Non-depolarizing Neuromuscular Blocking Activity of Bisquaternary Amino Di- and Tripeptide Derivatives

Leo H. D. J. Booij,[‡] Leon A. G. M. van der Broek,[†] Wilson Caulfield,[#] Brigitte M. G. Dommerholt-Caris,[†] John K. Clark,[#] Jan van Egmond,[‡] Ross McGuire,[#] Alan W. Muir,[#] Harry C. J. Ottenheijm,[†] and David C. Rees*[,#]

Departments of Medicinal Chemistry and Pharmacology, Organon Laboratories, Newhouse, Scotland ML1 5SH, U.K., Scientific Development Group, N V Organon, Molenstraat 110, 5342 CC Oss, The Netherlands, and Department of Anaesthesiology, University Medical Center, University of Nijmegen, Nijmegen, The Netherlands

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Herein we describe the synthesis of novel di- and tripeptide derivatives with two quaternary nitrogen groups attached and the biological testing of these compounds for neuromuscular blocking (NMB) activity in vitro and in vivo. The short peptide scaffold was selected because it offers potential for desired distance between the two pharmacophoric quaternary nitrogen groups, short duration of action, straightforward synthesis, and compatibility with an injectable formulation. From a small series of compounds **20c**,**e** are identified as effective non-depolarizing NMB agents in vitro and in vivo in anesthetized cats and Rhesus monkeys with potencies similar to those of the clinical reference compounds rocuronium (**4**) and suxamethonium (**2**) (monkey ED_{90} = 0.68, 0.23, 0.16, 5.04 μmol/kg, respectively). These new peptide derivatives **20c**,**e** have similar potency and onset time but longer duration and slower recovery than the clinically used reference compounds. The structure−activity relationships described for this chemical series lead to the conclusion that the di- or tripeptide fragment can be regarded as an alternative template to the steroid or aliphatic ester of previously reported NMBs and within this tripeptide-derived series clog *P* correlates well with in vitro NMB activity.

Introduction

General anesthesia is a state of reversible unconsciousness and pain suppression, which is induced to cause amnesia and prevent autonomic responses and to modulate muscle tone and cardiovascular reflexes during surgery. It consists of three separate components: hypnosis with amnesia, analgesia, and skeletal (peripheral) muscle relaxation, all of which are controlled by three different classes of drugs.[1] These drugs are administered by injection or inhalation and are used in combination with each other to achieve a fast onset of action (less than 2 min) followed by rapid and predictable recovery without serious side effects.

Neuromuscular blockers (NMBs) are one of the three classes of drugs used widely during surgical anesthesia. They are peripherally acting skeletal muscle relaxants that are used to paralyze laryngeal muscles prior to airway intubation and paralyze other skeletal muscles, predominantly those of the thorax and abdomen, during surgery. All currently used NMB drugs achieve this effect by blocking the pharmacological effects of acetylcholine (**1**), $CH_3CO_2CH_2CH_2N^+Me_3$, at membrane-bound ligand-gated nicotinic acetylcholine receptors on skeletal muscle.

Two classes of clinically used NMB drugs can be distinguished based upon the compounds' mechanisms of action: the depolarizing agents, e.g. suxamethonium (**2**), and the non-depolarizing agents, e.g. pancuronium (**3**), rocuronium (**4**), and atracurium (**5**). The non-depolarizing agents act as competitive antagonists for acetylcholine, and their action may be reversed by an anticholinesterase agent, e.g. neostigmine.[2] The depolarizing NMB suxamethonium (**2**) produces rapid peripheral skeletal muscle relaxation by nicotinic acetylcholine receptor (nAChR) desensitization (tachyphylaxis) but in addition causes (mechanism-related) undesired side effects such as potassium release and autonomic activation (through ganglion receptors).

The chemical structures of both classes of the clinically used NMBs possess two or three alkylated amino nitrogen atoms with at least one of these quaternized leading to a permanent charge (hence distinguishing these drugs from the widely researched centrally acting nAChR modulators). NMB activity is loosely related to the inter-nitrogen distance: approximately 1.0−1.4 nm is optimal and non-depolarizing agents tend to be more sterically bulky and less conformationally flexible than depolarizers.[3,4] This interonium distance can be maintained by various chemical moieties. Two extensively studied chemical series of non-depolarizing NMBs are the amino steroids (e.g. **3**, **4**)[3,5] where the distance is maintained by an androstane skeleton and the bisbenzylisoquinolinium diesters (e.g. **5**)[6] where a linear ester structure determines the distance. The conotoxin peptide muscle relaxants are chemically unrelated to the series described above but have been reported to demonstrate neuromuscular block in vitro.[7]

One of the challenges facing medicinal chemists researching new anesthetic agents is to achieve phar-

* To whom correspondence should be addressed. Current address: Medicinal Chemistry Department, AstraZeneca R&D Mölndal, S-431 83 Mölndal, Sweden. Tel: +46 31 7761350. Fax: +46 31 7763868. E-mail: David.C.Rees@astrazeneca.com.
[#] Organon Laboratories, Scotland.
[†] N V Organon, The Netherlands.
[‡] University of Nijmegen.

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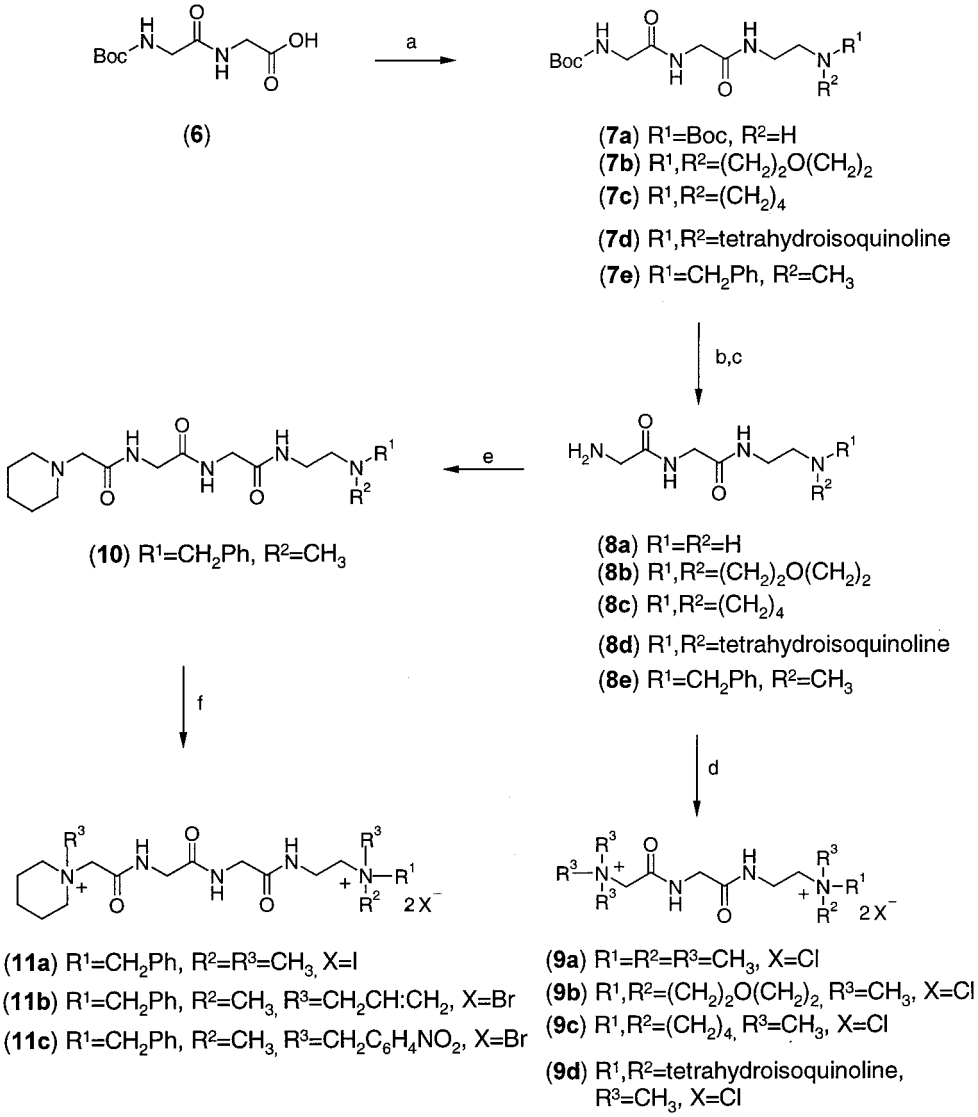
Scheme 1[a]



