Design, Synthesis, and Biological Evaluation of Symmetrically and Unsymmetrically Substituted Methoctramine-Related Polyamines as Muscular Nicotinic Receptor Noncompetitive Antagonists

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Received June 25, 1999

The universal template approach to drug design foresees that a polyamine can be modified in such a way to recognize any neurotransmitter receptor. Thus, hybrids of polymethylene tetraamines and philanthotoxins, exemplified by methoctramine (**1**) and PhTX-343 (**2**), respectively, were synthesized to produce novel inhibitors of muscular nicotinic acetylcholine receptors. Polyamines **3**−**25** were synthesized and their biological profiles were evaluated at frog rectus abdominis muscle nicotinic receptors and guinea pig left atria (M_2) and ileum longitudinal muscle (M_3) muscarinic acetylcholine receptors. All of the compounds, like prototypes **1** and **2**, were noncompetitive antagonists of nicotinic receptors while being, like **1**, competitive antagonists at muscarinic M_2 and M_3 receptor subtypes. Interestingly, polyamines bearing a low number of methylenes between the nitrogen atoms, as in **3**, **6**, and **7**, displayed a biological profile similar to that of **2**: a noncompetitive antagonism at nicotinic receptors in the 7−25 μM range while not showing any antagonism for muscarinic receptors up to 10 μM. Increasing the number of methylenes separating these nitrogen atoms in methoctramine-related tetraamines resulted in a significant improvement in potency at nicotinic receptors. The most potent tetraamine was **19**, bearing a 12 methylene spacer between the nitrogen atoms, which was 12-fold and 250-fold more potent than prototypes **1** and **2**, respectively. Tetraamines **9**−**11**, bearing a rather rigid spacer between the nitrogen atoms instead of the very flexible polymethylene chain, displayed a profile similar to that of **1** at nicotinic receptors, whereas a significant decrease in potency was observed at muscarinic M_2 receptors. This finding may have relevance in understanding the mode of interaction with these receptors. Similarly, the constrained analogue **12** of methoctramine showed a decrease in potency at nicotinic and muscarinic M_2 receptors, revealing that the tricyclic system, which incorporates the 2-methoxybenzylamine moiety of **1**, does not represent a good pharmacophore for activity at these sites. A most intriguing finding was the observation that the photolabile tetraamine **22** was more potent than methoctramine at nicotinic receptors and, what is more important, it inhibited a closed state of the receptor.

Introduction

Methoctramine (**1**, Figure 1) is the prototype polymethylene tetraamine for antagonism of muscarinic acetylcholine receptors (mAChR).[1] The development of polymethylene tetraamines as antagonists of mAChR has been the subject of several review articles.[2] Appropriate modifications of the chain lengths separating the nitrogen atoms and of the substituents on the terminal nitrogen atoms modulate both affinity and selectivity (specificity) for different receptors.[3,4] On the basis of these observations, it was suggested that a polyamine backbone represents a special feature in the polymethylene tetraamine−mAChR recognition process.[5] More recently, it has been suggested that such a structure may represent a universal template on which suitable pharmacophores can be inserted to achieve selectivity for any given receptor.[6] The discovery that **1**, at micromolar concentrations, antagonizes nicotinic acetylcholine receptors (nAChR) has provided the opportunity to apply the universal template approach to the design of polyamines as nAChR ligands.

Philanthotoxins, as exemplified by philanthotoxin-343 (PhTX-343) (**2**, Figure 1), a synthetic analogue of a wasp (*Philanthus triangulum*) toxin PhTX-433,[7] are noncompetitive antagonists of muscle[8,9] and neuronal nAChR,[10] although at submillimolar concentrations they may also competitively antagonize these receptors.[11] However, the mode of action of **2** on nAChR has not yet been fully evaluated. The affinity of **2** for nAChR is not high. However, by combining structural features of **2** and polymethylene tetraamines it may be possible to obtain ligands that have high affinities and selectivities for nAChR. The synthesis and pharmacological assay of these hybrid molecules is the subject of this report.

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10.1021/jm991110n CCC: $18.00 © 1999 American Chemical Society
Published on Web 11/23/1999

Methoctramine (**1**)

PhTX-343 (**2**)

Figure 1. Chemical structure of methoctramine (**1**) and PhTX-343 (**2**), prototypes of polymethylene tetraamines and polyamine amides, respectively. The numerals in PhTX-343 refers to the number of methylenes between the polyamine nitrogen atoms.

Structure–activity relationship (SAR) studies on the action of philanthotoxins on quisqualate-sensitive ionotropic glutamate receptors (qGluR) of insect skeletal muscle[12–17] and competition binding studies of these compounds on nAChR of *Torpedo* electroplax[11,18] have shown that an aromatic moiety at one terminus and a primary amine function at the other terminus are important for antagonism. Thus, a suitable starting point for the design of novel ligands for nAChR might be a tetraamine backbone to which may be attached either one or both terminal groups of **1** and **2**. To this end, compounds **3**–**8** were synthesized to verify the influence on potency of the butyryltyrosyl moiety of **2** and of the 2-methoxybenzyl group of **1**. Tetraamine **1** can assume many low-energy conformations in an aqueous environment because of its flexible polymethylene chain. Therefore, we have designed tetraamines **9**–**11** with less flexible chains to determine whether flexibility is an important determinant of potency with respect to nAChR antagonism. Also, the terminal 2-methoxybenzyl groups of **1** have been included into a tricyclic system (**12**) to determine whether the spatial relationship of the methoxy moiety relative to the amine function differently affects affinity for nAChR and mAChR. Previously, homologues of **1** have been investigated with the aim of assessing the structural requirements for optimum antagonism of mAChR.[1,19] In this study we have tested a number of these compounds (**13**–**21**) on nAChR in which the chain length between the inner nitrogen atoms varies between 4 and 14 methylenes. Finally, to determine which amino acid residues are involved in the binding of polymethylene tetraamines to nAChR, we have designed the photolabile analogue MR44 (**22**). During synthesis of the linear pentaamine required for **22**, the branched isomeric pentaamine was obtained, which gave the opportunity to synthesize the polyamines **23**–**25**. All of the compounds synthesized in this study were tested on peripheral M_2 and M_3 mAChR as well as on muscle-type nAChR.

Chemistry

All the compounds were synthesized by standard procedures (Schemes 1–7) and were characterized by IR, ^1H NMR, mass spectra, and elemental analysis.

Triamine amides **4** and **5** were synthesized by following an adapted procedure described for **2**.[7,16] Thus,

Scheme 1

$Me(CH_2)_2CONH$–CH(CH$_2$C$_6$H$_4$OBn)–COO–C$_6$H$_4$–NO_2 (**26**) + $H_2N(CH_2)_nNH(CH_2)_mNH(CH_2)_nNHR$

27: n = 3, m = 4, R = H
28: n = 6, m = 8, R = H
8: n = 6, m = 8, R = 2-MeOC$_6$H$_4$CH$_2$

↓ MeOH

$Me(CH_2)_2CONH$–CH(CH$_2$C$_6$H$_4$OBn)–$CONH(CH_2)_nNH(CH_2)_mNH(CH_2)_nNHR$

29: n = 3, m = 4, R = H
30: n = 6, m = 8, R = H
31: n = 6, m = 8, R = 2-MeOC$_6$H$_4$CH$_2$

↓ H$_2$/Pd, MeOH

$Me(CH_2)_2CONH$–CH(CH$_2$C$_6$H$_4$OH)–$CONH(CH_2)_nNH(CH_2)_mNH(CH_2)_nNHR$

2: n = 3, m = 4, R = H
4: n = 6, m = 8, R = H
5: n = 6, m = 8, R = 2-MeOC$_6$H$_4$CH$_2$
3: n = 3, m = 4, R = 2-MeOC$_6$H$_4$CH$_2$

(**2** → **3**: 1. 2-MeOC$_6$H$_4$CHO; 2. NaBH$_4$)

N-butyryl-*O*-benzyl-L-tyrosine *p*-nitrophenyl ester[16] (**26**) was allowed to react with tetraamines spermine (**27**), **28**,[1] and **8**. Intermediates **29**–**31** were debenzylated by catalytic hydrogenolysis to give **2**,[7] **4** and **5**, respectively. The condensation of **2** with 2-methoxybenzaldehyde and subsequent reduction with $NaBH_4$ of the intermediate Schiff base afforded **3** (Scheme 1).

Mono- and disubstituted spermine derivatives **6** and **7** were easily obtained through the condensation of spermine in a 1:2 or 10:1 molar ratio with 2-methoxybenzaldehyde and subsequent reduction with $NaBH_4$ of the intermediate Schiff bases.

Although monosubstituted tetraamine **8** was already known,[20] it was synthesized following the procedure shown in Scheme 2. *N*-(*tert*-Butoxycarbonyl)-6-aminocaproic acid was amidated with *N*-[(benzyloxy)carbonyl]-1,8-octanediamine[21] to give **32**. Removal of the *N*-(benzyloxy)carbonyl group was achieved by catalytic hydrogenation over 10% palladium on charcoal. Thus, hydrogenolysis of **32** gave **33**, which was reacted with *N*-[(benzyloxy)carbonyl]-6-aminocaproic acid to afford diamine diamide **34**, having the two terminal amine functions protected with different moieties. Removal of the *N*-(benzyloxy)carbonyl group of **34** by catalytic hydrogenolysis gave **35**, which was treated with 2-methoxybenzaldehyde followed by reduction with $NaBH_4$ of the intermediate Schiff base and subsequent hydrolysis with 3 N HCl to remove the *N*-*tert*-butoxycarbonyl group to afford the monosubstituted diamine diamide **36**. Reduction of **36** with borane–methyl sulfide complex gave **8** (Scheme 2).

Suberic acid and 4,4′-biphenyldicarboxylic acid were amidated with benzyl *N*-[4-(aminomethyl)benzyl]car-

Scheme 2[a]

Cbz—NH(CH$_2$)$_8$NH$_2$ + Boc—NH(CH$_2$)$_5$COOH → (Et$_3$N, EtOCOCl) Cbz—NH(CH$_2$)$_8$NHCO(CH$_2$)$_5$NH—Boc **32**

→ (H$_2$/Pd, MeOH) H$_2$N(CH$_2$)$_8$NHCO(CH$_2$)$_5$NH—Boc **33**

→ (+ Cbz—NH(CH$_2$)$_5$COOH) R—NH(CH$_2$)$_5$CONH(CH$_2$)$_8$NHCO(CH$_2$)$_5$NH—Boc

34: R = Cbz → (H$_2$/Pd, MeOH) **35**: R = H

→ (1. 2-MeOC$_6$H$_4$CHO; 2. NaBH$_4$; 3. 3 N HCl) 2-MeO-C$_6$H$_4$-CH$_2$NH(CH$_2$)$_5$XNH(CH$_2$)$_8$NHX(CH$_2$)$_5$NH$_2$

36: X = CO → (BH$_3$·MeSMe) **8**: X = CH$_2$

[a] Boc = (CH$_3$)$_3$COCO—; Cbz = C$_6$H$_5$CH$_2$OCO—

Scheme 3[a]

Cbz(H)NCH$_2$XCH$_2$NH$_2$ + HOOCYCOOH → (Et$_3$N, EtOCOCl) Cbz(H)NCH$_2$XCH$_2$N(H)C(O)YC(O)N(H)CH$_2$XCH$_2$N(H)Cbz

X = A or (CH$_2$)$_4$ Y = B or (CH$_2$)$_6$

37: X = A, Y = (CH$_2$)$_6$
38: X = (CH$_2$)$_4$, Y = B

→ (HBr, CF$_3$COOH) H$_2$NCH$_2$XCH$_2$N(H)C(O)YC(O)N(H)CH$_2$XCH$_2$NH$_2$

39: X = A, Y = (CH$_2$)$_6$
40: X = (CH$_2$)$_4$, Y = B

→ (1. 2-MeOC$_6$H$_4$CHO; 2. NaBH$_4$; 3. 2 N HCl) 2-MeO-C$_6$H$_4$-CH$_2$N(H)CH$_2$XCH$_2$N(H)C(O)YC(O)N(H)CH$_2$XCH$_2$N(H)CH$_2$-C$_6$H$_4$-2-OMe

41: X = A, Y = (CH$_2$)$_6$
42: X = (CH$_2$)$_4$, Y = B

→ (BH$_3$·MeSMe) 2-MeO-C$_6$H$_4$-CH$_2$N(H)CH$_2$XCH$_2$N(H)CH$_2$YCH$_2$N(H)CH$_2$XCH$_2$N(H)CH$_2$-C$_6$H$_4$-2-OMe

9: X = A, Y = (CH$_2$)$_6$
11: X = (CH$_2$)$_4$, Y = B

[a] Cbz = C$_6$H$_5$CH$_2$OCO—; A = —C$_6$H$_4$— (para); B = —C$_6$H$_4$—C$_6$H$_4$— (para)

bamate[21] and *N*-[(benzyloxy)carbonyl]-1,6-diamine,[22] respectively, to give **37** or **38**. Removal of the *N*-(benzyloxy)carbonyl group of **37** and **38** by hydrolysis with HBr gave **39** and **40**, respectively, which were treated with 2-methoxybenzaldehyde followed by the reduction of the formed Schiff bases to amine amides **41** and **42** that, in turn, were reduced with borane to **9** and **11**, respectively (Scheme 3).

