethenyl)-1-pyrrolidinecarboxylic Acid 1,1-Dimethylethyl Ester (7a). Successively, 16.0 g (74.4 mmol) of pyridinium chlorochromate, 36 g of 4A molecular sieve powder, and 4.5 mL of acetic acid were added to a water-cooled solution of 10.0 g (50 mmol) of alcohol **5a** in 250 mL of dry CH_2Cl_2. The resulting mixture was stirred at room temperature for 2 h. Celite (25 g) followed by 500 mL of ether was added and the precipitate was removed by filtration over Celite. The filtrate was diluted with 200 mL of toluene and concentrated in vacuo to dryness. The residue was chromatographed through a small plug (SiO_2, 50 g; eluent, ether) to give 7.7 g of **6a** as a colorless oil [IR (neat) 1737, 1696 cm^{-1}] which was used without purification directly in the next step.

A solution of 7.7 g of aldehyde **6a** in 20 mL of CH_2Cl_2 was added dropwise to a solution of 40 g (152 mmol) of triphenylphosphine and 26 g (78 mmol) of carbon tetrabromide in 700 mL of CH_2Cl_2, which was cooled to 0 °C. The resulting solution was stirred at

room temperature for 30 min. The solution was poured into an excess of saturated aqueous sodium bicarbonate, separated, dried, and concentrated in vacuo. The residue was purified by column chromatography (SiO_2, 300 g; eluents CH_2Cl_2 then 1% v/v MeOH–CH_2Cl_2) to give 9.90 g (57%) of **7a** as a colorless solid: mp 58–89 °C; IR (KBr) 1687 cm^{-1} (C=O); NMR ($CDCl_3$) δ 1.46 (s, 9, 3 CH_3), 1.74 (m, 1, CH), 2.16 (m, 1, CH), 1.85 (t, J = 7 Hz, 2, CH_2), 3.37 (m, 2, CH_2), 4.36 (m, 1, CHN), 6.36 (d, J = 7 Hz, 1, CH=); MS-LR m/z 256 (MH^+); $[\alpha]^{26}_D$ = +24° (c = 0.89, CH_2Cl_2). Anal. ($C_{11}H_{17}Br_2NO_2$) C, H, N.

(*R*)-2-(2,2-Dibromoethenyl)-1-pyrrolidinecarboxylic acid 1,1-dimethylethyl ester (7b): colorless crystals; mp 58–60 °C; $[\alpha]^{26}_D$ = −24° (c = 1.15, CH_2Cl_2). Anal. ($C_{11}H_{17}Br_2NO_2$) C, H, N.

(*S*)-2-Ethynyl-1-pyrrolidinecarboxylic Acid 1,1-Dimethylethyl Ester (8a). A solution of 66.3 mL (56.3 mmol, 0.85 M) of *sec*-butyllithium was added over a period of 30 min to a stirred solution of 10.0 g (28.2 mmol) of **7a** in 250 mL of dry THF, which was cooled to −78 °C. Stirring was continued at −78 °C for 30 min, followed by the addition of 200 mL of saturated aqueous NH_4Cl. The resulting mixture was allowed to warm to room temperature and diluted with 200 mL of ether. The layers were separated, and the organic phase was washed with 100 mL of saturated NaCl, dried, and concentrated in vacuo. The residue was purified by filtration of Magnesol to give 5.65 g of **8a** as a light yellow oil which was used without further purification in the next step. A sample of **8a** was purified by column chromatography (SiO_2; eluant, MeOH–CH_2Cl_2) to give **8a** as a pale yellow oil: IR (neat) 1698 cm^{-1} (C=O); NMR ($CDCl_3$) δ 1.48 (s, 9, 3 CH_3), 1.88 (m, 1, CH), 2.05 (m, 2, CH_2), 2.22 (m, 1, CH), 3.30 (m, 1, CH), 3.45 (m, 1, CH), 4.42 (m, 0.5) and 4.52 (m, 0.5) (CH); $[\alpha]^{26}_D$ = +84° (c = 1.05, CH_2Cl_2); MS-HR (EI) M^+ at m/z 195.1251 (calcd, 195.1259). Traces of the byproduct **17a** could not be removed and the material was used as is in the next step.

(*R*)-2-Ethynyl-1-pyrrolidinecarboxylic acid 1,1-dimethylethyl ester (8b): pale yellow oil; $[\alpha]^{26}_D$ = −81° (c = 0.97, CH_2Cl_2); MS-HR (EI) M^+ at m/z 195.1263 (calcd, 195.1259). Traces of the byproduct **17b** could not be removed and the material was used as is in the next step.