[a] Reagents and conditions: (a) DCC/HOBT/$H_2NCH_2CH_2NR^1R^2$/DMF; (b) TFA or HCl/EtOAc; (c) Dowex or KOH/MeOH; (d) R^3X/$NaHCO_3$/Sephadex/Dowex; (e) DCC/HOBT/piperidineacetic acid/DCM; (f) R^3X/MeCN.

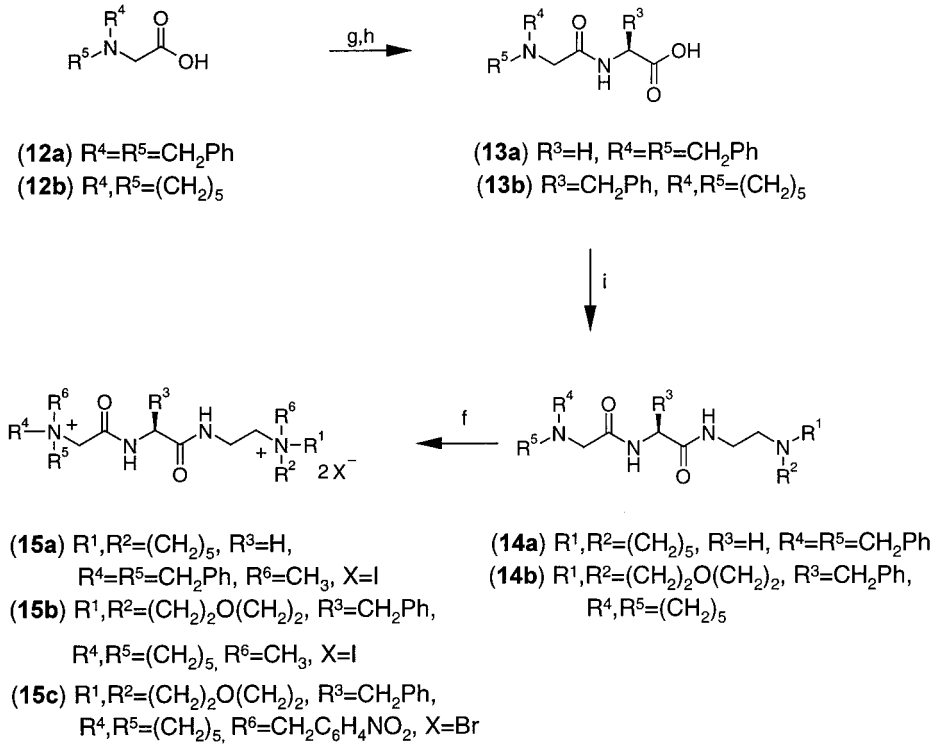
macokinetic properties leading to rapid onset and short duration of action. As a result anesthesia will become more controllable and can be rapidly adjusted to the clinical requirements. This challenge is created by the increasing trend of day care surgery and by the fact that nowadays more severely sick patients are operated upon and thus anesthetized. Attempts to fulfill this requirement have included: a soft-drug deactivation approach, e.g. incorporating metabolically labile ester groups into the opioid analgesic remifentanil,[8] incorporating chemically labile esters into the bistetrahydroisoquinolinium neuromuscular blockers,[9] and decreasing the potency of muscle relaxants to increase their speed of onset.[10,11] Another challenge in the search for new NMBs is the requirement for selectivity between blocking effects at the receptors on skeletal muscle and at other acetylcholine receptors, particularly those in the autonomic ganglia and the heart in order to avoid undesirable cardiovascular side effects.[12]

The aim of the research described in this manuscript is to investigate whether a short peptide fragment, decorated with two alkylated amino groups, would exhibit non-depolarizing NMB activity and to explore the structure−activity relationship (SAR) of such a series. It was envisaged that a peptide scaffold could confer a number of desirable properties in this context: e.g. rapid in vivo metabolism to nontoxic metabolites (the soft drug concept), low propensity to cross the blood−brain barrier, and straightforward synthesis from inexpensive starting materials. In designing which peptide fragments and which quaternary side chains to synthesize the following aspects were considered: mimic approximately the interonium distance of the steroidal NMBs, allow for some steric bulk/conformational constraints associated with non-depolarizing activity, and incorporate some quaternary side chains previously reported to confer NMB activity. It has been reported in the patent literature that certain bisquaternary peptide amines exhibit non-depolarizing NMB activity, e.g. $Me_3N^+PheNH(CH_2)_{10}NHPheN^+Me_3$, but the SARs of these compounds have not been reported.[13,14]

Chemistry

The di- and tripeptide derivatives linked to various diaminoethanes described in Schemes 1−3 were prepared by standard peptide synthesis techniques, es-

Scheme 2[a]