Alkylation of **44**, obtained through the reaction of **43**[20] with mesitylenesulfonyl chloride, with di[(*p*-bromomethyl)phenyl]methane[23] afforded **45**. Removal of the protecting groups of **45** by hydrolysis with 30% HBr gave **10** (Scheme 4).

The tricyclic compound **46** required for the synthesis of the constrained analogue **12** of methoctramine was synthesized by following an adapted procedure reported for its *N*-methyl analogue[24] starting from *N*-benzylglycine and 2-allyloxybenzaldehyde. Removal of the benzyl group of **46** by hydrogenolysis gave **47**, which was

Scheme 4

BrCH$_2$—C$_6$H$_4$—CH$_2$—C$_6$H$_4$—CH$_2$Br + 2-MeO-C$_6$H$_4$-CH$_2$N(R)(CH$_2$)$_6$N(R)H

43: R = H → (2,4,6-Me$_3$-C$_6$H$_2$SO$_2$Cl) **44**: R = 2,4,6-Me$_3$-C$_6$H$_2$SO$_2$—

→ 2-MeO-C$_6$H$_4$-CH$_2$N(R)(CH$_2$)$_6$N(R)CH$_2$—C$_6$H$_4$—CH$_2$—C$_6$H$_4$—CH$_2$N(R)(CH$_2$)$_6$N(R)CH$_2$-C$_6$H$_4$-2-OMe

45: R = 2,4,6-Me$_3$-C$_6$H$_2$SO$_2$— → (HBr, CH$_3$COOH) **10**: R = H

treated with *N*-[(benzyloxy)carbonyl]-6-aminocaproic acid to afford **48**. Hydrogenolysis of **48** gave **49**, which, upon reduction with borane−methyl sulfide complex, was transformed into **50** that was treated with suberic

Scheme 5

+ $C_6H_5CH_2OCONH(CH_2)_5COOH$, Et_3N, EtOCOCl

46: R = $C_6H_5CH_2$; **47**: R = H (H_2/Pd)

48: R = $C_6H_5CH_2OCO$—; **49**: R = H (H_2/Pd); $CO(CH_2)_5NHR$

BH_3·MeSMe

50 ($(CH_2)_6NH_2$)

+ $HOOC(CH_2)_6COOH$, Et_3N, EtOCOCl

$(CH_2)_6NHX(CH_2)_6XNH(CH_2)_6$

51: X = CO; **12**: X = CH_2 (BH_3·MeSMe)

Scheme 6

$C_6H_5CH_2OCO—NH(CH_2)_5COOH$ + $H_2N(CH_2)_4NH_2$

Et_3N, EtOCOCl

R—$NH(CH_2)_5CONH(CH_2)_4NHCO(CH_2)_5NH$—R

52: R = $C_6H_5CH_2OCO$; **53**: R = H (HBr, CH_3COOH)

1. 2-$MeOC_6H_4CHO$ 2. $NaBH_4$

$CH_2NH(CH_2)_5XNH(CH_2)_4NHX(CH_2)_5NHCH_2$ (OMe, MeO)

54: X = CO; **13**: X = CH_2 (BH_3·MeSMe)

acid to afford diamine diamide **51**. Reduction of amide functions of **51** gave **12** (Scheme 5).

Tetraamine **13** was synthesized by following an adapted procedure described for methoctramine.[25] Thus, *N*-[(benzyloxy)carbonyl]-6-aminocaproic acid was amidated with 1,4-diaminobutane to give **52** that, upon hydrolysis with HBr in acetic acid, afforded **53**, which was treated with 2-methoxybenzaldehyde followed by the reduction of the formed **54** into **13** (Scheme 6).

The addition of tetraamine **28**[1] to acrylonitrile gave a mixture of compounds: two of them (**55** and **56**) were isolated and characterized also through their transformation into **57** and **58**. Linear and branched nitriles **55** and **56** were transformed into **59** and **60** followed by reduction with $LiAlH_4$ or Raney Ni into the corresponding amines **61** and **62**. 4-Azidosalicylic acid *N*-hydroxysuccinimide ester[26] was amidate with **61** to give **63**, which, upon treatment with CF_3COOH to remove the protecting groups, afforded the photolabile compound MR44 (**22**). Condensation of **62** with 2-methoxybenzaldehyde followed by the reduction of the intermediate Schiff base and subsequent hydrolysis with HCl gave **23**. Finally, amidation of **26**[21] with **62** gave **64**, which, upon hydrolysis with CF_3COOH to **65**, was transformed into **24** through removal of the benzyl group. Condensation of **24** with 2-methoxybenzaldehyde afforded **25** (Scheme 7).

Biology

The effects of compounds **1**−**25** on M_2 mAChR were determined on guinea pig left atria stimulated electrically at 1 Hz.[4] The guinea pig ileum longitudinal muscle was used to study their effects on M_3 mAChR. In both cases the agonist was arecaidine propargyl ester (APE). The biological data are expressed in apparent dissociation constants (K_B) according to Furchgott.[27]

The effects of compounds **1**−**25** on muscle-type nAChR were studied on the frog rectus abdominis muscle with carbachol-induced contractions as the measured parameter.[28] The results are expressed as IC_{50} values, i.e., the concentrations required to inhibit the maximal response to carbachol by 50%.

In all of the experiments, **1** and **2** were used as standards.

Results and Discussion

Summaries of the results are presented in Table 1 and Figure 2. Over the concentration range investigated, all of the compounds were noncompetitive antagonists of muscle-type nAChR. The maximum response to carbachol was reduced and the magnitude of this reduction was dependent on the concentration of antagonist. In all cases, the antagonism was reversed by washing the muscle in drug-free saline. In contrast to the data for nAChR, compounds **1**−**25** competitively antagonized mAChR, as revealed by the parallelism of dose−response data for APE obtained in the presence and absence of the compounds.

Methoctramine (**1**) was a potent and selective antagonist of M_2 mAChR, whereas 10 μM PhTX-343 (**2**) was inactive on both M_2 and M_3 mAChR. Tetraamine **1** was 20-fold more potent than polyamine amide **2** at nAChR. As a consequence, it was necessary to determine whether the higher potency of **1** is due to the presence in the molecule of methoxybenzyl groups or to the different polyamine backbones of **1** and **2**. To this end, a series of methoctramine analogues bearing either one or both of the aromatic features of the two prototypes was investigated. Compound **8**, which lacks one of the methoxybenzyl groups of **1**, was a less potent antagonist of nAChR than **1** and, as reported previously,[20] a less potent antagonist of mAChR. This suggests that the methoxybenzyl group plays a role in the binding of **1** to nAChR and mAChR. Attachment of the butyryltyrosyl residue of **2** onto the unsubstituted tetraamine backbone of **1**, to give compound **4**, resulted in an increase in potency (to that of **8**) at nAChR. Again, the inclusion of a methoxybenzyl group on the terminal nitrogen atom

Scheme 7[a]

[a] Boc = $(CH_3)_3COCO$—; Bn = $C_6H_5CH_2$

of **4**, giving compound **5**, resulted in activities at nAChR and mAChR which were qualitatively similar to that of **1**. Analogously, methoxybenzyl and butyryltyrosyl moieties have been introduced onto the spermine backbone of **2**, affording **3**, **6**, and **7**. The inclusion of a methoxybenzylamine function in place of the terminal primary amino group of **2** and of **6** affording, respectively, **3** and **7** did not lead to the same improvement in activity observed for the methoctramine backbone at both nAChR and mAChR. This suggests the importance of the chain length separating the nitrogen atoms of a polyamine ligand in binding nicotinic and muscarinic receptors.

The influence on activity at nAChR of the distance between the two inner nitrogen atoms of **1** was investigated with compounds **13**−**21.** The number of carbon atoms in the alkyl chain separating these nitrogens has previously been shown to influence activity at M_2 but not at M_3 mAChR.[1,19] Apparently, the chain length effect was also important for the activity at nAChR (Table 1). Figure 2 shows graphically that optimum activity at nAChR occurred with a chain length of 12 carbon atoms as in **19**, whereas optimum activity at M_2 mAChR was obtained with **1**, which has a chain of eight carbon atoms. The pIC_{50} value for **19** was 250-fold higher than that for **2** and 12-fold higher than that for **1**.