(*S*)-2-[3-(1-Pyrrolidinyl)-1-propynyl]-1-pyrrolidinecarboxylic Acid 1,1-Dimethylethyl Ester (9a). A mixture of 5.30 g (27.1 mmol) of **8a**, 4.5 mL (54.3 mmol) of pyrrolidine, 2.20 g (67.8 mmol) of paraformaldehyde, 10 mL of glacial acetic acid, and 100 mg of CuCl in 80 mL of dry dioxane was stirred at room temperature under argon for 15 min and then heated to reflux for 1.5 h. The reaction mixture was concentrated in vacuo and the oily residue was partitioned between water and CH_2Cl_2. Following basification with dilute ammonium hydroxide, the two-phase mixture was extracted with CH_2Cl_2. The combined organic extracts were dried and concentrated in vacuo to leave 8.25 g of a dark brown oil. Purification of the crude product by column chromatography (Al_2O_3, activity grade 2.5; eluant, CH_2Cl_2 then 1% v/v MeOH–CH_2Cl_2) afforded 6.62 g (88%) of **9a** as a pale yellow oil: IR (neat) 1700 cm^{-1}; NMR ($CDCl_3$) δ 1.47 (s, 9, 3 CH_3), 1.79 (m, 4, 2 CH_2), 1.88 (m, 1, CH), 1.95–2.15 (m, 3, CH_2, CH), 2.60 (m, 4, 2 CH_2), 3.25–3.40 (m, 1, CH), 3.39 (d, J = 2 Hz, 2, CH_2), 3.4–3.5 (m, 1, CH), 4.4–4.6 (br s, 1, CH); MS-HR (FAB) M^+ at m/z 279.2073 (calcd, 279.2073); $[\alpha]^{26}_D$ = −100° (c = 1.04, CH_2Cl_2). Anal. ($C_{16}H_{26}N_2O_2$) C, H, N.

(*R*)-2-[3-(1-Pyrrolidinyl)-1-propynyl]-1-pyrrolidinecarboxylic acid 1,1-dimethylethyl ester (9b): pale yellow oil; $[\alpha]^{26}_D$ = +109° (c = 1.11, CH_2Cl_2); MS-HR (EI) M^+ at m/z 279.2071 (calcd, 279.2073). Anal. ($C_{16}H_{26}N_2O_2$) C, H, N.

(*S*)-2-[3-(Dimethylamino)-1-propynyl]-1-pyrrolidinecarboxylic acid 1,1-dimethylethyl ester (9c): pale yellow oil; $[\alpha]^{26}_D$ = −109° (c = 1.11, CH_2Cl_2); MS-HR (CI) MH^+ at m/z 252.1836 (calcd, 252.1838). Anal. ($C_{14}H_{24}N_2O_2 \cdot 0.2H_2O$) C, H, N.

(*R*)-2-[3-(Dimethylamino)-1-propynyl]-1-pyrrolidinecarboxylic acid 1,1-dimethylethyl ester (9d): pale yellow oil; $[\alpha]^{26}_D$ = +108° (c = 1.05, CH_2Cl_2); MS-HR (CI) MH^+ at m/z 252.1836 (calcd, 252.1838). Anal. ($C_{14}H_{24}N_2O_2 \cdot 0.2H_2O$) C, H, N.

(*S*)-1-[3-(2-Pyrrolidinyl)-2-propynyl]pyrrolidine Dihydrochloride (10a). A solution of 6.62 g (23.8 mmol) of **9a** in 60 mL of 2 N HCl and 60 mL of ethanol was refluxed for 0.5 h. The dark brown solution was concentrated in vacuo, washed with several portions of CH_2Cl_2, and concentrated to a minimal volume. The residue was basified with 10 N NaOH, extracted with CH_2Cl_2, dried, and filtered over Magnesol. Concentration of the filtrate in vacuo afforded 2.68 g of the free diamine **10a** as a yellow oil. Treatment of the oil with an excess of methanolic hydrogen chloride and crystallization from CH_3OH–ether afforded 2.5 g (42%) of **10a** as colorless crystals: mp 196–197 °C; NMR (DMSO-d_6) δ 1.8–2.1 (m, 7, 3 CH_2, CH), 2.23 (m, 1, CH), 3.2–3.6 (m, 6, 3 CH_2), 4.2 (s, 2, CH_2), 4.42 (m, 1, CH), 10.2 (br s, 2, NH_2); MS-HR (EI) M^+ at m/z 178.1461 (calcd, 178.1470); $[\alpha]^{26}_D$ = −20° (c = 1.05, MeOH). Anal. ($C_{11}H_{20}Cl_2N_2$) C, H, N.

(*R*)-1-[3-(2-Pyrrolidinyl)-2-propynyl]pyrrolidine dihydrochloride (10b): colorless crystals; mp 198–200 °C; $[\alpha]^{26}_D$ = +21° (c = 1.01, MeOH). Anal. ($C_{11}H_{20}Cl_2N_2$) C, H, N.

(*S*)-1-[3-(Dimethylamino)-2-propynyl]pyrrolidine dihydrochloride (10c): colorless crystals; mp 190–191 °C; $[\alpha]^{26}_D$ = −23 °C (c = 1.01, MeOH). Anal. ($C_9H_{18}Cl_2N_2 \cdot 0.5H_2O$) C, H, N.

(*R*)-1-[3-(Dimethylamino)-2-propynyl]pyrrolidine dihydrochloride (10d): colorless crystals; mp 190–191 °C; $[\alpha]^{26}_D$ = +22° (c = 1.04, MeOH). Anal. ($C_9H_{18}Cl_2N_2 \cdot 0.5H_2O$) C, H, N.