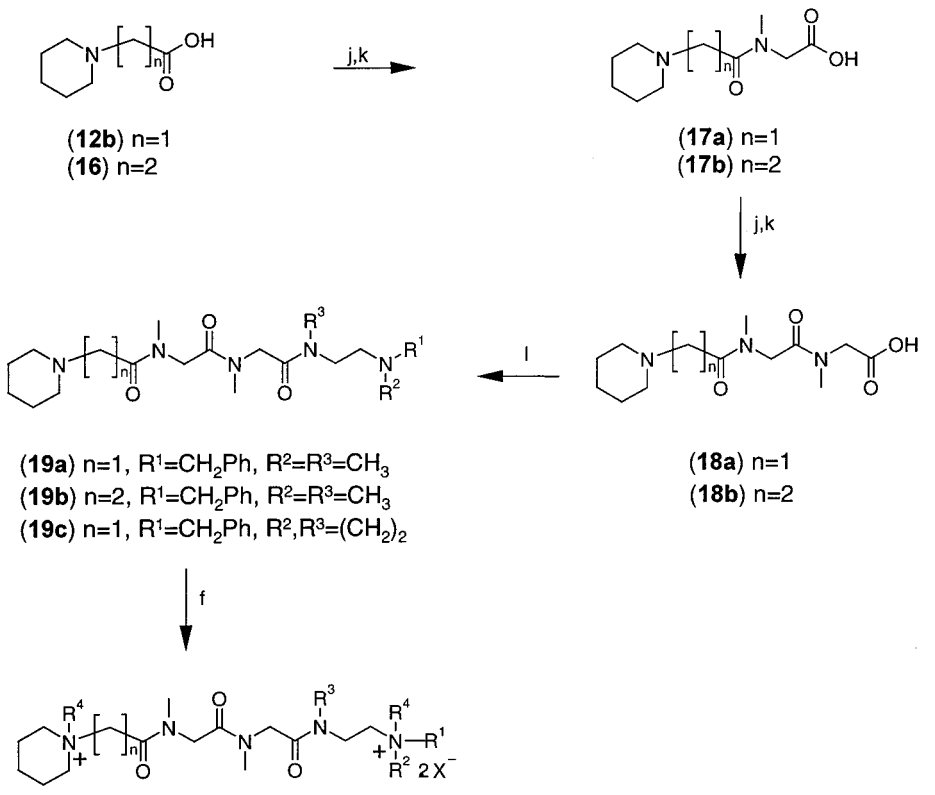
[a] Reagents and conditions: (g) DCC/HOBT/$H_2NCH(R^3)CO_2R$·HCl/Et_3N/DCM; (h) NaOH/MeOH; (i) DCC/HOBT/$H_2NCH_2CH_2NR^1R^2$/DMF; (f) R^6X/MeCN.

sentially following the methods employed by Konteatis et al.[15] Four of the substituted diaminoethanes were previously prepared according to known literature methods[16−18] as were piperidineacetic acid[19] and dibenzylglycine.[20] Commencing with a simple N-BOC-protected dipeptide **6**, as outlined in Scheme 1, couplings with various N,N-disubstituted ethylenediamine derivatives were carried out in DMF using 1-hydroxybenzotriazole hydrate (HOBT) and dicyclohexylcarbodiimide (DCC) to afford the ethylamides **7a**−**e**. The ethylamides **7a**−**d** could be deprotected using excess trifluoroacetic acid (TFA) giving the corresponding diamines **8a**−**d** after liberation from their TFA salts using ion-exchange resin, as highly water-soluble products. In the case of the benzylmethylamino derivative **7e** the deprotection was carried out by the method of Stahl et al.[21] which uses HCl in ethyl acetate, and the free base **8e** was obtained by standard extraction. The quaternary salts **9a**−**d** were then prepared from diamines **8a**−**d** by reaction with a large excess of iodomethane in the presence of sodium bicarbonate, a technique used in peptide quaternary salt preparation,[15,22] but the products proved insoluble in solvents such as ethyl acetate or DCM so the workup method in the above reference[22] could not be employed. Instead the products were isolated by an aqueous chromatographic method using Sephadex G-10 as adsorbent, which provided the quaternaries as the extremely hygroscopic ammonium hydroxides, which required treatment with ion-exchange resin to yield the corresponding chlorides. Although NMR spectra were consistent with the structures **9a**−**d**, elemental analysis values for the compounds indicated high water content and the possibility of some sodium ion contamination. Realizing this difficulty, the diamine **8e** was further coupled to piperidineacetic acid **12b**[19] giving the more lipophilic tripeptide derivative **10**, which could then be readily converted to quaternary compounds **11a**−**c** using the appropriate alkyl halides without the need for any scavenger, and the products could be obtained after isolation by precipitation from acetone/ether mixtures.

This finding significantly altered the methodology used in subsequent synthetic procedures. Beginning with an N,N-dialkylated terminal amino acid and coupling to amino acid ester derivatives followed by hydrolysis generated intermediates which were easier to isolate and handle than those of Scheme 1. Thus *N*,*N*-dibenzylglycine **12a**[20] (Scheme 2) was coupled to glycine ethyl ester and then hydrolyzed using a slight excess of sodium hydroxide to give the dipeptide **13a**. Further coupling to 2-aminoethylpiperidine gave ethylamide **14a**, which could be readily converted to quaternary dimethiodide **15a** again without contamination by scavenger. In a similar process, piperidineacetic acid **12b**[19] was reacted with L-phenylalanine methyl ester followed by hydrolysis to provide dipeptide **13b**. Coupling of **13b** to 4-(2-aminoethyl)morpholine gave ethylamide **14b**, which was alkylated to afford quaternary salts **15b,c**.

Scheme 3 indicates first an attempt to introduce some rigidity into the already interesting compounds **11a**−**c** of Scheme 1 by using sarcosine units in the backbone instead of glycine. This had two immediate synthetic effects, in that the coupling reactions using a secondary amine proved to be less facile and due to rotameric isomerization about the hindered amide bonds the NMR spectra of all the compounds proved to be difficult to interpret. Second, Scheme 3 shows an attempt to test the scope by the use of a chain-extended amino acid **16**. Piperidineacetic acid **12b**[19] and the corresponding propionic acid **16** were reacted with sarcosine ethyl ester

Scheme 3[a]



[a] Reagents and conditions: (j) DCC/HOBT/$CH_3NHCH_2CO_2Et$·HCl/DIPEA/DCM; (k) NaOH/MeOH or dioxane; (l) HBTU/$R^3NHCH_2CH_2NR^1R^2$/DIPEA/DMF; (f) R^4X/MeCN.

using standard procedures mentioned above and hydrolyzed to give dipeptide **17a** and amino acid **17b**. Further coupling to a second unit of sarcosine and hydrolysis provided **18a**,**b**, and finally addition of 2-(methylaminoethyl)benzylmethylamine[18] was accomplished using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), a reagent described by Knorr et al.[23] as being an excellent activating agent superior to DCC. The diamines **19a**−**c** could be chromatographically purified before alkylation to give the quaternary products **20a**−**e**.

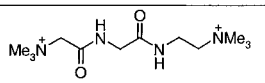
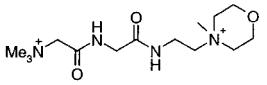
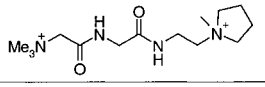
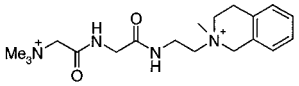
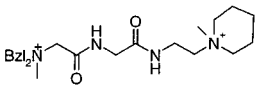
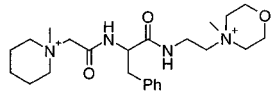
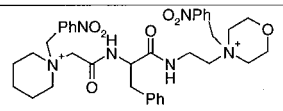
Results and Discussion

In selecting which length of peptide fragment to use, the aim was to approximately mimic the interonium distance of the steroidal NMBs. Analysis of the X-ray crystal structure[24] of pancuronium (**3**) shows that the interquaternary nitrogen distance is 1.11 nm. In this molecule, as in the close structural analogue rocuronium (**5**), there are no freely rotatable bonds between the two nitrogens. Thus, even allowing for some ring flexibility, the N−N distance will not alter significantly. X-ray data for various suxamethonium (**2**) crystals also exist, and these show that in the solid state the N−N distance can vary from 0.76 nm[25] to 1.2 nm.[26] Conformational analyses suggest that the likely distance between the quaternary nitrogens for the tripeptide derivative **11a** will be 1.15−1.49 nm, while the corresponding distance in the dipeptide derivative **9a** will be 0.91−1.13 nm. Thus both the di- and tripeptide derivatives have the potential to exhibit the favored interonium distance of the order of 1.1 nm.