The effect of reductions in the flexiblity of polymethylene tetraamines was studied with compounds **9**−**11**. In **9** the hexamethylene chain between the inner and outer nitrogen atoms of **1** was replaced with a *p*-xylene moiety. This did not influence activity at nAChR and M_3 mAChR (**9** was almost as potent as **1**), but there was a dramatic loss of potency at M_2 mAChR. It follows from these data that changes in flexibility may enable one to design polyamine-containing compounds with specifity for nAChR over mAChR. The tetraamines **10** and **11** contain a bis(4-methylphenyl)methane moiety or a 4,4′ -dimethylbiphenyl moiety, respectively, between the inner nitrogen atoms. Both compounds were similarly potent to **1** at nAChR and M_3 mAChR, but significantly less potent (39-fold and 24-fold, respectively) than **1** at M_2 mAChR. In **10** and **11,** the inner nitrogens are unlikely to be less than 11 Å apart. Since the biphenyl moiety of **11** can only rotate along the axis of the 4,4′-bond without altering the distance between the two amine functions it follows that this distance is important for its interaction with nAChR. Interestingly, this distance corresponds to that separating the inner nitrogens of **1** when it is in its fully extended conformation. This finding, in addition to the observed increase in potency at nAChR when the polyamine chain length is increased up to 12 methylenes, suggests that optimum

Table 1. Antagonist Affinities, Expressed as pIC_{50} or pK_B Values, in the Isolated Frog Rectus Abdominis Muscle (FRA) and Guinea Pig Left Atrium (M_2) and Longitudinal Ileum (M_3) unless Otherwise Specified

							pK_B	
no.[a]	R_1	R_2	R_3	*m*	*n*	pIC_{50} (FRA)	M_2	M_3
1				8		5.93 ± 0.03	7.91 ± 0.03	6.14 ± 0.06
2	H			4	3	4.62 ± 0.03	<5	<5
3	A			4	3	5.14 ± 0.04	<5	<5
4	H			8	6	5.27 ± 0.06	6.01 ± 0.02	5.81 ± 0.10
5	A			8	6	5.82 ± 0.05	7.49 ± 0.09	6.07 ± 0.03
6	A	H	H	4	3	4.60 ± 0.02	<5	<5
7	A	H	A	4	3	4.64 ± 0.01	<5	<5
8	A	H	H	8	6	5.27 ± 0.04	6.76 ± 0.01	5.81 ± 0.01
9						5.75 ± 0.03	5.44 ± 0.01	5.90 ± 0.02
10				0		6.23 ± 0.01	6.53 ± 0.08	5.97 ± 0.09
11				1		5.79 ± 0.01	6.32 ± 0.04	5.63 ± 0.09
12						5.28 ± 0.03	6.12 ± 0.05	6.11 ± 0.02
13				4		5.19 ± 0.07	6.01 ± 0.04	5.49 ± 0.01
14				5		5.22 ± 0.06	6.71 ± 0.15[b]	5.42 ± 0.07[b,c]
15				6		5.64 ± 0.02	6.87 ± 0.18[b]	5.29 ± 0.10[b,c]
16				7		5.61 ± 0.01	7.80 ± 0.08[b]	5.37 ± 0.07[b,c]
17				10		6.10 ± 0.01	7.71 ± 0.20[b]	5.73 ± 0.06[b,c]
18				11		6.35 ± 0.02	7.43 ± 0.08[d]	5.64 ± 0.04[c,d]
19				12		7.02 ± 0.04	7.35 ± 0.09[d]	5.98 ± 0.07
20				13		6.69 ± 0.02	7.02 ± 0.14[d]	5.91 ± 0.04[c,d]
21				14		5.96 ± 0.01	6.87 ± 0.11[d]	5.85 ± 0.04[c,d]
22	B	H	H	8	6	6.39 ± 0.02	7.01 ± 0.09	7.35 ± 0.03
23	H	C	H	8	6	5.60 ± 0.05	8.61 ± 0.01	7.61 ± 0.04
24	H	D	H	8	6	4.92 ± 0.04	7.18 ± 0.02	6.34 ± 0.06
25	A	D	A	8	6	5.90 ± 0.01	8.24 ± 0.04	7.45 ± 0.03

[a] Compounds **1**, **6**–**11**, **13**–**21**, and **25**, tetrahydrochlorides; **2**–**4**, trihydrochlorides; **5**, trioxalate; **12**, tetraoxalate; **22** and **24**, tetratrifluoroacetates; **23**, pentahydrochloride. [b] Data from ref 1. [c] Rat Ileum. [d] Data from ref 19.

potency is reached when two positively charged nitrogens match the distance between two anionic sites on the receptor surface rather than as a result of increasing hydrophobicity.

The tetraamine **12**, which has two terminal 2-methoxybenzylamine moieties incorporated in a rather rigid tricyclic system, was a weaker antagonist than **1** at both nAChR and M_2 mAChR while being similarly potent to **1** at M_3 mAChR. This indicates that the 2-methoxy group and the amine function are forced to assume a position that does not allow them to interact optimally with the receptor or, alternatively, the tricyclic system sterically hinders binding of the ligand to nAChR and M_2 mAChR.

The photoaffinity label MR44 (**22**) proved to be a most promising tool for studying the binding of a polyamine-containing ligand to nAChR. It was 3-fold and 59-fold more potent than **1** and **2**, respectively at nAChR. Studies by Mellor et al.[8] on nAChR of TE671 cells (expressing human, muscle-type nAChR), in which it was possible to determine the voltage dependence of antagonism and, thereby, whether the open channel state of nAChR was antagonized, have shown that **22** interacts exclusively with the closed channel conformation of this receptor. Compound **22** has been used to label nAChR of *Torpedo* electroplax; details of this study are published elsewhere.[29]

Finally, the potencies of branched polyamines (**23**–**25**) at nAChR depended on the substituent on their *N*-propylamino moiety. A comparison of **23** and **24** shows that a derivative containing a butyryltyrosyl substituent was less potent at nAChR and at both M_2 and M_3 mAChR as well. The inclusion of 2-methoxybenzyl groups on the terminal amino functions of **24**,

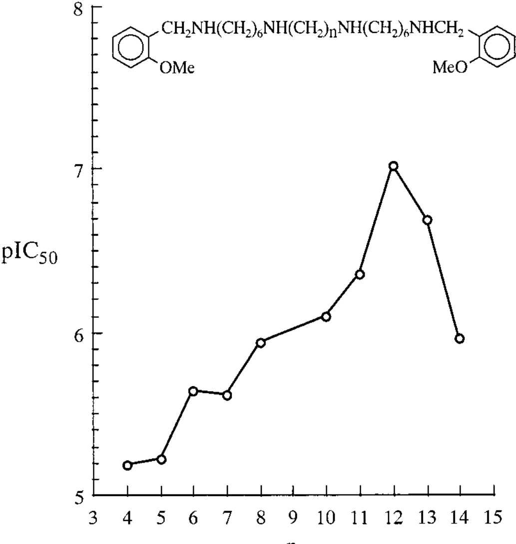

Figure 2. Effect of the carbon chain length of tetraamines **1** and **13**−**21** (n = 4−8 or 10−14) on blocking activity of frog rectus abdominis muscle nicotinic receptors. Data are from Table 1.

to give **25**, resulted, as expected, in a significant improvement in potency at nAChR and M_2 mAChR. However, rather surprisingly, this structural modification resulted in a significant increase in activity also for M_3 mAChR, as **25** was 13-fold and 20-fold more potent than **24** and **1**, respectively. Furthermore, **25** turned out to be almost as potent as **23** at nAChR and at both M_2 and M_3 mAChR as well. The high potency of **23** for M_2 mAChR deserves comment. In previous studies[1−6] tetraamines were shown to display a potency higher than that of **1** only when an appropriate substituent was present on at least one of the two terminal nitrogen atoms of the tetraamine backbone. Now we have demonstrated that this may not be necessary provided that one of the two inner nitrogen atoms carries an appropriate substituent as in **23**. It follows that the affinity for mAChR can be tuned by an appropriate substituent when it is present on any one of the four nitrogen atoms of the tetraamine template.

Philanthotoxins or polyamine amides, like **2**, have more than one antagonistic mode of action on muscle nAChR.[8,9] Polyamine amide **2** acts noncompetitively on the closed channel state of this receptor, although at high concentrations it may also cause open channel block and exhibit competitive antagonism.[11] Furthermore, a recent report from one of our laboratories shows that **2** potentiates nAChR by binding to a high-affinity site on this receptor.[8] This implies that the effect of **2** on this receptor is a balance between potentiation and antagonism. Interestingly, noncompetitive antagonism of neuronal nAChR by **2** is voltage-dependent, suggesting that it is the open channel state of this receptor that is targeted by the toxin.[10] The interactions of polymethylene tetraamines with nAChR may be similarly complex. The question of whether **1** and **2** act at the same site or sites on nAChR can only be addressed by ligand binding studies.

Experimental Section

Chemistry. Melting points were taken in glass capillary tubes on a Büchi SMP-20 apparatus and are uncorrected. IR and NMR spectra were recorded on Perkin-Elmer 297 and Varian VXR 300 instruments, respectively. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS), and spin multiplicities are given as s (singlet), br s (broad singlet), d (doublet), dd (double doublet), t (triplet), dt (double triplet), q (quartet), or m (multiplet). Although IR spectra data are not included (because of the lack of unusual features), they were obtained for all compounds reported and were consistent with the assigned structures. Fast atom bombardment (FAB) and electron impact (EI) mass spectra were obtained on VG707EH-F and VG7070E spectrometers, respectively. The elemental compositions of the compounds agreed to within ±0.4% of the calculated value. When the elemental analysis is not included, crude compounds were used in the next step without further purification. Chromatographic separations were performed on silica gel columns by flash (Kieselgel 40, 0.040−0.063 mm; Merck) or gravity column (Kieselgel 60, 0.063−0.200 mm; Merck) chromatography. Reactions were followed by thin-layer chromatography (TLC) on Merck (0.25 mm) glass-packed precoated silica gel plates (60 F_{254}) that were visualized in an iodine chamber. The term “dried” refers to the use of anhydrous sodium sulfate.

***N*1-{(1*S*)-2-{[6-({8-[(6-Aminohexyl)amino]octyl}amino)hexyl]amino}-1-[4-(benzyloxy)benzyl]-2-oxoethyl}butanamide (30).** A solution of **28**[1] (0.32 g, 0.94 mmol) in methanol (10 mL) was added dropwise with stirring at room temperature to a suspension of **26**[16] (0.36 g, 0.78 mmol) in methanol (10 mL). After 1 h, the reaction mixture was evaporated to give a residue that was purified by flash chromatography. Eluting with $CHCl_3$−MeOH−aqueous 28% ammonia (5:4.5:0.3) gave **30** as an oil: 31% yield; ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3), 1.19−1.82 (complex m, 30), 2.02−2.38 (m, 2 + 4 exchangeable with D_2O), 2.53−2.71 (m, 10), 2.96 (t, 2), 3.14 (q, 2), 4.57 (q, 1), 5.05 (s, 2), 6.16 (br s, 1, exchangeable with D_2O), 6.44 (d, 1, exchangeable with D_2O), 6.91 (d, 2), 7.15 (d, 2), 7.28−7.46 (m, 5).

***N*1-[(1*S*)-2-{[6-({8-[(6-Aminohexyl)amino]octyl}amino)hexyl]amino}-1-(4-hydroxybenzyl)-2-oxoethyl]butanamide Trihydrochloride (4).** A solution of **30** (0.16 g, 0.24 mmol) in methanol (15 mL) was hydrogenated over 10% Pd on charcoal (wet, Degussa type E101 NE/W) (16 mg) for 3 h. Following catalyst removal, the solvent was evaporated, yielding **4** as free base, which was converted into the trihydrochloride salt: 75% yield; mp 159−161 °C; ^{1}H NMR (CD_3OD) δ 0.84 (t, 3), 1.19−1.69 (complex m, 30), 2.15 (t, 2), 2.79−2.99 (m, 10), 3.07−3.14 (m, 2), 3.55−3.67 (m, 2), 4.44 (t, 1), 6.68 (d, 2), 7.04 (d, 2); MS (FAB) calcd for $C_{33}H_{61}N_5O_3$ 576.5 [M + H$^+$], found 576.5.

***N*1-{(1*S*)-1-[4-(Benzyloxy)benzyl]-2-[(6-{[8-({6-[(2-methoxybenzyl)amino]hexyl}amino)octyl]amino}hexyl)amino]-2-oxoethyl}butanamide (31).** It was synthesized from **26** (0.2 g, 0.43 mmol) and **8** (0.2 g, 0.48 mmol) following the procedure described for **30** and purified by flash chromatography. Eluting with $CHCl_3$−MeOH−aqueous 28% ammonia (8.5:1.5:0.15) gave **31** as an oil: 18% yield; ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3), 1.23−1.90 (complex m, 30), 2.15 (t, 2), 2.52−2.67 (complex m, 10), 2.97 (d, 2), 3.15 (q, 2), 3.66 (br s, 3, exchangeable with D_2O), 3.79 (s, 2), 3.84 (s, 3), 4.64 (q, 1), 5.02 (s, 2), 6.71−6.94 (m, 6), 7.25 (d, 2), 7.29−7.43 (m, 5).