(*S*)-2-[3-(1-Pyrrolidinyl)-1-propynyl]-1-pyrrolidinecarboxaldehyde (11a). Acetic anhydride (5.2 mL, 55 mmol) was added dropwise to a solution of 1.3 g (7.3 mmol) of diamine **10a** in 16 mL of 88% formic acid. The resulting solution was stirred at room temperature for 2 h. The solution was diluted with 20 mL of water and concentrated in vacuo to dryness. The residue was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$. The CH_2Cl_2 solution was dried and concentrated in vacuo to dryness. The residue (2.35 g) was purified by column chromatography (Al_2O_3, 125 g; eluant, CH_2Cl_2 then 1% v/v MeOH–CH_2CL_2) to give **11a** as a pale yellow oil: IR (neat) 1673 cm^{-1} (C=O); NMR ($CDCl_3$) δ 1.81 (m, 4, 2 CH_2), 1.85–2.25 (m, 4, 2 CH_2), 3.38 (d, J = 2 Hz, 0.5) and 3.40 (d, J = 2 Hz, 1.5) (CH_2N), 3.4 (m, 1.5) and 3.60 (m, 0.5) (CH_2N), 4.46 (m, 0.75) and 4.72 (m, 0.25) (CHN), 8.23 (s, 0.25) and 8.43 (s, 0.75)(CHO); MS-HR (FAB) MH^+ at m/z 207.1493 (calcd, 207.1497); $[\alpha]^{26}_D$ = −126° (c = 1.17, CH_2Cl_2). Anal. ($C_{12}H_{18}N_2O \cdot H_2O$) C, H, N.

The hydrochloride salt of **11a** was prepared by the addition of a sample of **11a** to an excess of 1.0 N methanolic hydrogen chloride. The resulting solution was concentrated in vacuo to give 320 mg of a yellow gum: IR KBr (neat) 1665 cm^{-1} (C=O); NMR (DMSO-d_6) δ 1.95 (m, 7, 3 CH_2, CH), 2.15 (m, 1, CH), 3.31 (m, 1, CH), 3.34 (br s, 4, 2 CH_2), 3.53 (m, 1, CH), 4.09 (d, J = 1.5 Hz, 0.8) and 4.14 (d, J = 1.5 Hz, 1.2) (CH_2), 4.58 (m, 0.4) and 4.79 (m, 0.6) (CH), 8.20 (s, 0.4) and 8.33 (s, 0.6) (CHO); MS-HR (FAB) MH^+ at m/z 207.1491 (calcd, 207.1497); $[\alpha]^{26}_D$ = −104° (c = 1.11 MeOH). Anal. ($C_{12}H_{19}ClN_2O \cdot H_2O$) H, N, C: calcd, 55.27; found, 55.92.

(*R*)-2-[3-(1-Pyrrolidinyl)-1-propynyl]-1-pyrrolidinecarboxaldehyde (11b): pale yellow oil; $[\alpha]^{26}_D$ = +131° (c = 1.05, CH_2Cl_2); MS-HR (FAB) MH^+ at 207.1499 (calcd, 207.1497). Anal. ($C_{12}H_{18}N_2O \cdot 2H_2O$) C, H, N.

The hydrochloride salt of **11b** was prepared by the addition of a sample of **11b** to an excess of 1.0 N methanolic hydrogen chloride. The resulting solution was concentrated in vacuo to give the hydrochloride salt of **11b** as a yellow gum: $[\alpha]^{26}_D$ = +114° (c = 1.10, MeOH).

(*S*)-1-Acetyl-2-[3-(1-pyrrolidinyl)-1-propynyl]pyrrolidine (12a). A sample of the dihydrochloride salt of **10a** (1.0 g, 4 mmol) was partitioned between 5 N NaOH and CH_2Cl_2. The organic solution was dried and concentrated in vacuo to give 0.71 g of the free base of **10a** as a pale yellow oil. A solution of the diamine **10a** in 30 mL of CH_2Cl_2 was cooled to 0 °C and 1.5 mL (16 mmol) of acetic anhydride was added. After stirring the mixture at 0 °C for 2 h, 5 mL of MeOH was added. The solution was washed with 30 mL of saturated aqueous $NaHCO_3$, dried, and concentrated in vacuo to give 1.19 g of a yellow oil. Purification by column chromatography (Al_2O_3, activity grade 2.5; eluant, 1% v/v CH_3OH–CH_2Cl_2) afforded 0.80 g (90%) of a pale yellow oil: IR (neat) 1654 cm^{-1} (C=O); NMR ($CDCl_3$; 25 °C) δ 1.7–2.3 (m, 4, 2 CH_2), 1.80 (m, 4, 2 CH_2), 2.04 (s, 1.2) and 2.19 (s, 1.8) ($COCH_3$), 2.58 (m, 4, 2 CH_2), 3.37 (d, J = 2 Hz, 0.8) and 3.39 (d, J = 2 Hz, 1.2) (CH_2N), 3.3–3.5 (m, 1, CHN), 3.5–3.7 (m, 1, CH), 4.47, (m, 0.6) and 4.81 (m, 0.4) (CHN); NMR (DMSO-d_6; 25 °C) δ 1.94 (s, 1.2) and 2.06 (s, 1.8) (CH_3), 4.58 (d, J = 6 Hz, 0.4) and 4.68 (d, J = 7.5 Hz, 0.6) (CH); NMR (DMSO-d_6; 120 °C) δ 2.02 (s, 3, CH_3),

4.63 (d, J = 6 Hz, 1); MS-HR (FAB) MH^+ at m/z 221.1652 (calcd, 221.1654); $[\alpha]^{26}_D$ = −115° (c = 1.14, CH_2Cl_2). Anal. ($C_{13}H_{20}N_2O$) H, N; C: calcd, 70.87; found, 70.19.