In addition to this conformational guideline, an element of bulk and/or lipophilicity has also been postulated as important for non-depolarizing ability.[3,5] Structural variations have been made to test this hypothesis by making changes to the lipophilicity of the molecules in the series of tripeptides. The initial biological screen was in an in vitro isolated chick biventer muscle preparation. Compounds were tested to determine the dose required to block the indirectly elicited electrically stimulated muscle twitch. The doses of the dipeptide bisquaternary compounds, tripeptide bisquaternary compounds, and reference compounds producing 50% twitch block are shown in Tables 1−3, respectively.

The most active compounds identified in the chick biventer assay were then evaluated in vivo in anesthetized cats. These experiments determined the potency (ED_{50}) of the inhibition of the twitch response of the electrically indirectly stimulated tibialis anterior muscle and the time course in terms of onset, recovery, and duration of action. The selectivity of test compounds for undesirable effects on autonomic system was investigated by measuring inhibition of the stimulated vagus

Table 1. Structure and in Vitro NMB Activity of Dipeptide Bisquaternary Derivatives

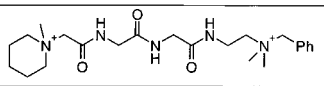
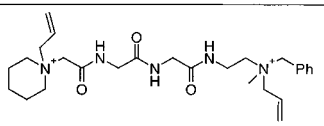
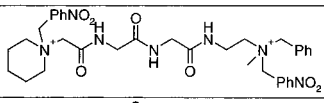
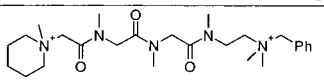
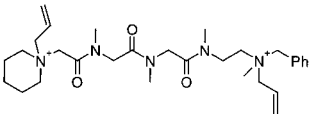
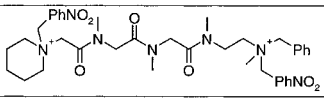
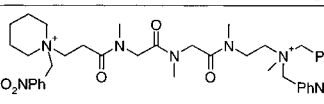
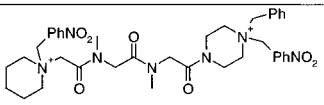
Compound No.	Structure	Chick Biventer[a]
9a		>390[br](2)
9b		>390[b](2)
9c		>390[b](2)
9d		>955(2)
15a		134 ± 19(2)
15b		407 ± 29(2)
15c		19.8 ± 2.8[B](2)

[a] Dose causing 50% block of muscle twitch (μM); number of determinations in parentheses. [B] Developed slow increase in baseline tension. [b] Decrease in baseline tension. [r] Poor recovery or no recovery following washing.

nerve-induced bradycardia and superior cervical nerve-induced contractions of the nictitating membrane. These results are shown in Table 4, also in comparison with reference NMBs. Two of the tripeptide derivatives are evaluated in anesthetized Rhesus monkeys (Table 5).

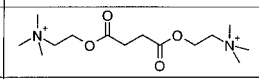
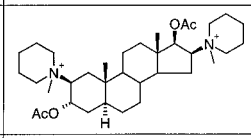
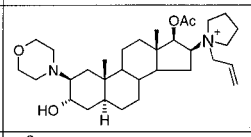
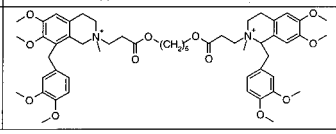
The first compound in Table 1 is the parent Gly-Gly dipeptide derivative **9a** possessing an ethylenediamine moiety which confers potential for similar 'through-bond' distance between the two quaternary nitrogens as in the reference NMB steroid, pancuronium. This compound, **9a**, is inactive in the chick biventer assay (>390 μM) so the next three compounds introduce cyclic quaternary groups present in known NMBs from chemical series with different scaffolds (e.g. steroids and the bisbenzylisoquinolinium diesters): i.e. the morpholine **9b**, the pyrrolidine **9c**, and the tetrahydroisoquinoline **9d**. Modification of these structures failed to increase NMB activity (>390 μM); however when both of the quaternary groups of **9a** are changed, as in **15a**, the NMB activity (134 μM) was modestly increased. Increasing the lipophilicity and conformational restraint within the dipeptide scaffold by incorporating a Phe residue and introducing cyclic quaternary groups at both nitrogens leads to **15b** (407 μM) and **15c** (19.8 μM) which is the most active compound prepared in this series. The *p*-nitrobenzyl quaternary substituent in **15c** has previously been incorporated into a different chemical series where it is reported to convert depolarizing NMB activity into non-depolarizing NMB activity.[27] Compound **15c** was tested in the anesthetized cat model where it demonstrates NMB activity ED_{50} = 10 μmol/kg (Table 4) albeit at approximately 2 orders of magnitude higher doses than the clinically used reference compounds (**2**−**5**). For comparison, an inactive representative from the dipeptide series, **9c**, was tested in the cat and, as expected, is inactive (ED_{50} > 10 μmol/kg).

Table 2. Structure and in Vitro NMB Activity of Tripeptide Bisquaternary Derivatives

Compound No.	Structure	Chick Biventer[a]
11a		345 ± 58
11b		283 ± 11
11c		47 ± 5
20a		140 ± 3.5
20b		69 (n=1)
20c		0.4 ± 0.005
20d		4.2 ± 1.1
20e		0.7 ± 0.2

[a] Dose causing 50% block of the muscle twitch (μM).

Table 3. Structure and in Vitro NMB Activity of Reference Compounds

Name Compound	Structure	Chick Biventer
Suxamethonium **2**		0.54 ± 0.07(4)*
Pancuronium **3**		0.2 ± 0.02(4)
Rocuronium **4**		0.76 ± 0.07(5)
Atracurium **5**		0.41 ± 0.1(4)

*Immediate concentration-dependent increase in baseline tension showing a depolarizing action.