***N*1-{(1*S*)-1-(4-Hydroxybenzyl)-2-[(6-{[8-({6-[(2-methoxybenzyl)amino]hexyl}amino)octyl]amino}hexyl)amino]-2-oxoethyl}butanamide Trioxalate (5).** It was obtained from **31** (0.05 g, 0.064 mmol) following the procedure described for **4** and purified by flash chromatography. Eluting with CH_2Cl_2−EtOH−aqueous 28% ammonia (7.5:2.5:0.2) gave the free base that was converted into the trioxalate salt: 68% yield; mp 212−214 °C; ^{1}H NMR (CD_3OD) δ 0.85 (t, 3), 1.27−1.90 (complex m, 30), 2.17 (t, 2), 2.71−3.22 (complex m, 14), 3.94 (s, 3), 4.22 (s, 2), 4.41−4.51 (m, 1), 6.71 (d, 2), 6.92−7.13 (m, 4), 7.40−7.51 (m, 2), 7.88−8.06 (m, 1, exchangeable with D_2O). Anal. ($C_{47}H_{75}N_5O_{16}$) C, H, N.

***N*1-{(1*S*)-1-(4-Hydroxybenzyl)-2-[(3-{[4-({3-[(2-methoxybenzyl)amino]propyl}amino)butyl]amino}propyl)amino]-2-oxoethyl}butanamide Trihydrochlo-**

ride (3). A mixture of **2**[16] (0.03 g, 0.07 mmol), molecular sieves (3 Å), and 2-methoxybenzaldehyde (10.5 mg, 0.07 mmol) in ethanol (10 mL) was stirred for 30 min at room temperature, and then $NaBH_4$ (2.9 mg, 0.07 mmol) was added and the stirring was continued overnight. Following removal of molecular sieves, the solution was made acidic with 6 N HCl (2 mL). Removal of the solvent gave a residue, which was dissolved in water (20 mL). The solution was washed with ether (3 × 20 mL) to remove nonbasic materials and then was made basic with 2 N NaOH, and finally extracted with chloroform (3 × 20 mL). Removal of washed (brine) and dried solvent gave **3** as the free base that was converted into the trihydrochloride salt: quantitative yield; ^1H NMR (CD_3OD) δ 0.85 (t, 3), 1.29−1.80 (complex m, 10), 2.16 (t, 2), 2.40−2.72 (complex m, 10), 2.75−3.01 (m, 2), 3.15 (q, 2), 3.77−3.85 (m, 5), 4.45 (t, 1), 6.68 (d, 2), 6.81−7.09 (m, 4), 7.20−7.29 (m, 2). Anal. ($C_{31}H_{52}Cl_3N_5O_4$) C, H, N.

***N*1-(3-Aminopropyl)-*N*4-{3-[(2-methoxybenzyl)amino]propyl}-1,4-butanediamine Tetrahydrochloride (6).** It was obtained from **27** (1.5 g, 7.41 mmol) and 2-methoxybenzaldehyde (0.1 g, 0.74 mmol) following the procedure described for **3**. The free base was transformed into the tetrahydrocloride salt: 30% yield; mp 233−235 °C (from EtOH); ^1H NMR (D_2O) δ 1.67−1.76 (m, 4), 1.93−2.15 (m, 4), 2.96−3.12 (m, 12), 3.81 (s, 3), 4.21 (s, 2), 6.97−7.12 (m, 2), 7.30−7.52 (m, 2). Anal. ($C_{18}H_{38}Cl_4N_4O$) C, H, N.

***N*1,*N*4-Di{3-[(2-methoxybenzyl)amino]propyl}-1,4-butanediamine Tetrahydrochloride (7).** It was obtained in a quantitative yield from **27** (0.5 g, 2.47 mmol) and 2-methoxybenzaldehyde (0.74 g, 5.43 mmol) following the procedure described for **3**. The free base was transformed into the tetrahydrocloride salt: mp 205−208 °C; ^1H NMR (D_2O) δ 1.67−1.76 (m, 4), 2.03−2.12 (m, 4), 2.96−3.12 (m, 12), 3.85−3.95 (m, 6), 4.18−4.26 (m, 4), 7.02−7.18 (m, 4), 7.32−7.52 (m, 4). Anal. ($C_{26}H_{46}Cl_4N_4O_2$) C, H, N.

***N*1-(*tert*-Butoxycarbonyl)-*N*15-[(benzyloxy)carbonyl]-6-oxo-7-aza-1,15-pentadecanediamine (32) and *N*1-(*tert*-Butoxycarbonyl)-*N*22-[(benzyloxy)carbonyl]-6,17-dioxo-7,16-diaza-1,22-docosanediamine (34).** The procedure used for the synthesis of **32** is described. Ethyl chlorocarbonate (1.72 mL, 18.0 mmol) in dry dioxane (5 mL) was added dropwise to a stirred and cooled (5 °C) solution of *N*-(*tert*-butoxycarbonyl)-6-aminocaproic acid (4.16 g, 18.0 mmol) and triethylamine (2.5 mL, 18.0 mmol) in dioxane (100 mL), followed after standing for 30 min by the addition of *N*1-[(benzyloxy)carbonyl]-1,8-octanediamine[21] (4.9 g, 18.0 mmol) in dioxane (20 mL). After being stirred at room temperature overnight, the mixture was evaporated, affording a residue that was suspended in water (150 mL). The precipitate was filtered and washed with 2 N $KHSO_4$, aqueous $NaHCO_3$ satured solution, and water to give **32**: 80% yield; mp 97−99 °C; ^1H NMR ($CDCl_3$) δ 1.29−1.70 (complex m, 27), 2.12−2.19 (t, 2), 3.08−3.24 (m, 6), 4.54 (br s, 1, exchangeable with D_2O), 4.78 (br s, 1, exchangeable with D_2O), 5.09 (s, 1), 5.49 (br s, 1, exchangeable with D_2O), 7.26−7.36 (m, 5).

Similarly, **34** was obtained from *N*-[(benzyloxy)carbonyl]-6-aminocaproic acid (3.32 g, 12.5 mmol) and **33** (4.70 g, 13.0 mmol): 64% yield; mp 107−109 °C (from THF); ^1H NMR ($CDCl_3$) δ 1.29−1.69 (complex m, 33), 2.15 (t, 4), 3.06−3.25 (m, 8), 4.60 (br s, 1, exchangeable with D_2O), 4.91 (br s, 1, exchangeable with D_2O), 5.08 (s, 2), 5.60 (br s, 2, exchangeable with D_2O), 7.30−7.35 (m, 5).

***N*1-(*tert*-Butoxycarbonyl)-6-oxo-7-aza-1,15-pentadecanediamine (33).** A solution of **32** (7.10 g, 14 mmol) in methanol (110 mL) was hydrogenated over 10% Pd on charcoal (0.7 g) for 45 min at room temperature and a pressure of 15 psi. Following catalyst removal, the solvent was evaporated, affording **33** in a quantitative yield: mp 69−71 °C; ^1H NMR ($CDCl_3$) δ 1.29−1.68 (complex m, 29), 2.15 (t, 2), 2.66 (t, 2), 3.09−3.28 (m, 4), 4.61 (br s, 1, exchangeable with D_2O), 5.58 (br s, 1, exchangeable with D_2O).

***N*1-(*tert*-Butoxycarbonyl)-6,17-dioxo-7,16-diaza-1,22-docosanediamine (35).** It was obtained from **34** (1.85 g, 3.1 mmol) as an oil in a quantitative yield following the procedure described for **33**: ^1H NMR ($CDCl_3$) δ 1.20−1.69 (complex m, 33), 1.88 (br s, 2, exchangeable with D_2O), 2.13−2.16 (m, 4), 2.69 (t, 2), 3.06−3.22 (m, 6), 4.68 (br s, 1, exchangeable with D_2O), 5.82 (br s, 2, exchangeable with D_2O).

***N*1-(2-Methoxybenzyl)-6,17-dioxo-7,16-diaza-1,22-docosanediamine (36).** A solution of **35** (1.41 g, 3.0 mmol) and 2-methoxybenzaldehyde (0.45 g, 3.33 mmol) in toluene (80 mL) was refluxed and the water formed was continuously removed for 3 h. The cooled mixture was filtered and the filtrate was evaporated to give the corresponding Schiff base that was dissolved in ethanol (50 mL) and treated with $NaBH_4$ (0.125 g, 3.33 mmol). The mixture was stirred at room temperature overnight, made acidic with 3 N HCl, and stirred for a further 5 h. The solution was washed with ether (2 × 50 mL) and chloroform (2 × 50 mL), and then it was made basic with 40% NaOH and finally extracted with chloroform. Removal of dried solvents gave **36** as an oil: 67% yield; ^1H NMR ($CDCl_3$) δ 1.30−1.63 (complex m, 27), 2.14−2.20 (m, 4), 2.59 (t, 2), 2.69 (t, 2), 3.18−3.27 (m, 4), 3.78 (s, 2), 3.84 (s, 3), 5.61 (br s, 2, exchangeable with D_2O), 6.86−6.93 (m, 2), 7.22−7.28 (m, 2).

***N*1-(2-Methoxybenzyl)-7,16-diaza-1,22-docosanediamine Tetrahydrochloride (8).** A solution of 10 M $BH_3 \cdot CH_3SCH_3$ (0.09 mL) in dry diglyme (4 mL) was added dropwise at room temperature to a solution of **36** (0.85 g, 1.73 mmol) in dry diglyme (40 mL) with stirring under a stream of dry nitrogen. When the addition was completed, the reaction mixture was heated at 120 °C for 18 h. After cooling at 0 °C, excess borane was destroyed by cautious dropwise addition of MeOH (7 mL). The resulting mixture was left to stand for 4 h at room temperature, cooled at 0 °C, treated with HCl gas for 10 min, and then heated at 120 °C for 4 h. After cooling, ether was added and the resulting mixture was stirred overnight at room temperature, yielding a solid that was filtered: 55% yield; mp 221−223 °C (from EtOH); ^1H NMR (DMSO-d_6) δ 1.29−1.64 (complex m, 28), 2.70−2.81 (complex m, 12), 3.84 (s, 3), 4.07 (m, 2), 6.96−7.11 (m, 2), 7.37−7.54 (m, 2), 8.06 (br s, 2, exchangeable with D_2O), 9.04 (br s, 3, exchangeable with D_2O).

***N*1,*N*8-Di[4-{[*N*-(benzyloxy)carbonyl]aminomethyl}benzyl]octanediamide (37).** It was obtained as a white solid from suberic acid (0.52 g, 2.9 mmol) and benzyl *N*-[4-(aminomethyl)benzyl]carbamate[21] (1.6 g, 5.9 mmol) following the procedure described for **32**: 75% yield; mp 225−226 °C; ^1H NMR (DMSO-d_6) δ 1.18−1.24 (m, 4), 1.41−1.48 (m, 4), 2.08 (t, 4), 4.14 (dd, 4), 5.0 (s, 4), 7.05−7.16 (m, 10), 7.21−7.33 (m, 8), 7.80 (t, 1, exchangeable with D_2O), 8.22 (t, 1, exchangeable with D_2O).

***N*,*N*′-Di[6-{[(benzyloxy)carbonyl]amino}hexyl]-1,1′-biphenyl-4,4′-diyldiamide (38).** It was obtained as a white solid from 4,4′-biphenylcarboxylic acid (0.1 g, 0.42 mmol) and *N*-[(benzyloxy)carbonyl]-1,6-hexanediamine[21] (0.21 g, 0.84 mmol) following the procedure described for **32**: 12% yield; mp 244−246 °C (from DMSO); ^1H NMR (DMSO-d_6) δ 1.20−1.68 (m, 16), 2.90 (dt, 4), 3.25 (dt, 4), 4.98 (s, 4), 7.23 (t, 2), 7.28−7.40 (m, 10), 7.81 (d, 4), 7.91 (d, 4), 8.52 (t, 2).