The hydrochloride salt of **12a** was prepared by the addition of a sample of **12a** to an excess of 1.0 N methanolic hydrogen chloride and the resulting solution was concentrated in vacuo to give 320 mg of a yellow gum: IR (KBr) 1642 cm^{-1} (C=O); NMR (DMSO-d_6) δ 1.8–2.2 (m, 8, 4 CH_2), 1.96 (s, 1.5) and 2.08 (s, 1.5) ($COCH_3$), 2.8–3.2 (m, 2, CH_2), 3.2–3.3 (m, 0.5) and 3.3–3.6 (m, 3.5) (2 CH_2), 4.06 (m, 1) and 4.12 (m, 1) (CH_2N), 4.63 (m, 0.5) and 4.80 (m, 0.5) (CHN), 11.7 (br d, 1, NH^+); MS-HR (FAB) (MH^+ − HCl) at m/z 221.1650 (calcd, 221.1654); $[\alpha]^{26}_D$ = −103° (c = 1.06, MeOH). Anal. ($C_{13}H_{20}N_2O$·HCl·0.8H_2O) C, N; H: calcd, 8.40; found, 7.86.

(*R*)-1-Acetyl-2-[3-(1-pyrrolidinyl)-1-propynyl]pyrrolidine (12b): pale yellow oil; MS-HR (FAB) MH^+ at m/z 221.1657 (calcd, 221.1654); $[\alpha]^{26}_D$ = +113° (c = 1.87, CH_2Cl_2). Anal. ($C_{13}H_{20}N_2O$) H, N; C: calcd, 70.87; found, 70.25.

The hydrochloride salt of **12a** was prepared to give a yellow gum; $[\alpha]^{26}_D$ = +85° (c = 1.14, MeOH); MS-HR (CI) MH^+ − HCl at m/z 221.1654 (calcd, 221.1654). Anal. ($C_{13}H_{20}N_2O$·HCl·1.2H_2O) C, H, N.

(*S*)-1-Acetyl-2-[3-(dimethylamino)-1-propynyl]pyrrolidine (12c): pale yellow oil; MS-HR (FAB) MH^+ at m/z 195.1495 (calcd, 195.1497); $[\alpha]^{26}_D$ = −124° (c = 1.15, CH_2Cl_2). Anal. ($C_{11}H_{18}N_2O$·H_2O) C, H, N.

(*R*)-1-Acetyl-2-[3-(dimethylamino)-1-propynyl]pyrrolidine (12d): pale yellow oil; MS-HR (FAB) MH^+ at m/z 195.1497 (calcd, 195.1497); $[\alpha]^{26}_D$ = +141° (c = 1.87, CH_2Cl_2). Anal. ($C_{11}H_{18}N_2O$·H_2O) C, H, N.

(*S*)-1-Acetyl-2-[3-(trimethylammonio)-1-propynyl]-pyrrolidine (12e): pale yellow crystals; mp 137–138 °C; $[\alpha]^{26}_D$ = −90° (c = 1.09, MeOH). Anal. ($C_{12}H_{21}IN_2O$) C, H, N.

(*R*)-1-Acetyl-2-[3-(trimethylammonio)-1-propynyl]-pyrrolidine (12f): pale yellow crystals; mp 137–138 °C; $[\alpha]^{26}_D$ = +90° (c = 1.02, MeOH). Anal. ($C_{12}H_{21}IN_2O$) C, H, N.

(*S*)-2-[3-(1-Pyrrolidinyl)-1-propynyl]-1-(trifluoroacetyl)pyrrolidine (13a). A sample of the dihydrochloride salt of **10a** (1.0 g, 3.98 mmol) was partitioned between 5 N NaOH and CH_2Cl_2. The organic layer was separated, dried, and concentrated in vacuo to give the free base of **10a** (0.71 g) as a pale yellow oil. A solution of **10a** in 20 mL of CH_2Cl_2 was cooled to 0 °C and 2.25 mL (15.9 mmol) of trifluorocetic anhydride was added dropwise. After stirring at 0 °C for 1.5 h, 5 mL of MeOH was added and stirring continued at room temperature for 20 min. The reaction mixture was washed with 30 mL of saturated $NaHCO_3$, dried, and concentrated in vacuo to give 1.36 g of pale yellow oil. Purification by column chromatography (Al_2O_3, activity grade 2.5, 140 g; eluant, CH_3OH–CH_2Cl_2) afforded 0.81 g (74%) of **13a** as a pale yellow oil: IR (neat) 1695 cm^{-1} (C=O); NMR ($CDCl_3$) δ 1.79 (m, 4, 2 CH_2), 2.08 (m, 2, CH_2), 2.0–2.3 (m, 2, CH_2), 2.58 (m, 4, 2 CH_2), 3.38 (d, J = 2 Hz, 2, CH_2N), 3.60 (m, 1.8) and 3.80 (m, 1.2) (CH_2), 4.83 (m, 0.6) and 4.88 (m, 0.4) (CHN); MS-HR (FAB) MH^+ at 275.1375 (calcd, 275.1371); $[\alpha]^{26}_D$ = −125° (c = 1.091, CH_2Cl_2). Anal. ($C_{13}H_{17}F_3N_2O$) C, H, N.

The hydrochloride salt of **13a** was prepared to give colorless crystals: mp 156–157 °C; $[\alpha]^{26}_D$ = −102° (c = 1.16, MeOH). Anal. ($C_{13}H_{17}ClF_3N_2O$) C, H, N.