Homologation of the Gly backbone gives the tripeptide series in Table 2. The piperidinobenzyl bisquaternary **11a** displayed only weak NMB activity (ED_{50} = 345 μM), but increasing the bulk of the quaternary group with bisallyl or *p*-nitrobenzyl slightly increases activity, in line with the SAR of previously reported NMB chemical

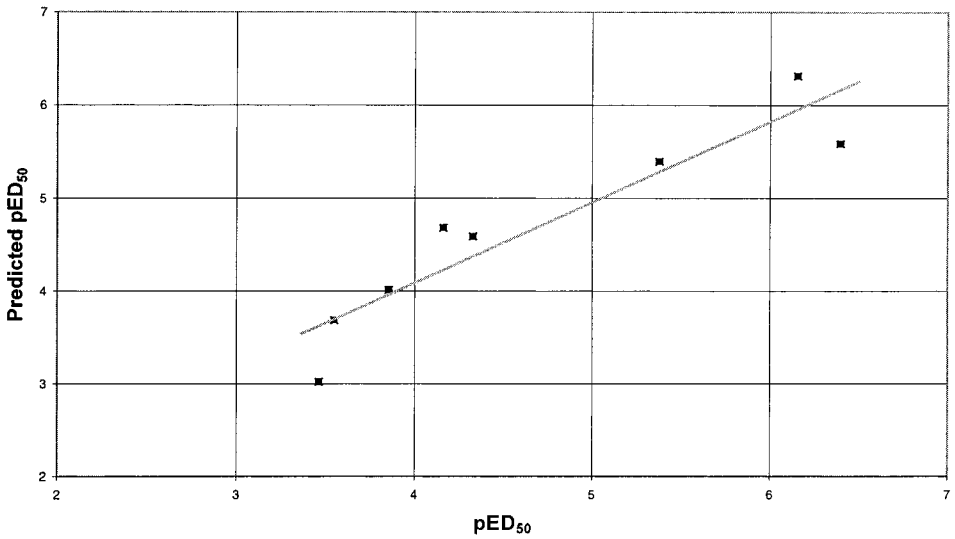


Figure 1. Plot of pED_{50} (chick biventer data from Tables 1 and 2) versus predicted pED_{50} according to eq 1.

Table 4. Potency and Time Course of NMB Activity in Anesthetized Cats

compd ($n = 1$)	potency ED_{50} (μmol/kg)	onset (min)	recovery$_{25-75\%}$ (min)	duration$_{90\%}$ (min)	vagal ED_{50} (μmol/kg)	nic memb ED_{50} (μmol/kg)
9c	>10.0				>10.0	>10.0
15a	>19.2				~12.8	~19.2
15c	10.1				~0.8	>11.2
11c	7.3				0.7	5.3
20a	>12.8				~1.6	>12.8
20c	1.2	3.3	3.8	12.7	<0.4	>1.8
20e	~0.1	2.6	3.9	10.4	~0.2	≫0.2
suxamethonium, **2**	0.11	1.7	1.9	5.7		*a*
pancuronium, **3**	0.03	4.1	4.0	11.7	0.07	
rocuronium, **4**	0.25	2.0	2.9	8.3	2.17	
atracurium, **5**	0.10	5.8	5.9	19.4	1.6	

[a] Pre- and post-block increases in twitch tension and short-lived muscle fasciculations 20−30 s after dosing.

Table 5. Potency and Time Course of NMB Profile in Anesthetized Monkeys

compd	potency ED_{90} (μmol/kg)	onset (min)	recovery 25−75% (min)	duration 90% (min)
20c ($n = 2$)	0.68	1.7	17.6	27.7
20e ($n = 2$)	0.23	2.9	15.6	42.5
rocuronium, **4** ($n = 5$)	0.16	3.1 ± 0.5	5.0 ± 0.9	13.6 ± 2.4
suxamethonium, **2** ($n = 5$)	5.04	1.5 ± 0.2	2.9 ± 0.6	9.7 ± 2.0[a]

[a] Data from Muir et al. *Eur. J. Anaesthesiol.* **1998**, *15*, 467−479.

series, to within 2 orders of magnitude of the clinically used reference compounds in the same assay (Table 3) (**11b**,**c**: ED_{50} = 283, 47 μM, respectively).

In the cat model **11c** shows weak NMB potency (ED_{50} = 7.3 μmol/kg) but is more active at inhibiting the vagal nerve (ED_{50} = 0.7 μmol/kg) indicating undesirable selectivity for the autonomic system. N-Methylation of the glycine backbone of **11a** gives a modest increase in NMB activity in vitro (**11a**, **20a**: ED_{50} = 345, 140 μM, respectively) which is further enhanced by changing the quaternary groups from methyl to allyl and *p*-nitrobenzyl (**20a**−**c**: ED_{50} = 140, 69, 0.4 μM respectively, Table 2). This latter compound **20c** is potent in vivo (cat ED_{50} = 1.2 μmol/kg, Table 4) and has a time course that compares favorably with some of the reference compounds, being faster in onset (3.3 min), recovery (3.8 min), and duration (12.7 min) than atracurium (**5**) but slower than suxamethonium (**2**) and rocuronium (**4**). This result encouraged us to make additional analogues. Homologation of the backbone with a single methylene unit at the N-terminus to give the β-Ala-Gly(Me)-Gly-(Me) derivative **20d** decreases activity in vitro compared to **20c** (ED_{50} = 4.2, 0.4 μM); however, rigidifying the C-terminal region by incorporating a piperazine group retains in vitro activity at similar dose levels as the reference compounds (**20e**: ED_{50} = 0.7 μM) and gives approximately 10-fold improved potency with a similar or possibly improved onset time in vivo (cat ED_{50} = ~0.1 μmol/kg; onset time 2.6 min). Although selectivity with respect to the autonomic system is improved with **20e** compared to other peptides, it is less attractive in this respect than the reference compounds (Table 4).

The initial working hypothesis that lipophilicity might be an important factor in determining non-depolarizing NMB activity was quantified retrospectively by a QSAR analysis. Figure 1 and eq 1 show that in the tripeptide series (the first eight compounds in Table 6) activity at the chick biventer varies with calculated lipophilicity[28] with an r^2 of 0.86:

$$\text{predicted } pED_{50} = 0.56(\text{clog } P) + 5.40 \qquad (1)$$

$$n = 8;\ r^2 = 0.86;\ F = 38.8;\ s = 0.46$$

In eq 1, n is the number of observations, r is the correlation coefficient, F is the F-value, and s is the standard error.

The dataset used in Figure 1 may be extended to include the non-depolarizing reference compounds pan-

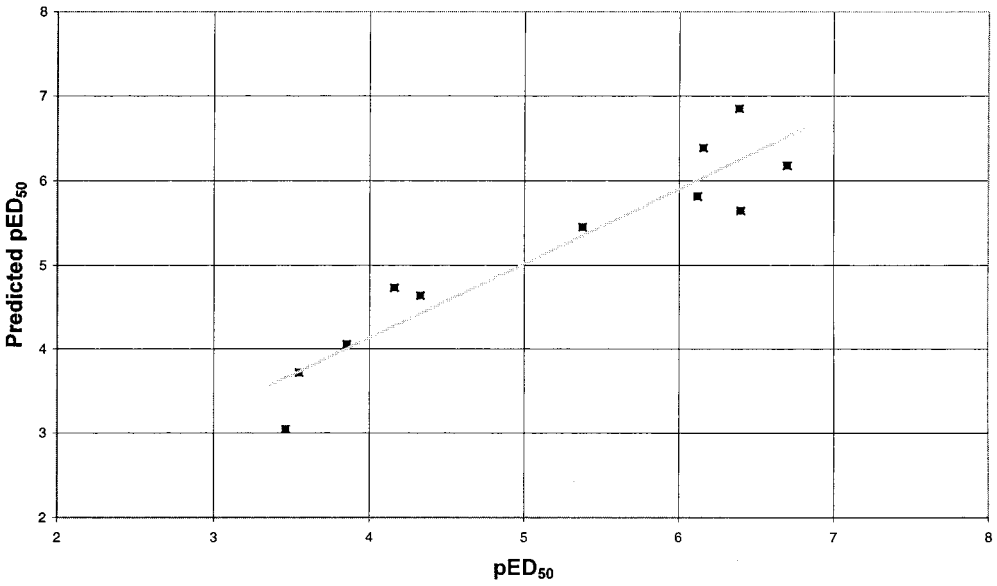


Figure 2. Plot of pED_{50} (chick biventer) versus predicted pED_{50} according to eq 2.