***N*1,*N*8-Di[4-(aminomethyl)benzyl]octanediamide (39).** A solution of **37** (1.4 g, 2.1 mmol) and 30% HBr in acetic acid (12 mL) in CF_3COOH (30 mL) was stirred for 1 h. Ether (30 mL) was then added, yielding a solid that was dissolved in water. The solution was made basic with NaOH pellets and extracted with chloroform (400 mL) for 10 h. The organic phase was evaporated to give in a quantitative yield **39** as a white solid: mp 240−243 °C; ^1H NMR (DMSO-d_6) δ 1.18−1.28 (m, 4), 1.41−1.52 (m, 4), 2.12 (t, 4), 3.66 (s, 4), 4.22 (s, 4), 7.05−7.16 (m, 10), 7.12−7.28 (m, 8).

***N*,*N*′-Di(6-aminohexyl)-1,1′-biphenyl-4,4′-diyldiamide (40).** It was obtained in a quantitative yield as a foam solid from **38** (0.01 g, 0.10 mmol) as described for **39**. It was not possible to obtain an NMR spectrum owing to the insolubility of the compound. Anal. ($C_{26}H_{38}N_4O_2$) C, H, N.

***N*1,*N*8-Di(4-{[(2-methoxybenzyl)amino]methyl}benzyl)octanediamide (41)**. A mixture of **39** (0.28 g, 0.68 mmol), 2-methoxybenzaldehyde (0.2 g, 1.5 mmol), $NaBH_4$ (56 mg, 1.5

mmol), and molecular sieve (3 Å) in dry methanol (60 mL) was stirred at room temperature for 18 h. After cooling, the mixture was cautiously made acidic with 6 N HCl, filtered, and evaporated. The residue was dissolved in water, and the resulting solution was washed with ether, made basic with 2 N NaOH, and extracted with CH_2Cl_2. Removal of the solvent gave a residue that was purified by flash chromatography. Eluting with CH_2Cl_2−EtOH−aqueous 28% ammonia (9:1:0.1) afforded **41**: 75% yield; mp 152−155 °C; ^{1}H NMR ($CDCl_3$) δ 1.18−1.35 (m, 4), 1.53−1.66 (m, 4), 2.05 (br s, 2, exchangeable with D_2O), 2.16 (t, 4), 3.73−3.82 (d + s, 14), 4.38 (d, 4), 6.00 (t, 2, exchangeable with D_2O), 6.90 (q, 4), 7.19−7.32 (m, 12).

***N,N'*-Di{6-[(2-methoxybenzyl)amino]hexyl}-1,1′-biphenyl-4,4′-diyldiamide (42).** It was obtained as a solid from **40** (0.05 g, 0.11 mmol) and 2-methoxybenzaldehyde (0.05 g, 0.36 mmol) in refluxing MeOH following the procedure described for **41**: 26% yield; mp 164−166 °C; ^{1}H NMR (CD_3OD) δ 1.28−1.77 (m, 16), 2.19 (br s, 2, exchangeable with D_2O), 2.52 (t, 4), 3.45−3.55 (m, 4), 3.82 (s, 4), 3.90 (s, 6), 6.42 (t, 2), 6.88−7.02 (m, 4), 7.23−7.40 (m, 4), 7.69 (d, 4), 7.89 (d, 4).

***N*1,*N*8-Di(4-{[(2-methoxybenzyl)amino]methyl}benzyl)-1,8-octanediamine Tetrahydrochloride (9).** This compound was obtained by reduction of **41** with BH_3·MeSMe as described for **8**: 60% yield; mp > 280 °C (from MeOH/2-PrOH); ^{1}H NMR (DMSO-d_6) δ 1.19−1.38 (m, 4), 1.55−1.75 (m, 4), 2.71−2.93 (m, 4), 3.80 (s, 6), 3.91−4.23 (m, 14), 6.93−7.11 (m, 4), 7.37−7.49 (m, 4) 7.57−7.71 (m, 8), 9.50 (br s, 8, exchangeable with D_2O). Anal. ($C_{40}H_{58}Cl_4N_4O_2$) C, H, N.

***N,N'*-Di{6-[(2-methoxybenzyl)amino]hexyl}-1,1′-biphenyl-4,4′-diyldimethylamine Tetrahydrochloride (11).** This compound was obtained by reduction of **42** with BH_3·MeSMe as described for **8**: 47% yield; mp 245−250 °C (from EtOH/ether); ^{1}H NMR (DMSO-d_6) δ 1.38−1.52 (m, 8), 1.65−1.87 (m, 8), 2.95−3.15 (m, 8), 3.91 (s, 6), 4.20 (s, 4), 4.25 (s, 4), 6.98−7.12 (m, 4), 7.34−7.51 (m, 4), 7.62 (d, 4), 7.76 (d, 4). Anal. ($C_{42}H_{62}Cl_4N_4O_2$) C, H, N.

***N*1-{6-[(Mesitylsulfonyl)amino]hexyl}-*N*1-(2-methoxybenzyl)-2,4,6-trimethyl-1-benzenesulfonamide (44).** A solution of mesitylenesulfonyl chloride (0.41 g, 1.9 mmol) in CH_2Cl_2 (6 mL) was added dropwise to a vigorously stirred solution of **43**[20] dihydrochloride (0.31 g; 1.0 mmol) in 1 N NaOH (6 mL). After the addition was complete, the biphasic mixture was stirred for 24 h (0 °C to room temperature). The layers were separated and the aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL). The combined organic extracts were washed with 0.5 N HCl and then water and evaporated to give **44**: 95% yield; mp 125−126 °C; ^{1}H NMR ($CDCl_3$) δ 0.92−1.52 (complex m, 8), 2.33 (s, 6), 2.65 (s, 6), 2.66 (s, 6), 2.84 (q, 2), 3.09 (t, 2), 3.73 (s, 3), 4.38 (s, 2), 4.57 (t, 1, exchangeable with D_2O), 6.81−6.92 (m, 2), 6.97 (d, 4), 7.09 (d, 1), 7.25 (t, 1).

***N*1-{6-[(Mesitylsulfonyl)(4-{4-[((mesitylsulfonyl){6-[(mesitylsulfonyl)(2-methoxybenzyl)amino]hexyl}amino)methyl]benzyl}benzyl)amino]hexyl}-*N*1-(2-methoxybenzyl)-2,4,6-trimethyl-1-benzenesulfonamide (45)**. Sodium hydride (60% in mineral oil, 16 mg, 0.38 mmol) was added to a solution of **44** (0.16 g, 0.27 mmol) in dry DMF (15 mL). The resulting suspension was stirred for 1 h at room temperature under nitrogen. A solution of di[(*p*-bromomethyl)phenyl]methane[23] (46 mg, 0.13 mmol) in dry DMF (5 mL) was added dropwise and the reaction mixture was heated at 85 °C for 20 h. After cooling to 0 °C, H_2O (30 mL) was cautiously added, followed by extraction with CH_2Cl_2 (3 × 30 mL). Removal of the solvent gave a residue that was purified by flash chromatography. Eluting with cyclohexanes−EtOAc (8:2) afforded **45** as an oil: 70% yield; ^{1}H NMR ($CDCl_3$) δ 0.92−1.52 (complex m, 8), 2.33 (s, 6), 2.65 (s, 6), 2.66 (s, 6), 2.84 (q, 2), 3.09 (t, 2), 3.73 (s, 3), 4.38 (s, 2), 4.57 (t, 1, exchangeable with D_2O), 6.81−6.92 (m, 2), 6.97 (d, 4), 7.09 (d, 1), 7.25 (t, 1).

***N*1-(2-Methoxybenzyl)-*N*6-(4-{4-[({6-[(2-methoxybenzyl)amino]hexyl}amino)methyl]benzyl}benzyl)-1,6-hexanediamine Tetrahydrochloride (10).** HBr in acetic acid (30%; 4 mL) was added to a solution of **45** (72 mg, 0.09 mmol) and phenol (0.3 g, 0.4 mmol) in CH_2Cl_2 (4 mL) at 0 °C. The reaction mixture was stirred overnight (0 °C to room temperature). Water was added, followed by extraction with ether. The aqueous layer was made basic with 2 N NaOH and extracted with CH_2Cl_2. Removal of the dried solvent gave crude **10** that was transformed into the tetrahydrochloride salt: 70% yield; mp 187−191 °C (from EtOH/MeOH); ^{1}H NMR ($CDCl_3$) δ 1.05−1.60 (complex m, 16), 2.17 (br s, 4, exchangeable with D_2O), 2.58 (t, 8), 3.73 (s, 6), 3.81 (s, 8), 3.95 (s, 2), 6.89 (q, 4), 7.08−7.40 (m, 12). Anal. ($C_{48}H_{74}Cl_4N_4O_2$) C, H, N.

***cis*-1-Benzyl-1,2,3,3a,4,9b-hexahydrochromeno[4,3-*b*]pyrrole Hydrochloride (46).** A mixture of *N*-benzylglycine hydrochloride (2.4 g, 11.9 mmol) and chlorotrimethylsilane (15 mL, 118 mmol) was gently refluxed under nitrogen for 3 h. After cooling, the formed solid was collected by filtration and washed with dry ether. To a suspension of this solid in dry toluene (70 mL) were added diisopropylethylamine (2.77 mL, 15.9 mmol) and 2-allyloxybenzaldehyde (0.41 mL, 2.7 mmol), and the reaction mixture was heated under reflux and the water formed was continuously removed for 18 h. Removal of the solvent gave an oil that was partitioned between aqueous $NaHCO_3$−CH_2Cl_2. The organic phase was dried and evaporated to give a residue that was purified by flash chromatography. Elution with a gradient of 0−2% MeOH−CH_2Cl_2 afforded **46** as the free base that was transformed in a quantitative yield into the hydrochloride salt: mp 175−176 °C (from 2-PrOH/ether); ^{1}H NMR (CD_3OD) δ 1.95−2.15 (m, 1), 2.44−2.56 (m, 1), 3.00−3.19 (m, 1), 3.30−3.52 (m, 2), 4.10−4.14 (m, 2), 4.50−4.57 (m, 2), 4.80−4.97 (m, 2), 6.93−7.10 (m, 2), 7.23−7.42 (m, 2), 7.45−7.60 (m, 5).

***cis*-1,2,3,3a,4,9b-Hexahydrochromeno[4,3-*b*]pyrrole (47)**. A solution of **46** (0.31 g, 1.03 mmol) in MeOH (15 mL) was hydrogenated over 10% Pd on charcoal (wet, Degussa type E101 NE/W) (250 mg) overnight at 40 °C and a pressure of 75 psi. Following catalyst removal, the solvent was evaporated to give a residue that was purified by flash chromatography. Eluting with CH_2Cl_2−EtOH−aqueous 28% ammonia (9.1:1.0:0.01) afforded **47**: 85% yield; mp 210−215 °C (HCl salt from EtOH/ether); ^{1}H NMR ($CDCl_3$) δ 1.40−1.60 (m, 1), 2.00−2.20 (m, 1), 2.27 (br s, 1, exchangeable with D_2O) 2.38−2.58 (m, 1), 2.82−3.15 (m, 2), 3.52 (t, 1), 3.95 (d, 1) 4.03−4.11 (m, 1), 4.80−4.97 (m, 2), 6.83−6.95 (m, 2), 7.15 (t, 1), 7.36 (d, 1).