(*R*)-2-[3-(1-Pyrrolidinyl)-1-propynyl]-1-(trifluoroacetyl)pyrrolidine (13b): pale yellow oil; $[\alpha]^{26}_D$ = 124° (c = 1.098, MeOH). Anal. ($C_{13}H_{17}F_3N_2O$) C, H, N. The hydrochloride salt of **13b** was prepared to give colorless crystals: mp 156–157 °C; $[\alpha]^{26}_D$ = +103° (c = 1.16, CH_2Cl_2). Anal. ($C_{13}H_{17}ClF_3N_2O$) C, H, N.

(*S*)-1-(Methylsulfonyl)-2-[3-(1-pyrrolidinyl)-1-propynyl]pyrrolidine (14a). A sample of the dihydrochloride salt of **10a** (0.82 g, 3.25 mmol) was partitioned between 5 N NaOH and CH_2Cl_2. The organic layer was separated, dried, and concentrated in vacuo to give 0.58 g of the free base of **10a** as a pale yellow oil. A solution of the diamine **10a** in 20 mL of CH_2Cl_2 was cooled to 0 °C and 0.28 mL (3.58 mmol) of methanesulfonyl chloride was added dropwise. After stirring at 0 °C for 2 h, 5 mL of MeOH was added and the mixture was diluted with CH_2Cl_2. The mixture was washed with saturated aqueous $NaHCO_3$, dried, and filtered through a pad of Magnesol. The filtrate was concentrated in vacuo to give 0.82 g of **14a** as a pale yellow oil: IR (neat) 1372 cm^{-1} (SO_2); NMR ($CDCl_3$) δ 1.81 (m, 4, 2 CH_2), 2.05 (m, 1, CH), 2.16 (m, 1, CH), 2.58 (m, 4, 2 CH_2), 2.99 (s, 3, CH_3), 3.36 (m, 1, CH), 3.41 (d, J = 2 Hz, 2, CH_2), 3.51 (m, 1, CH), 4.61 (q, J = 2 Hz, 1, CH); MS-HR (FAB) MH^+ m/z 257.1323 (calcd, 257.1324); $[\alpha]^{26}_D$ = −85° (c = 1.22, CH_2Cl_2). Anal. ($C_{12}H_{20}N_2O_2S$) C, H, N, S.

The hydrochloride salt of **14a** was prepared by the addition of a sample of **14a** to an excess of 1.0 M methanolic hydrogen chloride followed by the addition of ether. The salt was allowed to crystallize at 0 °C. The crystals were collected by filtration, washed with ether, and dried to afford 0.30 g of the hydrochloride salt of **14a** as colorless crystals: mp 198–199 °C dec; IR (KBr) 1362 cm^{-1} (SO_2); NMR (DMSO-d_6) δ 1.8–2.1 (m, 7, 3 CH_2 CH), 2.16 (m, 1, CH), 3.00 (s, 3, CH_3), 3.05 (m, 1, CH), 3.34 (m, 4, 2 CH_2), 3.45 (m, 1, CH), 4.14 (s, 2, CH_2), 4.58 (m, 1, CHN), 11.5 (br s, 1, NH^+); MS-HR (FAB) MH^+ − HCl at 257.1328 (calcd, 257.1324); $[\alpha]^{26}_D$ = −81° (c = 1.14, MeOH). Anal. ($C_{12}H_{21}ClN_2O_2S$) C, H, Cl, N, S.

(*R*)-1-(Methylsulfonyl)-2-[3-(1-pyrrolidinyl)-1-propynyl]pyrrolidine (14b): pale yellow oil; $[\alpha]^{26}_D$ = +81° (c = 1.17, CH_2Cl_2); MS-HR (CI) MH^+ at m/z 257.1309 (calcd, 257.1324). Anal. ($C_{12}H_{20}N_2O_2S$) H, N, S; C: calcd, 56.22; found, 55.79.

The hydrochloride salt of **14b** was prepared to give colorless crystals: mp 198–199 °C; $[\alpha]^{26}_D$ = +78° (c = 1.02, MeOH). Anal. ($C_{12}H_{21}ClN_2O_2S$) C, H, Cl, N, S.

(*S*)-2-[3-(1-Pyrrolidinyl)-1-propynyl]-1-pyrrolidinecarboxylic Acid Methyl Ester (15a). A solution of 0.24 mL (2.8 mmol) of methyl chloroformate in 5 mL of ether was added dropwise to a solution of 0.5 g (from 0.75 g of the dihydrochloride salt of **10a**, 2.8 mmol) in 10 mL of ether which was stirred at 0 °C. After approximately 50% of the methyl chloroformate was added, a solution of 0.6 mL of 5 N NaOH was added dropwise. The mixture was stirred for 45 min and diluted with ether, and the ether layer separated. The ether solution was dried and concentrated in vacuo. Purification of the residue by column chromatography (Al_2O_3, activity grade 2.5, 100 g; eluant, 1% v/v MeOH–CH_2Cl_2) gave 0.5 g (76%) of **15a** as a pale yellow oil: IR (neat) 1706 cm^{-1} (CO_2); NMR ($CDCl_3$) δ 1.80 (m, 4, 2 CH_2), 1.9 (m, 1, CH), 2.05 (m, 3, CH_2, CH), 2.59 (m, 4, 2 CH_2), 3.35 (m, 1, CH), 3.38 (d, J = 2 Hz, 2, CH_2), 3.5 (m, 1, CH), 3.72 (s, 3, CH_3), 4.5 (br s, 0.5) and 4.6 (br s, 0.5) (CH); MS-HR (EI) M^+ at m/z 236.1521 (calcd, 236.1525); $[\alpha]^{26}_D$ = −131° (c = 1.04, CH_2Cl_2). Anal. ($C_{13}H_{20}N_2O_2$·0.25H_2O) C, H, N.