Table 6. Chick Biventer Activity Data and Clog *P* Values for Regression Equations

compd	chick biventer ED_{50} (μM)	pCB[a]	clog *P*[b]
11a	345	3.46	−4.12
11b	283	3.55	−2.97
11c	47	4.33	−1.41
20a	140	3.85	−2.4
20c	0.4	6.40	0.31
20d	4.2	5.38	−0.02
20e	0.7	6.15	1.57
20b	69	4.16	−1.25
15a	134	3.87	−2.93
15b	407	3.39	−1.81
15c	19.8	4.70	2.01
pancuronium, **3**	0.2	6.70	1.21
rocuronium, **4**	0.76	6.12	0.6
atracurium, **5**	0.41	6.39	2.36

[a] $-\log(ED_{50})$. [b] Calculated log *P* (ref 28).

curonium (**3**), rocuronium (**4**), and atracurium (**5**). Regression analysis on this dataset gives the QSAR shown in Figure 2 and eq 2:

$$\text{predicted } pED_{50} = 0.59(\text{clog } P) + 5.46 \qquad (2)$$

$$n = 11;\ r^2 = 0.88;\ F = 69.2;\ s = 0.46$$

In eq 2, n is the number of observations, r is the correlation coefficient, F is the F-value, and s is the standard error.

Equation 1 shows that in a homologous series of NMBs, increasing the overall lipophilicity results in increased activity in vitro. Equation 2 demonstrates that this relationship between clog *P* and activity is a more general trend, which applies to different structural classes of non-depolarizing NMBs.

Applying eq 2 to the dipeptide compounds **15a**−**c** gives mixed results. In the case of **15c**, the data are complicated by an increase in baseline tension. Equation 2 would predict a pED_{50} of 3.73 for **15a**, in good agreement with the experimentally determined value of 3.87. The predicted pED_{50} values for **15b**,**c** would be 4.39 and 6.64, compared to experimental values of 3.39 and 4.7, respectively. Thus eq 2 is less predictive in these two cases, and it could be that the presence of bulky benzyl groups in the center of the last two dipeptides causes unexpected effects on activity or perhaps the effects of the morpholine fragments are being poorly modeled by the clog *P* descriptor.

The mechanism of the NMB activity of the new peptide-derived compounds described above appears to be non-depolarizing (competitive). In contrast to the depolarizing agent, suxamethonium (**2**), the new compounds do not produce either muscle fasciculation or pre-/post-twitch augmentation in the cat or monkey in vivo. In the chick biventer preparation some compounds produced slowly developing small increases or decreases in the baseline tension, the mechanism of which is unknown, but which indicates a change in basic muscle tone. In contrast, suxamethonium (**2**) produced an immediate concentration-dependent increase in the baseline tension which in a multifocally innervated chick muscle is indicative of a depolarizing action.

Upon the basis of the above biological data, two tripeptide derivatives were selected for evaluation in anesthetized Rhesus monkeys. Both **20c**,**e** are effective NMBs ($ED_{50} = 0.68, 0.23\ \mu$M/kg) with a rapid onset (1.7, 2.9 min, Table 5). The duration of action of NMBs is a critical factor in determining their clinical applications, and in comparison with the reference compounds rocuronium (**4**) and suxamethonium (**2**) these peptides are of similar potency and onset time but have longer duration and slower recovery, which may be a less attractive profile for short-duration surgery.

Conclusions

Di- and tripeptide moieties are shown to be suitable scaffolds for designing compounds with potent in vitro and in vivo NMB activity by appending two quaternary nitrogen atoms. In this context the peptide scaffold can be regarded as an alternative to previously reported ones based, for example, on steroids, alkaloids, or aliphatic esters. The dipeptide based bisquaternary derivative **15c** demonstrates NMB activity in vitro and in an anesthetized cat assay at doses approximately 100-fold higher than clinically used reference com-

pounds. The tripeptide derivative **20e** is more potent than **15c** in vitro and in vivo in cats, and in Rhesus monkeys **20e** exhibits comparable potency and onset/time course profile as atracurium (**5**) and rocuronium (**4**). Within this series clog *P* appears to correlate well with the observed in vitro NMB activity in the chick biventer assay. Taken together these results contribute to the design of potentially short-acting NMB drugs.

Experimental Section

General Experimental − Chemistry. All reagent chemicals were obtained from commercial suppliers and used without further purification except those described in the text which were prepared by published procedures. Elemental analyses of the final products were performed using a Perkin-Elmer 2400 CHN analyzer and counterions were evaluated by Dionex ion chromatography. All ^{1}H NMR spectra were recorded at 200 or 400 MHz on Bruker AM200 or 400 spectrometers, and chemical shifts are reported in ppm relative to TMS or a soluble sodium derivative. Mass spectra, where quoted, were recorded with a Finnigan MAT 90 mass spectrometer for fast atom bombardment spectra using glycerol or thioglycerol as matrix and an HP 5989 MS engine in positive mode for electrospray spectra, more suitable for the bisquaternary products. HPLC data were obtained using a Perkin-Elmer Integral 4000LC system run on Turbochrom Workstation software version 6.1.1 and a Phenomenex Aqua C18 25 × 0.46 cm reverse-phase column. Elution solvents were 0.1% formic acid (or trifluoroacetic acid) in water (A) and 0.1% acid in acetonitrile (B) using a gradient elution method of 95/5 A/B for 5 min, then up to 10/90 over 15 min, and finally 10/90 for 10 min. Detection was carried out by a Sedex 55 evaporative light scattering detector or Perkin-Elmer UV detector at 230 nM.

***N-tert*-Butyloxycarbonyl-glycyl-glycine-2-(*tert*-butyloxycarbonylamino)ethylamide (7a).** This compound was prepared by reacting *N-tert*-butyloxycarbonyl-glycyl-glycine (**6**) (3.3 g, 14 mmol) in dry DMF (50 mL) with *N-tert*-butyloxycarbonylethylenediamine[16] (1.6 g, 10 mmol) in the presence of HOBT (2 g, 15 mmol) and DCC (**caution! irritant, sensitizer, and potential carcinogen**; 2 g, 10 mmol) stirring for 24 h at room temperature. Dicyclohexylurea (DCU) was removed by filtration, and the DMF was evaporated under reduced pressure. The residue was partitioned between ethyl acetate and 0.4 N HCl then 5% $NaHCO_3$ and finally brine. The product was isolated by drying over Na_2SO_4 and evaporation to give an oil: 2.8 g (76% yield; ^{1}H NMR ($CDCl_3$) δ 7.37, 7.35 (2s, 2H), 5.75 (s, 1H), 5.45 (s, 1H), 3.95 (d, 2H, J = 8 Hz), 3.84 (d, 2H, J = 8 Hz), 3.35 (m, 2H), 3.26 (m, 2H), and 1.43 (s, 9H); MS M + H 375.2.