Benzyl *N*-{6-[*cis*-1,2,3,3a,4,9b-Hexahydrochromeno-[4,3-*b*]pyrrol-1-yl]-6-oxohexyl}carbamate (48). It was synthesized from **47** (0.45 g, 2.57 mmol) and *N*-[(benzyloxy)carbonyl]-6-aminocaproic acid following the procedure reported for **32** and purified by flash chromatography. Elution with a step gradient system of EtOAc−cyclohexane (5:5) and CH_2Cl_2−EtOAc−EtOH (8.8:1:0.2) gave **48** as a clear oil: 70% yield; ^{1}H NMR ($CDCl_3$) δ 1.38−1.72 (complex m, 6), 1.93−2.59 (complex m, 5), 3.14 (q, 2) 3.35−3.52 (m, 2), 4.06−4.19 (m, 2), 5.04 (br s, 2 + 1 exchangeable with D_2O), 5.52 (d, 1), 6.71 (d, 1), 6.83 (t, 1), 7.04 (t, 1), 7.16−7.35 (m, 5), 7.64 (d, 1).

1-[*cis*-1,2,3,3a,4,9b-Hexahydrochromeno[4,3-*b*]pyrrol-1-yl]-6-amino-1-hexanone (49). It was synthesized from **48** (0.76 g, 1.8 mmol) following the procedure described for **33**. The crude compound was taken up in 2 N HCl, and the resulting solution was washed with ether, made basic with 2 N NaOH, and extracted with CH_2Cl_2. Removal of the dried solvents gave **49** as a clear oil: 93% yield; ^{1}H NMR ($CDCl_3$) δ 1.32−1.74 (complex m, 6 + 2 exchangeable with D_2O), 1.93−2.68 (complex m, 7), 3.39−3.50 (m, 2), 4.06−4.21 (m, 2), 5.54 (d, 1), 6.71 (d, 1), 6.82 (t, 1), 7.07 (t, 1), 7.63 (d, 1).

6-[*cis*-1,2,3,3a,4,9b-Hexahydrochromeno[4,3-*b*]pyrrol-1-yl]-1-hexanamine (50). It was synthesized from **49** (0.48 g, 1.66 mmol) following the procedure described for **8**: 75% yield; mp 192−195 °C (HCl salt from EtOH/ether); ^{1}H NMR ($CDCl_3$) δ 1.20−1.56 (complex m, 8 + 2 exchangeable with D_2O), 1.90−2.45 (complex m, 7), 2.63 (t, 2), 2.91−3.19 (m, 3), 3.50−3.75 (m, 1), 3.92−4.04 (m, 2), 6.81−6.93 (m, 2), 7.10−7.24 (m, 2).

***N*1,*N*8-Di[6-(*cis*-1,2,3,3a,4,9b-hexahydrochromeno[4,3-*b*]pyrrol-1-yl)-1-hexyl]octanediamide (51).** It was synthesized from **50** (0.34 g, 1.24 mmol) and suberic acid (0.11 g, 0.62 mmol) following the procedure described for **32** and purified by gravity chromatography. Elution with CH_2Cl_2−

MeOH–aqueous 28% ammonia (9.4:0.6:0.06) gave **51** as a clear oil: 45% yield; mp 175–180 °C (oxalate salt from EtOH/ether); ^{1}H NMR ($CDCl_3$) δ 1.08–1.70 (complex m, 24), 1.83–2.27 (complex m, 12), 2.30–2.44 (m, 2), 2.90–3.25 (m, 10), 3.85–4.05 (m, 4), 5.45–5.57 (m, 2, exchangeable with D_2O), 6.78–6.93 (m, 4), 7.25–7.50 (m, 4).

***N*1,*N*8-Di[6-(*cis*-1,2,3,3a,4,9b-hexahydrochromeno[4,3-*b*]pyrrol-1-yl)-1-hexyl]-1,8-octanediamine Tetraoxalate (12).** It was synthesized from **51** (0.09 g, 0.13 mmol) following the procedure described for **8** and transformed into the tetraoxalate salt: 70% yield; mp 192–195 °C (EtOH/MeOH); ^{1}H NMR (CD_3OD) δ 1.32–1.45 (complex m, 16), 1.60–1.83 (m, 12),1.93–2.10 (m, 2), 2.40–2.57 (m, 2), 2.94–3.04 (m, 12), 3.20–3.34 (m, 2), 3.47–3.56 (m, 2), 3.61–3.68 (m, 2), 4.00 (dt, 2), 4.15 (dd, 2), 4.65 (d, 2), 6.97 (d, 2), 7.07 (t, 2), 7.36 (t, 2), 7.49 (d, 2). Anal. ($C_{50}H_{74}N_4O_{18}$) C, H, N.

***N*1,*N*4-Di(6-{[(benzyloxy)carbonyl]amino}caproyl)-1,4-butanediamine (52).** It was synthesized from 1,4-butanediamine (0.30 g, 3.36 mmol) and *N*-[(benzyloxy)carbonyl]-6-aminocaproic acid following the procedure described for **32**: 75% yield; mp 165–166 °C; ^{1}H NMR (DMSO-d_6) δ 1.18–1.24 (m, 4), 1.30–1.48 (m, 12), 1.99 (t, 4), 2.92–3.11 (m, 8), 4.97 (s, 4), 7.18–7.33 (m, 10 + 2 exchangeable with D_2O), 7.73 (t, 2, exchangeable with D_2O).

***N*1,*N*4-Di(6-aminocaproyl)-1,4-butanediamine Dihydrobromide (53).** It was synthesized in quantitative yield from **52** (1.40 g, 2.4 mmol) and 30% HBr in acetic acid (20 mL) following the procedure described for **39**: mp 204–205 °C; ^{1}H NMR (DMSO-d_6) δ 1.13–1.60 (m, 16), 2.03 (t, 4), 2.65–2.75 (m, 4), 2.93–3.12 (m, 4), 7.78 (br s, 8, exchangeable with D_2O).

***N*1,*N*4-Di[6-(2-methoxybenzyl)amino}caproyl]-1,4-butanediamine (54).** It was synthesized from **53** (0.69 g, 2.19 mmol) and 2-methoxybenzaldehyde (0.66 g, 4.83 mmol) following the procedure described for **41**: 80% yield; mp 78–80 °C; ^{1}H NMR ($CDCl_3$) δ 1.12–1.69 (m, 16), 1.80 (br s, 2, exchangeable with D_2O), 2.13 (t, 4), 2.60 (t, 4), 3.18 (q, 4), 3.74 (s, 4), 3.81 (s, 6), 6.25 (t, 2, exchangeable with D_2O), 6.87 (q, 4), 7.17–7.27 (m, 4).

***N*1,*N*4-Di[6-{(2-methoxybenzyl)amino}hexyl]-1,4-butanediamine Tetrahydrochloride (13).** This compound was obtained by reduction of **53** with BH_3·MeSMe following the procedure described for **8**: 60% yield; mp 240 °C (from EtOH/MeOH); ^{1}H NMR (DMSO-d_6) δ 1.22–1.40 (m, 8), 1.55–1.79 (m, 12), 2.73–2.95 (m, 12), 3.85 (s, 6), 4.08 (s, 4), 6.93–7.12 (m, 4), 7.35–7.49 (m, 4) 7.57–7.71 (m, 8), 9.10 (br s, 8, exchangeable with D_2O). Anal. ($C_{36}H_{58}Cl_4N_4O_2$) C, H, N.

3-{[6-({8-[(6-Aminohexyl)amino]octyl}amino)hexyl]amino}propanenitrile Tetrahydrochloride (55) and 3-((6-Aminohexyl){8-[(6-aminohexyl)amino]octyl}amino)propanenitrile Tetrahydrochloride (56). A solution of acrylonitrile (0.58 mL, 8.86 mmol) in MeOH (3 mL) was added dropwise to a cooled (0 °C) and stirred solution of **28** (3.04 g, 8.86 mmol) in MeOH (10 mL). Stirring and cooling were continued for 8 h, and then removal of the solvent gave a complex mixture of compounds that was purified by flash chromatography. Elution with a step gradient system of $CHCl_3$–MeOH–aqueous 28% ammonia (5:4.5:0.5 to 5:4.5:0.7) afforded a main fraction that was transformed into the tetrahydrochloride salts. They were separated by fractional crystallization from MeOH. The precipitate was **55**: 17% yield; R_f 0.48 [eluent system $CHCl_3$–MeOH–aqueous 28% ammonia (5:4.5:1)]; mp 280 °C (dec); ^{1}H NMR (free base) ($CDCl_3$) δ 1.25–1.63 (complex m, 33), 2.49–2.69 (m, 14), 2.91 (t, 2); EI MS *m*/*z* 395 (M^+).

Evaporation of mother liquor afforded **56** as an oil: 15% yield; R_f 0.42 [eluent system $CHCl_3$–MeOH–aqueous 28% ammonia (5:4.5:1)]; ^{1}H NMR (free base) ($CDCl_3$) δ 1.27–1.46 (complex m, 33), 2.40–2.45 (m, 6), 2.58–2.82 (complex m, 10); EI MS *m*/*z* 395 (M^+).

3-[(6-{[8-({6-[(2-Methoxybenzyl)amino]hexyl}amino)octyl]amino}hexyl)amino]propanenitrile (57). It was obtained from **55** (0.19 g, 0.48 mmol) and 2-methoxybenzaldehyde (0.131 g, 1.06 mmol) following the procedure described for **3** as an oil: 90% yield; ^{1}H NMR ($CDCl_3$) δ 1.22–1.41 (complex m, 32), 2.40–2.56 (m, 14), 2.83 (t, 2), 3.69 (s, 2), 3.75 (s, 3), 6.77–6.87 (m, 2), 7.12–7.19 (m, 2).

3-{{6-[(2-Methoxybenzyl)amino]hexyl}[8-({6-[(2-methoxybenzyl)amino]hexyl}amino)octyl]amino}propanenitrile (58). It was obtained from **56** (0.22 g, 0.55 mmol) and 2-methoxybenzaldehyde (0.150 g, 1.21 mmol) following the procedure described for **3** as an oil: 70% yield; ^{1}H NMR ($CDCl_3$) δ 1.29–1.69 (complex m, 31), 2.35–2.42 (m, 6), 2.53–2.56 (m, 8), 2.75 (t, 2), 3.76 (s, 4), 3.82 (s, 6), 6.82–6.92 (m, 4), 7.17–7.28 (m, 4).

3-{{6-[(*tert*-Butoxycarbonyl)amino]hexyl}[8-(*tert*-butoxycarbonyl)({6-[(*tert*-butoxycarbonyl)amino]hexyl}amino)octyl]amino}propanenitrile (60). A solution of **56** as free base (0.1 g, 0.25 mmol) in chloroform (10 mL) and di-*tert*-butyl dicarbonate (0.182 g, 0.83 mmol) was stirred overnight and then washed with aqueous satured $NaHCO_3$ solution (2 × 30 mL) and brine (2 × 30 mL). Removal of dried solvents gave in a quantitative yield **60** as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.20–1.51 (complex m, 55), 2.40 (t, 6), 2.76 (t, 2), 3.04–3.10 (m, 8), 4.62 (br s, 2, exchangeable with D_2O).

***N*1,16,*N*22-Tri(*tert*-butoxycarbonyl)-7-(3-aminopropyl)-7,16-diaza-1,22-docosanediamine (62).** A suspension of **60** (0.95 g, 1.36 mmol), aqueous 40% NaOH (0.75 mL) and Raney Ni (nickel sponge; suspension in water) (0.25 g) in EtOH (15 mL) was hydrogenated for 28 h at room temperature and a pressure of 15 psi. Following catalyst removal, the solvent was evaporated, yielding a residue that was dissolved in water (50 mL) and extracted with $CHCl_3$ (3 × 20 mL). Removal of dried solvents gave **62** as an oil: 90% yield; ^{1}H NMR ($CDCl_3$) δ 1.12–1.43 (complex m, 55), 1.60 (t, 2), 2.31 (t, 4), 2.44 (t, 4), 2.80 (t, 2), 2.98–3.08 (m, 8), 4.70 (br s, 2, exchangeable with D_2O).