(*R*)-2-[3-(1-Pyrrolidinyl)-1-propynyl]-1-pyrrolidinecarboxylic acid methyl ester (15b): pale yellow oil; $[\alpha]^{26}_D$ = +128° (c = 1.19, CH_2Cl_2); MS-HR (EI) M^+ at m/z 236.1532 (calcd, 236.1525). Anal. ($C_{13}H_{20}N_2O_2$·0.25H_2O) C, H, N.

(*S*)-2-[3-(1-Pyrrolidinyl)-1-propynyl]-1-pyrrolidine carboxylic acid ethyl ester (16a): pale yellow oil; $[\alpha]^{26}_D$ = −126° (c = 1.10, CH_2Cl_2); MS-HR (EI) M^+ at 250.1675 (calcd, 250.1682). Anal. ($C_{14}H_{22}N_2O_2$·0.25H_2O) C, H, N.

(*R*)-2-[3-(1-Pyrrolidinyl)-1-propynyl]-1-pyrrolidinecarboxylic acid ethyl ester (16b): pale yellow oil; $[\alpha]^{26}_D$ = +125° (c = 1.06, CH_2Cl_2); MS-HR (EI) M^+ at 250.1689 (calcd, 250.1682). Anal. ($C_{14}H_{22}N_2O_2$·0.25H_20) C, H, N.

(*S*)-2-(2,2-Dibromoethenyl)-1-pyrrolidine (18a). A solution of 3.5 g (10 mmol) of **7a** and 7.7 mL (100 mmol) of trifluoroacetic acid in 100 mL of CH_2Cl_2 was stirred at room temperature for 1.5 h. The solution was washed with saturated aqueous Na_2CO_3, dried, and concentrated in vacuo. Purification of the residue by column chromatography (Al_2O_3, activity grade 2.5, 100 g; eluant, 1% v/v MeOH–CH_2Cl_2) gave **18a** as a yellow oil: IR (neat) 3269 cm^{-1} (NH); NMR ($CDCl_3$) δ 1.52 (m, 1, CH), 1.83 (m, 2, CH_2), 1.90 (s, 1, NH), 2.05 (m, 1, CH), 2.97 (m, 2, CH_2), 3.81 (q, J = 7 Hz, 1, CHN), 7.27 (d, J = 9 Hz, 1, CH=); MS-LR m/z 256 (MH^+); $[\alpha]^{26}_D$ = +2.0° (c = 1.10, MeOH). Anal. ($C_6H_9Br_2N$) C, H, Br, N.

The hydrochloride salt of **18a** was prepared by the addition of an excess of methanolic hydrogen chloride to a sample of **18a** followed by the addition of ether. The resulting precipitate was recrystallized from MeOH–ether to give the hydrochloride salt of **18a** as colorless crystals: mp 211–213 °C; NMR ($CDCl_3$) δ 1.59 (br s 2, NH^+), 1.90 (m, 1, CH), 2.05 (m, 1, CH), 2.15 (m, 1, CH), 2.36 (m, 1, CH), 3.45 (m, 2, CH_2), 4.32 (q, J = 7 Hz, 1 CH), 6.96 (d, J = 7 Hz, 1, CH=); MS LR MH^+ m/z 254; $[\alpha]^{26}_D$ = +12° (c

= 1.10, MeOH). Anal. ($C_6H_{10}Br_2ClN$) C, H, Br, Cl, N.

(*R*)-2-(2,2-Dibromoethenyl)-1-pyrrolidine (18b): pale yellow oil; $[\alpha]^{26}_D = -30°$ (c = 1.13, CH_2Cl_2). Anal. ($C_6H_9Br_2N$) C, H, Br, N.

The hydrochloride salt of **18b** formed colorless crystals: mp 210–211 °C; $[\alpha]^{26}_D = -14°$ (c = 1.00, MeOH). Anal. ($C_6H_{10}Br_2ClN$) C, H, Br, Cl, N.

[*S*-(*R,*R**)-2-(2,2-Dibromoethenyl)-1-(3,3,3-trifluoro-2-methoxy-1-oxo-2-phenylpropyl)pyrrolidine (19a)**.[30] A solution of 0.4 g (1.6 mmol) of **18a** and 0.6 g (2.3 mmol) of (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride and 0.3 mL of pyridine in 10 mL of CH_2Cl_2 was stirred at room temperature for 3 h. The solution was diluted with saturated aqueous Na_2CO_3, and the solution was separated, dried, diluted with 100 mL of toluene, and concentrated in vacuo to dryness. The residue was filtered through a plug (Al_2O_3, activity grade 2.5; eluant 2% v/v MeOH–CH_2Cl_2) to give 0.53 g of **19a** as a pale yellow oil: NMR (DMSO-d_6; 25 °C) δ 1.33 (m, 1, CH), 1.56 (m, 1, CH), 1.73 (m, 1, CH), 2.00 (m, 1, CH), 2.69 (m, 1, CH), 3.30 (m, 1, CH), 3.33 (s, 2.7) 3.62 (s, 0.3), (OCH_3), 4.61 (q, J = 7 Hz, 1, CH), 5.86 (d, J = 8 Hz, 0.1, CH), 6.70 (d, J = 8 Hz, 0.9, CH), 7.44 (m, 5, arom H); NMR (DMSO-d_6; 120 °C) δ 3.6 2 (s, 3, CH_3), 4.65 (q, J = 8 Hz, 1, CH), 6.62 (br s, 1, CH); MS LR MH^+ m/z 472. The sample of **19a** crystallized on standing and was triturated with hexane to give an off-white solid: mp 110–112 °C; $[\alpha]^{26}_D = -111°$ (c = 1.03, CH_2Cl_2). Anal. ($C_{16}H_{16}Br_2F_3NO_2$) C, H, N, F.