***N*-Glycyl-glycine-2-aminoethylamide (8a).** Compound **7a** (2.2 g, 5.9 mmol) was dissolved in TFA (22 mL). After 30 min at room temperature the excess TFA was evaporated and the residue was partitioned between diethyl ether and water. The aqueous layer contains the product as the TFA salt, which can be liberated by treatment with Dowex Ira 400 resin, 20−50 mesh. The product was obtained by evaporation as a gum: 1 g (98% yield); ^{1}H NMR (D_2O) δ 3.9 (s, 2H), 3.4 (s, 2H), 3.35 (m, 2H), 2.86 (t, 2H, J = 8 Hz); MS M + H 175.1.

***N,N,N*-Trimethyl-glycyl-glycine-2-(*N′*,*N′*,*N′*-trimethylammonio)ethylamide Dichloride (9a).** Compound **8a** (250 mg, 1.44 mmol) was dissolved in MeOH (10 mL) and reacted with iodomethane (1.4 mL, 23 mmol) in the presence of $NaHCO_3$ (1.6 g, 9.5 mmol), stirring the mixture at room temperature for 72 h. The reaction was filtered, the filtrate evaporated and the residue dissolved in the minimum of water and chromatographed on a column of Sephadex G-10 (170 g, previously swollen in water). The fractions containing the product were evaporated under reduced pressure giving the very hygroscopic quaternary ammonium hydroxide, which was converted to the corresponding dichloride by ion exchange using Dowex 1 × 8 200−400 mesh resin: 165 mg (34% yield); ^{1}H NMR (D_2O) δ 4.25 (s, 2H), 4.03 (s, 2H), 3.78 (t, 2H, J = 6 Hz), 3.55 (t, 2H, J = 6 Hz), 3.36 (s, 9H), 3.2 (s, 9H); MS M − H 259.7; HPLC >98%. Anal. ($C_{12}H_{28}N_4Cl_2O_2$·2.5H_2O·1.5NaCl) C, H, N; Cl: calcd, 26.74; found, 29.85.

***N-tert*-Butyloxycarbonyl-glycyl-glycine-2-(4-morpholino)ethylamide (7b).** Compound **6** (2.3 g, 10 mmol) was reacted with 4-(2-aminoethyl)morpholine (1.9 mL, 10 mmol) using conditions and reagent quantities similar to those described for the synthesis of **7a**, giving 2.1 g of the product (61% yield): ^{1}H NMR ($CDCl_3$) δ 7.47 (m, 1H), 6.95 (m, 1H), 5.74 (m, 1H), 3.95 (m, 4H), 3.85 (d, 2H, J = 7 Hz), 3.38 (m,-4H), 2.45 (m, 6H), 1.45 (s, 9H).

***N*-Glycyl-glycine-2-(4-morpholino)ethylamide (8b).** Compound **7b** (2 g, 5.8 mmol) was deprotected using conditions and reagent quantities similar to those described during the synthesis of **8a**, giving 1.4 g of the product (99% yield): ^{1}H NMR (D_2O) δ 3.96 (s, 2H), 3.82 (m, 6H), 3.45 (t, 2H, J = 6 Hz), 2.85 (m, 6H); MS M + H 245.1.

***N,N,N*-Trimethyl-glycyl-glycine-2-[4-(4-methylmorpholino)]ethylamide Dichloride (9b).** Prepared from compound **8b** (250 mg, 1.02 mmol) using conditions and reagent quantities similar to those described for the synthesis of **9a**, giving 209 mg of the product (55% yield): ^{1}H NMR (D_2O) δ 4.25 (s, 2H), 4.08 (m, 4H), 4.04 (s, 2H), 3.75 (m, 2H), 3.69 (m, 2H), 3.60 (m, 4H), 3.35 (s, 9H), 3.29 (s, 3H); MS M − H 301.7; HPLC >95%. Anal. ($C_{14}H_{30}N_4Cl_2O_3$·2H_2O·NaCl) C, H, N, Cl.

***N-tert*-Butyloxycarbonyl-glycyl-glycine-2-(1-pyrrolidino)ethylamide (7c).** Compound **6** (2.3 g, 10 mmol) was reacted with 1-(2-aminoethyl)pyrrolidine (1.14 g, 10 mmol) using conditions and reagent quantities similar to those described for the synthesis of **7a**. The product proved to be soluble in water, and the aqueous layer was basified with 4 N NaOH then extracted with DCM giving after drying over Na_2SO_4 and evaporation 1.3 g of the product (40% yield): ^{1}H NMR ($CDCl_3$) δ 7.27 (s, 1H), 7.05 (s, 1H), 5.72 (s, 1H), 3.96 (d, 2H, J = 6 Hz), 3.85 (d, 2H, J = 6 Hz), 3.42 (q, 2H, J = 6 Hz), 2.65 (t, 2H, J = 6 Hz), 2.56 (m, 4H), 1.78 (m, 4H), 1.45 (s, 9H).

***N*-Glycyl-glycine-2-(1-pyrrolidino)ethylamide (8c).** Compound **7c** (1.3 g, 4 mmol) was deprotected using conditions and reagent quantities similar to those described for the synthesis of **8a**, giving 700 mg of the product (78% yield): ^{1}H NMR (D_2O) δ 3.88 (s, 2H), 3.35 (m, 4H), 2.6 (m, 6H), 1.74 (m, 4H).

***N,N,N*-Trimethyl-glycyl-glycine-2-[1-(1-methylpyrrolidino)]ethylamide Dichloride (9c).** Prepared from compound **8c** (170 mg, 0.75 mmol) using conditions and reagent quantities similar to those described for the synthesis of **9a**, giving 101 mg of the product (38% yield): ^{1}H NMR (D_2O) δ 4.24 (s, 2H), 4.0 (s, 2H), 3.75 (t, 2H, J = 8 Hz), 3.5 (m, 6H), 3.35 (s, 9H), 3.11 (s, 3H), 2.2 (m, 4H); MS M − H 285.7; HPLC >97%. Anal. ($C_{14}H_{30}N_4Cl_2O_2$·3H_2O·NaCl) C, H, N; Cl: calcd, 22.63; found, 21.58.

***N-tert*-Butyloxycarbonyl-glycyl-glycine-2-(1,2,3,4-tetrahydro-2-isoquinolino)ethylamide (7d).** This compound was prepared by reacting **6** (2.3 g, 10 mmol) in dry DMF (50 mL) with 2-(2-aminoethyl)-1,2,3,4-tetrahydroisoquinoline[17] (1.72 g, 9.75 mmol) in the presence of HOBT (1.35 g, 10 mmol) and DCC (2 g, 10 mmol) stirring for 24 h at room temperature. DCU was removed by filtration, and the DMF was evaporated under reduced pressure. The residue was partitioned between ethyl acetate and 5% $NaHCO_3$ and finally brine. The product proved to be soluble in water, and the aqueous layer was basified with 4 N NaOH then extracted with DCM. The organic layers were combined and dried over Na_2SO_4 then evaporated to give 2.44 g of the product (63% yield): ^{1}H NMR ($CDCl_3$) δ 7.14−7.05 (m, 5H), 6.88 (s, 1H), 5.38 (s, 1H), 3.9 (d, 2H, J = 7 Hz), 3.74 (d, 2H, J = 8 Hz), 3.63 (s, 2H,), 3.47 (q, 2H, J = 6 Hz), 2.9 (m, 2H), 2.76 (m, 2H), 2.55 (t, 2H, J = 6 Hz), 1.45 (s, 9H).