***N*1,*N*8-Di(6-aminohexyl)-*N*1-{3-[(2-methoxybenzyl)amino]propyl}-1,8-octanediamine Pentahydrochloride (23).** A mixture of **62** (0.1 g, 0.15 mmol), 2-methoxybenzaldehyde (0.023 g, 0.17 mmol), $NaBH_4$ (7 mg, 0.17 mmol) and molecular sieve (3 Å) in dry ethanol (15 mL) was stirred at room temperature for 18 h. After cooling, the mixture was cautiously made acidic with 3 N HCl and stirred for 2 h at room temperature, then filtered and evaporated. The residue was dissolved in water, and the resulting solution was washed with ether, made basic with 2 N NaOH, and extracted with CH_2Cl_2. Removal of the dried solvents gave **23** as an oil: 25% yield; ^{1}H NMR ($CDCl_3$) δ 1.27–1.51 (complex m, 36), 2.32–2.47 (m, 6), 2.54–2.69 (m, 10), 3.77 (s, 2), 3.83 (s, 3), 6.81–6.94 (m, 2), 7.19–7.28 (m, 2). It was transformed into the pentahydrochloride salt as a hygroscopic solid. Anal. ($C_{31}H_{66}Cl_5N_5O$) C, H, N.

***N*1-{(1*S*)-2-{[3-((6-[*tert*-Butoxycarbonyl]aminohexyl){8-[(6-(*tert*-butoxycarbonyl]aminohexyl)amino]octyl}amino)propyl]amino}-1-[4-(benzyloxy)benzyl]-2-oxoethyl}butanamide (64)**. It was obtained from **26** (0.28 g, 0.6 mmol) and **62** (0.42 g, 0.6 mmol) following the procedure described for **30** and purified by flash chromatography. Elution with CH_2Cl_2–MeOH (9:1) gave **64** as an oil: 65% yield; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3), 1.24–1.45 (complex m, 57), 1.58–1.62 (m, 2), 2.17 (t, 2), 2.38–2.50 (m, 6), 2.96 (t, 2), 3.05–3.16 (m, 8), 3.20–3.26 (m, 2), 4.52–4.59 (m, 1), 4.75 (br s, 1, exchangeable with D_2O), 5.00 (s, 2), 6.28 (br s, 1, exchangeable with D_2O), 6.85 (d, 2), 7.08 (d, 2), 7.23 (br s, 1, exchangeable with D_2O), 7.30–7.38 (m, 5).

***N*1-{(1*S*)-2-{[3-((6-Aminohexyl){8-[(6-aminohexyl)amino]octyl}amino)propyl]amino}-1-[4-(benzyloxy)benzyl]-2-oxoethyl}butanamide Tetratrifluoroacetate (65)**. A solution of **64** (0.39 g, 0.39 mmol) and CF_3COOH (0.9 mL) in dry CH_2Cl_2 (10 mL) was stirred at room temperature for 2 h. Removal of the solvent gave **65** in a quantitative yield: ^{1}H NMR (CD_3OD) δ 0.83 (t, 3), 1.38–1.85 (complex m, 32), 2.16 (t, 2), 2.80–3.08 (m, 16), 3.14–3.24 (m, 2), 4.42 (t, 1), 5.02 (s, 2), 6.91 (d, 2), 7.16 (d, 2), 7.28–7.41 (m, 5).

***N*1-[(1*S*)-2-{[3-((6-Aminohexyl){8-[(6-aminohexyl)amino]octyl}amino)propyl]amino}-1-(4-hydroxybenzyl)-2-oxoethyl]butanamide Tetratrifluoroacetate (24).** It was obtained in a quantitative yield as a hygroscopic solid from **65** (0.39 g, 0.39 mmol) following the procedure described for **4**.

^{1}H NMR (CD_3OD) δ 0.85 (t, 3), 1.38−1.85 (complex m, 32), 2.16 (t, 2), 2.80−3.08 (m, 16), 3.18−3.24 (m, 2), 4.38 (t, 1), 6.70 (d, 2), 7.05 (d, 2). Anal. ($C_{44}H_{72}F_{12}N_6O_{11}$) C, H, N.

***N*1-{(1*S*)-1-(4-Hydroxybenzyl)-2-[(3-{{6-[(2-methoxybenzyl)amino]hexyl}[8-({6-[(2-methoxybenzyl)amino]hexyl}amino)octyl]amino}propyl)amino]-2-oxoethyl}-butanamide Tetrahydrochloride (25).** It was obtained from **24** (free base, 0.14 g, 0.22 mmol) following the procedure described for **3** and purified by flash chromatography. Elution with a step gradient system of $CHCl_3$−MeOH−aqueous 28% ammonia (9:1:0.1 to 8:2:0.17) gave **25** as the free base that was converted into the tetrahydrochloride salt as a hygroscopic solid: 72% yield; ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3), 1.24−1.46 (complex m, 30), 1.61 (q, 2), 2.14 (t, 2), 2.18−2.31 (m, 6), 2.50−2.59 (m, 8), 2.70−3.05 (m, 2), 3.10−3.30 (m, 2), 3.75−3.81 (m, 10), 4.33−4.48 (m, 1), 6.35 (d, 1, exchangeable with D_2O), 6.63 (d, 2), 6.83−7.01 (m, 6), 7.18−7.27 (m, 4). Anal. ($C_{52}H_{88}Cl_4N_6O_5$) C, H, N.

3,11,20,*N*26-Tetra(*tert*-butoxycarbonyl)-4,11,20-triaza-1,26-hexacosanediamine (61). Compound **59** was obtained in a quantitative yield as a yellow oil from **55** as free base (0.60 g, 1.51 mmol) and di-*tert*-butyl dicarbonate (1.45 g, 6.64 mmol) following the procedure described for **60**: ^{1}H NMR (free base) ($CDCl_3$) δ 1.08−1.53 (complex m, 64), 2.43−2.59 (m, 2), 2.94−3.27 (complex m, 12), 3.40 (t, 2), 4.58 (br, 1, exchangeable with D_2O).

A solution of **59** (1.15 g, 1.45 mmol) in anhydrous ether (30 mL) was added to a stirred and cooled (0 °C) suspension of $LiAlH_4$ (0.2 g, 5.33 mmol) in anhydrous ether (100 mL) under a nitrogen atmosphere. After 30 min of stirring, 1 N NaOH (30 mL) was added to the mixture to destroy the excess of $LiAlH_4$. Following filtration through Celite, the organic phase was separated and the aqueous phase was extracted with ether (2 × 40 mL). Combined organic layers were washed with 5% citric acid (2 × 20 mL) and brine (2 × 20 mL). Removal of the dried solvent gave a residue that was purified by flash column. Elution with $CHCl_3$−MeOH (9:1) afforded **61** as an oil: 25% yield; ^{1}H NMR (free base) ($CDCl_3$) δ 1.15−1.67 (complex m, 66), 1.71−1.82 (m, 2), 2.67 (t, 2), 3.01−3.29 (complex m, 14), 4.59 (br s, 1, exchangeable with D_2O).

***N*1-(3-{[6-({8-[(6-Aminohexyl)amino]octyl}amino)hexyl]-amino}propyl)-4-azido-2-hydroxybenzamide Tetratrifluoroacetate (22).** A solution of 4-azidosalicylic acid *N*-hydroxysuccinimide ester[26] (0.08 g, 0.29 mmol) in dry chloroform (5 mL) was added dropwise to a stirred solution of **61** (0.21 g, 0.26 mmol) in distilled chloroform (10 mL). After stirring for 2 h at room temperature, the solvent was evaporated to give a residue that was purified by flash chromatography. Eluting with petroleum ether−acetone (7.5:2.5) afforded **63** as an oil: 0.1 g (40% yield);^{1}H NMR (free base) ($CDCl_3$) δ 1.24−1.47 (complex m, 64), 1.69−1.77 (m, 2), 3.10−3.18 (m, 12), 3.35−3.39 (m, 4), 4.61 (br s, 1, exchangeable with D_2O), 6.49−6.59 (m, 2), 7.62 (d, 2), 8.33−8.43 (br s, 1, exchangeable with D_2O).

CF_3COOH (0.25 mL) was added to a stirred solution of **63** (0.1 g, 0.10 mmol) in dry CH_2Cl_2 (3 mL) under a nitrogen atmosphere. After stirring for 2 h at room temperature, the solvent was removed to give an oil that was triturated with ether (3 × 30 mL) to remove the excess CF_3COOH, yielding in a quantitative yield **22** as a foam solid: ^{1}H NMR (CD_3OD) δ 1.27−1.34 (complex m, 16), 1.53−1.60 (m, 12), 1.82−1.93 (m, 2), 2.80−2.94 (complex m, 14), 3.40 (t, 2), 6.45−6.50 (m, 2), 7.68 (d, 2); MS (FAB) calcd for $C_{30}H_{56}N_8O_2$ 561.8 [M + H$^+$], found 561.8.

Biology. Functional Antagonism: Guinea pigs of either sex (200−400 g) and frogs (10−20 g) were sacrificed under ketamine or ether anesthesia, respectively, and the organs required were set up rapidly under a suitable resting tension in 15-mL organ baths containing physiological salt solution kept at appropriate temperature (see below) and aerated with 5% CO_2−95% O_2 at pH 7.4. Concentration−response curves were constructed by cumulative addition of the agonist. The concentration of agonist in the organ bath was increased approximately 5-fold at each step, with each addition being made only after the response to the previous addition had attained a maximal level and remained steady. Contractions were recorded by means of a force displacement transducer (FT.03 Grass and 7003 Basile) connected to a four-channel pen recorder (Battaglia-Rangoni KV 380). In all cases, parallel experiments in which tissues did not receive any antagonist were run in order to check for variations in sensitivity.

Guinea Pig Left Atria: The guinea pig heart was rapidly removed and washed by perfusion through the aorta with oxygenated physiological salt solution, and right and left atria were separated out. The left atria were mounted under 0.2−0.3 g tension at 35 °C in Tyrode solution of the following composition (mM): NaCl, 136.9; KCl, 5.4; $MgSO_4 \cdot 7H_2O$, 1.0; $CaCl_2$, 2.52; NaH_2PO_4, 0.4; $NaHCO_3$, 11.9; glucose, 5.5. Tissues were stimulated through platinum electrodes by square-wave pulses (0.6−0.8 ms, 1 Hz, 1−5 V). Inotropic activity was recorded isometrically. Tissues were equilibrated for 1 h, and cumulative concentration−response curves to arecaidine propargyl ester (APE) (0.01−1 μM) were constructed. Following incubation with the antagonist for 60 min, a new concentration−response curve to APE was obtained.

Guinea Pig Ileum Longitudinal Muscle: The terminal portion of the ileum was excised after the 8−10 cm nearest to the ileo−caecal junction was discarded. The tissue was cleaned, and segments 2−3 cm long of ileum longitudinal muscle were separated from the underlying circular muscle and set up under 1*g* tension at 37 °C in organ baths containing Tyrode solution of the following composition (mM): NaCl, 118; KCl, 4.75; $CaCl_2$, 2.54; $MgSO_4$, 1.2; $KH_2PO_4 \cdot 2H_2O$, 1.19; $NaHCO_3$, 25; glucose, 11. Tension changes were recorded isotonically. Tissues were allowed to equilibrate for at least 30 min during which time the bathing solution was changed every 10 min. Concentration−response curves to APE (0.01−0.5 μM) were obtained at 30-min intervals, the first one being discarded and the second one taken as control. Following incubation with the antagonist for 60 min, a new concentration−response curve to the agonist was obtained.