[*S*-(*R,*S**)]-2-(2,2-Dibromoethenyl)-1-(3,3,3-trifluoro-2-methoxy-1-oxo-2-phenylpropyl)pyrrolidine (19b)**:[30] pale yellow oil; NMR (DMSO-d_6; 25 °C) δ 1.4–1.9 (m, 4, 2 CH_2), 2.10 (m, 1, CH), 2.49 (m, 1, CH), 3.49 (s, 0.6) and 3.59 (s, 2.4) (OCH_3), 3.92 (t, J = 7 Hz, 0.2) and 4.60 (dt, J = 4, 8 Hz, 0.8) (CH), 6.50 (d, 0.8, J = 10 Hz) and 6.95 (d, J = 8 Hz, 2) (CH), 7.5 (m, 5, arom H); NMR (DMSO-d_6; 120 °C) δ 3.60 (s, 3, OCH3), 4.65 (br s, 1, CH), 6.45 (br s, 1, CH); MS LR MH^+ m/z 472. The sample crystallized on standing and was triturated with hexane to give an off-white solid: mp 85–87 °C; $[\alpha]^{26}_D = -157°$ (c = 1.12, CH_2Cl_2). Anal. ($C_{16}H_{16}Br_2F_3NO_2$) C, H, N, F.

[*S*-(*R,*R**)]-2-[3-(1-Pyrrolidinyl)-1-propynyl]-1-(3,3,3-trifluoro-2-methoxy-1-oxo-2-phenylpropyl)pyrrolidine (20a)**:[30] NMR (DMSO-d_6; 25 °C) δ 1.50 (m, 1, CH), 1.66 (m, 4, 2 CH_2), 1.6–2.0 (m, 4, 2 CH_2), 2.43 (m, 4, 2 CH_2), 3.35 (d, J = 2 Hz, 0.9) and 3.42 (d, J = 2 Hz, 0.1), 3.55 (s, 0.3) and 3.62 (s, 2.7) (OCH_3), 4.77 (m, 0.9) and 4.82 (m, 0.1) (CH), 3.36 (br s, 2, CH_2), 3.65 (s, 3, CH_3), 4.82 (br s, 1, CH); NMR (DMSO-d_6; 140 °C) δ 3.37 (s, 2, CH_2), 3.62 (s, 3, OCH_3), 4.79 (br s, 1, CH); $[\alpha]^{26}_D = -104°$ (c = 1.15, CH_2Cl_2); MS-HR (EI) M^+ at m/z 394.1871 (calcd, 394.1869). Anal. ($C_{21}H_{25}F_3N_2O_2$) H, N; C: calcd, 63.95; found, 63.42.

[*S*-(*R,*S**)]-2-[3-(1-Pyrrolidinyl)-1-propynyl]-1-(3,3,3-trifluoro-2-methoxy-1-oxo-2-phenylpropyl)pyrrolidine (20b)**:[30] NMR (DMSO-d_6; 25 °C) δ 1.7 (m, 4, 2 CH_2), 1.8–2.0 (m, 4, 2 CH_2), 2.50 (m, 4, 2 CH_2), 3.1–3.3 (m, 2, CH_2), 3.58 (s, 2.7) and 3.65 (s, 0.3) (OCH_3), 4.01 (d, J = 8 Hz, 0.1) and 4.79 (d, J = 8 Hz, 0.9) (CH); NMR (DMSO-d_6; 140 °C) δ 3.60 (s, 3, OCH_3), 4.80 (br s, 1, CH); $[\alpha]^{26}_D = -67°$ (c = 0.73, CH_2Cl_2); MS-HR (EI) M^+ at 394.1867 (calcd, 394.1869). Anal. ($C_{21}H_{25}F_3N_2O_2$) H, N; C: calcd, 63.95; found, 63.43.

[*S*-(*R,*R**)]-2-[3-(Dimethylamino)-1-propynyl]-1-(3,3,3-trifluoro-2-methoxy-1-oxo-2-phenylpropyl)pyrrolidine (20c)**:[30] NMR (DMSO-d_6; 25 °C) δ 1.52 (m, 1, CH), 1.7–2.0 (m, 3, CH, CH_2), 2.13 (s, 6, 2 CH_3), 2.45 (m, 4, 2 CH_2), 3.19 (s, 2, CH_2), 3.3–3.5 (m, 2, CH_2), 3.56 (s, 0.3) and 3.63 (s, 2.7) (OCH_3), 4.80 (m, 0.9) and 4.81 (m, 0.1) (CH); NMR (DMSO-d_6; 140 °C) δ 3.60 (s, 3, OCH_3), 4.80 (m, 1, CH); $[\alpha]^{26}_D = -140°$ (c = 1.01, CH_2Cl_2); MS-HR (EI) M^+ at 368.1712 (calcd, 368.1711). Anal. ($C_{19}H_{23}F_3N_2O_2$) C, H, N.