Frog Rectus Abdominis Muscle: The rectus abdominis muscle of frogs was set up at room temperature in Clark frog Ringer solution of the following composition (mM): NaCl, 111; KCl, 1.88; $CaCl_2$, 1.08; NaH_2PO_4, 0.08; $NaHCO_3$, 2.38; glucose, 11.1. The tissues were equilibrated under 1*g* tension for 60 min. Two cumulative concentration−response curves to carbachol (1−100 μM) were constructed at 45 min intervals, the first one being discarded and the second one taken as control. Following incubation with the antagonist for 30 min a new concentration−response curve to the agonist was obtained.

Data Analysis: Antagonism of mAChR, expressed as pK_B values, was estimated according to the Furchgott equation: K_B = [antagonist]/(DR − 1), where DR is the ratio between individual EC_{50} values in the presence and in the absence of antagonists.[27] The potency of the agonist, i.e., the concentration resulting in 50% of the maximum response (EC_{50}), was estimated graphically from the individual concentration−response curves after checking for parallelism of the curves. Antagonism of nAChR was estimated by determining the concentration of the noncompetitive antagonist that inhibited 50% of the maximum response to the agonist. Three different antagonist concentrations were used and each concentration was tested at least four times. Data were analyzed by a pharmacological computer program.[30]

Acknowledgment. This research was supported by grants from the University of Bologna, the European Community (BMH4-CT97-2395), and MURST.

References

(1) Melchiorre, C.; Cassinelli, A.; Quaglia, W. Differential Blockade of Muscarinic Receptor Subtypes by Polymethylene Tetraamines. Novel Class of Selective Antagonists of Cardiac M-2 Muscarinic Receptors. *J. Med. Chem.* **1987**, *30*, 201−204.
(2) (a) Melchiorre, C. Polymethylene Tetraamines: a New Generation of Selective Muscarinic Antagonists. *Trends Pharmacol. Sci.* **1988**, *9*, 216−220. (b) Melchiorre, C.; Minarini, A.; Angeli, P.; Giardinà, D.; Gulini, U.; Quaglia, W. Polymethylene Tet-

raamines as Muscarinic Receptor Probes. *Trends Pharmacol. Sci.* **1989**, Suppl. IV, 55−59. (c) Melchiorre, C. Polymethylene Tetraamines: a Novel Class of Cardioselective M_2 Antagonists. *Med. Res. Rev.* **1990**, *3*, 327−349.

(3) (a) Melchiorre, C. Tetramine Disulfides: a New Tool in α-Adrenergic Pharmacology. *Trends Pharmacol. Sci.* **1981**, *2*, 209−211. (b) Melchiorre, C.; Recanatini, M.; Bolognesi, M. L.; Filippi, P.; Minarini, A. Polyamines: a Case of Multiple Yet Specific Receptor Recognition. *Curr. Top. Med. Chem.* **1993**, *1*, 43−65.

(4) (a) Melchiorre, C.; Bolognesi, M. L.; Chiarini, A.; Minarini, A.; Spampinato, S. Synthesis and Biological Activity of Some Methoctramine-Related Tetraamines Bearing an 11-Acetyl-5,-11-dihydro-6*H*-pyrido[2,3-*b*][1,4]-benzodiazepin-6-one Moiety as Antimuscarinics: a Second Generation of Highly Selective M_2 Muscarinic Receptor Antagonists. *J. Med. Chem.* **1993**, *36*, 3734−3737. (b) Minarini, A.; Bolognesi, M. L.; Budriesi, R.; Canossa, M.; Chiarini, A.; Spampinato, S.; Melchiorre, C. Design, Synthesis, and Biological Activity of Methoctramine-Related Tetraamines Bearing an 11-Acetyl-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one Moiety: Structural Requirements for Optimum Occupancy of Muscarinic Receptor Subtypes As Revealed by Symmetrical and Unsymmetrical Polyamines. *J. Med. Chem.* **1994**, *37*, 3363−3372.

(5) Melchiorre, C.; Angeli, P.; Brasili, L.; Giardinà, D.; Pigini, M.; Quaglia, W. Polyamines: a Possible "Passe-Partout" for Receptor Characterization. *Actual. Chim. Thér. − 15e série* **1988**, 149−168.

(6) Bolognesi, M. L.; Minarini, A.; Budriesi, R.; Cacciaguerra, S.; Chiarini, A.; Spampinato, S.; Tumiatti, V.; Melchiorre, C. Universal Template Approach to Drug Design: Polyamines as Selective Muscarinic Receptor Antagonists. *J. Med. Chem.* **1998**, *41*, 4150−4160.

(7) Eldefrawi, A. T.; Eldefrawi, M. E.; Konno, K.; Mansour, N. A.; Nakanishi, K.; Oltz, E.; Usherwood, P. N. R. Structure and Synthesis of a Potent Glutamate Receptor Antagonist in Wasp Venom. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4910−4913.

(8) Mellor, I. R.; et al. Unpublished results.

(9) Jayaraman, V.; Usherwood, P. N. R.; Hess, G. P. Inhibition of the Nicotinic Acetylcholine Receptor by Philanthotoxin-343: Kinetic Investigations in the Microsecond Time Region Using a Laser-Pulse Photolysis Technique. *Biochem. J.* **1999** (in press).

(10) Liu, M.; Nakazawa, K.; Inoue, K.; Ohno, Y. Potent and Voltage-Dependent Block by Philanthotoxin-343 of Neuronal Nicotinic Receptor Channels in PC-12 Cells. *Br. J. Pharmacol.* **1997**, *122*, 379−385.

(11) Rozental, R: Giles, G.; Scoble, T.; Albuquerque, E. X.; Idriss, M.; Sherby, S.; Satelle, D. B.; Nakanishi, K.; Konno, K.; Eldefrawi, A. T.; Eldefrawi, M. E. Allosteric Inhibition of Nicotinic Acetylcholine Receptors of Vertebrates and Insects by Philanthotoxin. *J. Pharmacol. Exp. Ther.* **1989**, *249*, 123−130.

(12) Usherwood, P. N. R.; Blagbrough, I. S. Electrophysiology of Polyamines and Polyamine Amides. In *Neuropharmacology of Polyamines*; Carter, C. J., Ed.; Academic Press: London, 1994; pp 185−204.

(13) Usherwood, P. N. R.; Blagbrough, I. S. Spider Toxins Affecting Glutamate Receptors: Polyamines in Therapeutic Neurochemistry. *Pharmacol. Ther.* **1991**, *52*, 245−268.

(14) Sudan, H. L.; Kerry, C. J.; Mellor, I. R.; Choi, S.-K.; Huang, D.; Nakanishi, K.; Usherwood, P. N. R. The Action of Philanthotoxin-343 and Pholabile Analogues on Locust (*Schistocerca gregaria*) Muscle. *Invertebrate Neurosci.* **1995**, *1*, 159−172.

(15) Choi, S.-K.; Kalivretenos, A. G.; Usherwood, P. N. R.; Nakanishi, K. Labeling Studies of Photolabile Philanthotoxins with Nicotinc Acetylcholine Receptors: Mode of Interaction Between Toxin and Receptor. *Chem. Biol.* **1995**, *2*, 23−32.

(16) Goodnow, R., Jr.; Konno, K.; Niwa, M.; Kallimopoulos, T.; Bukonwik, R.; Lenares, D.; Nakanishi, K. Synthesis of Glutamate Receptor Antagonist Philanthotoxin-433 (PhTX-433) and Its Analogues. *Tetrahedron* **1990**, *46*, 3267−3286.

(17) Choi, S.-K.; Goodnow, R. A.; Kalivretenos, A.; Chiles, G. W.; Fushiya, S.; Nakanishi, K. Synthesis of Novel and Photolabile Philanthotoxin Analogues: Glutamate Receptor Antagonists. *Tetrahedron* **1992**, *48*, 4793−4822.

(18) Nakanishi, K.; Huang, X.; Jiang, H.; Liu, Y.; Fang, K.; Huang, D.; Choi, S.-K.; Katz, E.; Eldefrawi, M. Structure−Binding Relation of Philanthotoxins from Nicotinic Acetylcholine Receptor Binding Assay. *Biorg. Med. Chem.* **1997**, *5*, 1969−1988.

(19) Melchiorre, C.; Quaglia, W.; Picchio, M. T.; Giardinà, D.; Brasili, L.; Angeli, P. Structure−Activity Relationships among Methoctramine-Related Polymethylene Tetraamines. Chain-Length and Substitution Effects on M_2 Muscarinic Receptor Blocking Activity. *J. Med. Chem.* **1989**, *32*, 79−84.

(20) Quaglia, W.; Giardinà, D.; Marucci, G.; Melchiorre, C.; Minarini, A.; Tumiatti, V. Structure−Activity Relationships Among Methoctramine-Related Polymethylene Tetraamines. 3. Effect of the Four Nitrogens on M_2 Muscarinic Blocking Activity as Revealed by Symmetrical and Unsymmetrical Polyamines. *Farmaco* **1991**, *46*, 417−434.

(21) *N*-[(Benzyloxy)carbonyl]-1,8-octanediamine and benzyl *N*-[4-(aminomethyl)benzyl]carbamate were synthesized from 1,8-octanediamine and *p*-xylenediamine, respectively, following the procedure reported in ref 22.

(22) Atwell, G. J.; Denny, W. A. Monoprotection of α,ω-Alkanediamines with the *N*-Benzyloxycarbonyl Group. *Synthesis* **1984**, *27*, 1032−1033.

(23) Steinberg, H.; Cram, D. J. Macro Rings. II. Polynuclear Paracyclophanes. *J. Org. Chem.* **1952**, *17*, 5388−5391.

(24) Confalone, P. N.; Huie, E. M. The Stabilized Iminium Ylide−Olefin [3+2] Cycloaddition Reaction. Total Synthesis of Sceletium Alkaloid A_4. *J. Am. Chem. Soc.* **1984**, *106*, 7175−7178.

(25) Minarini, A.; Quaglia, W.; Tumiatti, V.; Melchiorre, C. An Improved Synthesis of *N,N*-Bis[6-*o*-methoxybenzyl methyl hexylamino]-1,8-octanediamine (Methoctramine). *Chem. Ind.* **1989**, 652−653.

(26) Dupuis, G. An Asymmetrical Disulfide-Containing Photoreactive Heterobifunctional Reagent Designed to Introduce Radioactive Labeling Into Biological Receptors. *Can. J. Chem.* **1987**, *65*, 2450−2453.

(27) Furchgott, R. F. The Classification of Adrenoceptors (Adrenergic Receptors). An Evaluation from the Standpoint of Receptor Theory. In *Catecholamines*; Blaschko, H., Muscholl, E., Eds.; Springer-Verlag: Berlin, 1972; pp 283−335.

(28) Teodori, E.; Gualtieri, F.; Angeli, P.; Brasili, L.; Giannella, M.; Pigini, M. Resolution, Absolute Configuration, and Cholinergic Enantioselectivity of (+)- and (−)-*cis*-2-Methyl-5-[(dimethylamino)methyl]-1,3-oxathiolane Methiodide. *J. Med. Chem.* **1986**, *29*, 1610−1615.

(29) Bixel, M. G.; et al. unpublished results.

(30) Tallarida, R. J.; Murray, R. B. *Manual of Pharmacological Calculations with Computer Programs, Version 4.2*; Springer-Verlag: New York, 1991.

JM991110N