[*S*-(*R,*S**)]-2-[3-(Dimethylamino)-1-propynyl]-1-(3,3,3-trifluoro-2-methoxy-1-oxo-2-phenylpropyl)pyrrolidine (20d)**:[30] NMR (DMSO-d_6; 25 °C) δ 1.6–2.1 (m, 4, 2 CH_2), 2.17 (s, 6, 2 CH_3), 3.20 (d, J = 2 Hz, 0.2) and 3.25 (d, J = 2 Hz, 1.8), 3.59 (s, 2.7) and 3.63 (s, 3, OCH_3), 4.03 (d, J = 6 Hz, 0.1) and 4.79 (d, J = 6 Hz, 0.9); NMR (DMSO-d_6; 140 °C) δ 3.18 (s, 2, CH_2), 3.30 (s, 3, OCH_3), 4.80 (s, 1, CH); $[\alpha]^{26}_D = -73°$ (c = 1.03, CH_2Cl_2); MS-HR (EI) M^+ at m/z 368.1705 (calcd, 368.1711). Anal. ($C_{19}H_{23}F_3N_2O_2$) C, H, N.

Muscarinic Receptor Binding Assays. The receptor binding assays were adapted from published procedures.[21a,b,22] The cerebral cortex from male Wistar rats (200–250 g of body weight) was homogenized in 50 volumes (wet wt/v) of ice-cold 10 mM (8.1 mM Na_2HPO_4, 1.9 mM KH_2PO_4) sodium–potassium phosphate buffer (NaKPB), pH 7.4. The resulting homogenate was then diluted 1:3000, 1:300, or 1:400 (original wet wt/v) with ice-cold NaKPB for use in the [^{3}H]QNB, [^{3}H]CD, and [^{3}H]PZ assays, respectively. The final protein content per 2.0 mL of incubation mixture was ~0.1, 0.75 and 0.55 mg, respectively.

The heart from male Wistar rats (200–250 g body weight) was placed in ice-cold 10 mM (8.1 mM Na_2HPO_4, 1.9 mM KH_2PO_4) sodium–potassium phosphate buffer (NaKPB), pH 7.4, and gently squeezed to flush blood from the ventricles. Excess buffer was blotted from the heart which was weighed and placed into 50 volumes with wet wt/v) of ice-cold buffer where it was minced with a scissor. The mince was then homogenized with a Polytron (setting 8 with PT-10 saw-tooth generator) for 60 s. The homogenate was then centrifuged at 48000g for 15 min at 4 °C. The supernatant was discarded and the pellet was resuspended in 50 volumes (wet wt/v) of fresh buffer with the Polytron as above. The homogenate was then filtered through two layers of gauze and diluted to a final tissue concentration 1:750 (wet wt/v), giving a final protein concentration of 0.15–0.2 mg/2.0 mL in the assay tubes.

Binding assays were initiated by adding 1.80 mL of the appropriate tissue homogenate to tubes containing 100 μL of tritiated ligand, 50 μL of unlabeled compound (final concentration 1×10^{-3}–1×10^{-12} M), and 50 μL of buffer or atropine (final concentration = 10 μM) to define nonspecific binding. For [^{3}H]QNB binding to cardiac membranes, Gpp(NH)p was added to the diluted homogenate to give a final concentration of 10 μM. The concentrations of radioligand were as follows: 250 pM, [^{3}H]QNB (30.0 Ci/mmol), 1 nM [^{3}H]CD (55.5 Ci/mmol), and 0.1 nM [^{3}H]PZ (84.1 Ci/mmol). Samples were incubated at 25 °C for 120 min ([^{3}H]QNB and [^{3}H]CD) or 60 min ([^{3}H]PZ). [^{3}H]CD and [^{3}H]PZ adheres readily to both glass and plastic surfaces. To eliminate this problem (and the chance for introducing artifacts into the results), stock vials, pipet tips, and all glass tubes were routinely treated with Prosil-28, a siliconizing agent, and oven dried prior to use in an assay. Additionally, the GF/B glass-fiber filters were presoaked in an aqueous polyethylenimine (PEI) solution (0.1%, pH 7.0) prior to use. All points in the inhibition curve (including total and nonspecific binding) were always measured on single PEI-treated filter strips to minimize filter-to-filter variability. The [^{3}H]CD and [^{3}H]PZ were prepared fresh in buffer just prior to use in the assay to avoid decomposition. The incubations were terminated by rapid filtration (Brandel 24-sample cell harvester) and tubes were rinsed twice with 5 mL of ice-cold NaKPB buffer. Receptor-bound radioligand was determined by liquid scintillation spectrometry. The data were analyzed by nonlinear regression (LIGAND) and apparent K_i values determined by fitting a one-site competitive model to the competition data.[23b]

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(30) The nomenclature is based on rules 1d and 2d of Chemical Abstracts stereochemical nomenclature rules (Blackwood, J. E.; Giles, P. M. *J. Chem. Inf. Comput. Sci.* **1975**, *15*, 67). (When two chiral centers are not in the same ring system, *R** and *S** are employed without locants. Two centers of like chirality are indicated by the relative descriptor *R**,*R** while two centers of unlike chirality are described by *R**,*S**. The absolute configuration is designated by the center which has the highest ranking substituent according to the sequence rule).