***N*-Glycyl-glycine-2-(1,2,3,4-tetrahydro-2-isoquinolino)-ethylamide (8d).** Compound **7d** (500 mg, 1.28 mmol) was deprotected using conditions and reagent quantities similar to those described during the synthesis of **8a**, giving 322 mg of the product (87% yield): ^{1}H NMR (D_2O) δ 7.18 (m, 3H), 7.1 (m, 1H), 3.9 (s, 2H), 3.7 (s, 2H), 3.45 (t, 2H, J = 8 Hz), 3.44 (s, 2H), 2.85 (m, 4H), 2.73 (t, 2H, J = 8 Hz).

***N,N,N*-Trimethyl-glycyl-glycine-2-(2-methyl-1,2,3,4-tetrahydro-2-isoquinolino)ethylamide Dichloride (9d).** Prepared from compound **8d** 150 mg, 0.52 mmol) using conditions and reagent quantities similar to those described for the synthesis of **9a**, giving 101 mg of the product (83% yield): ^{1}H NMR (D_2O) δ 7.46 (m, 3H), 7.2 (m, 1H), 4.81 (s, 2H), 4.62 (d, 2H, J = 6 Hz), 4.17 (s, 2H), 3.95 (s, 2H), 3.77 (2t, 4H, J = 6 Hz), 3.55 (t, 2H, J = 6 Hz), 3.27 (s, 9H), 3.23 (s, 3H); MS M − H 347.2; HPLC >89%. Anal. ($C_{19}H_{32}N_4Cl_2O_2 \cdot 7H_2O \cdot NaCl$) C, Cl; H: calcd, 8.07; found, 6.22; N: calcd, 9.75; found, 9.26.

***N*-*tert*-Butyloxycarbonyl-glycyl-glycine-2-[(methylphenylmethyl)amino]ethylamide (7e).** Compound **6** (2.3 g, 10 mmol) was reacted with (2-aminoethyl)benzylmethylamine[17] (1.6 g, 9.75 mmol) using conditions and reagent quantities similar to those described for the synthesis of **7d**, giving 3.5 g of the product (92% yield): ^{1}H NMR ($CDCl_3$) δ 7.29 (m, 5H), 7.2 (s, 1H), 6.7 (s, 1H), 5.55 (s, 1H), 3.92 (d, 2H, J = 7 Hz), 3.83 (d, 2H, J = 7 Hz), 3.5 (s, 2H), 3.35 (q, 2H, J = 8 Hz), 2.5 (t, 2H, J = 8 Hz), 2.2 (s, 3H), 1.45 (s, 9H).

***N*-Glycyl-glycine-2-[(methylphenylmethyl)amino]ethylamide (8e).** HCl gas (1.1 g, 30.2 mmol) was dissolved in dry ethyl acetate (25 mL) and this solution was added to compound **7e** (3.5 g, 9.25 mmol) in ethyl acetate (35 mL). The solution was stirred for 24 h at room temperature after which the product as its HCl salt could be removed by filtration. The salt (2.04 g, 5.8 mmol) was dissolved in MeOH (20 mL) and treated with a solution of KOH (766 mg, 5.8 mmol) in MeOH (20 mL) with stirring at room temperature for 15 min, then the solvent was removed under reduced pressure. The residue was extracted with DCM and filtered, giving on evaporation 1.3 g of product (81% yield): ^{1}H NMR ($CDCl_3$) δ 7.83 (s, 1H), 7.29 (m, 5H), 6.48 (s, 1H), 3.94 (d, 2H, J = 5 Hz), 3.51 (s, 2H), 3.41 (s, 2H), 3 37 (q, 2H, J = 5 Hz), 2.5 (t, 2H, J = 5 Hz), 2.21 (s, 3H), 1.8 (s, 2H).

***N*-(1-Piperidinomethylcarbonyl)glycyl-glycine-2-[(methylphenylmethyl)amino]ethylamide (10).** Piperidineacetic acid **12b**[19] (700 mg, 4.9 mmol) was dissolved with slight heating in DCM (50 mL) and treated with compound **8e** (1.3 g, 4.7 mmol) followed by HOBT (635 mg, 4.7 mmol) and DCC (968 mg, 4.7 mmol). The mixture was stirred at room temperature for 60 h, then the solvent was reduced to low volume and DCU was removed by filtration. The filtrate was shaken with 5% Na_2CO_3 (3 × 20 mL) dried over Na_2SO_4 and evaporated, giving 1.5 g of the product (80% yield): ^{1}H NMR ($CDCl_3$) δ 7.9 (m, 1H), 7.29 (m, 5H), 6.85 (m, 1H), 6.45 (m, 1H), 4.0 (d, 2H, J = 5 Hz), 3.9 (d, 2H, J = 4 Hz), 3.54 (s, 2H), 3.45 (q, 2H, J = 5 Hz), 3.0 (s, 2H), 2.5 (m, 6H), 2.22 (s, 3H), 1.6 (m, 5H), 1.5 (m, 1H).

1-[1-[2-[*N*-(Phenylmethyl)-*N*,*N*-dimethylammonio]ethylamino]-*N*-(1-methyl piperidinomethylcarbonyl)glycyl]-glycine Diiodide (11a). Compound **10** (300 mg, 0.74 mmol) was dissolved in acetonitrile (15 mL) and stirred at room temperature for 60 h with iodomethane (0.6 mL, 9.6 mmol). The solvent was removed under reduced pressure and the residue was triturated with acetone and diethyl ether giving 370 mg of the product as an amorphous solid (73% yield): ^{1}H NMR (DMSO) δ 8.85 (m, 1H), 8.44 (m, 1H), 8.24 (m, 1H), 7.55 (s, 5H), 4.6 (s, 2H), 4.15 (s, 2H), 3.88 (d, 2H, J = 4 Hz), 3.75 (d, 2H, J = 4 Hz), 3.64 (m, 2H), 3.5 (m, 6H), 3.25 (s, 3H), 3.02 (s, 6H), 1.85 (m, 4H), 1.57 (m, 2H); MS M − H 433.4; HPLC >98%. Anal. ($C_{23}H_{39}N_5I_2O_3 \cdot 0.5H_2O$) C, H, I; N: calcd, 10.34; found, 9.64.

1-[1-[2-[*N*-Methyl-*N*-(phenylmethyl)-*N*-(2-propenyl)-ammonio]ethylamino]-*N*-[1-(2-propenyl)piperidinomethylcarbonyl]glycyl]glycine Dibromide (11b). Compound **10** (300 mg, 0.74 mmol) was dissolved in acetonitrile (15 mL) and stirred at room temperature for 60 h with allyl bromide (0.3 mL, 3.5 mmol), excluding light from the reaction. The solvent was removed under reduced pressure and the residue was triturated with acetone and diethyl ether giving 400 mg of the product as an amorphous solid (83% yield): ^{1}H NMR (DMSO) δ 8.94 (m, 1H), 8.45 (m, 1H), 8.32 (m, 1H), 7.54 (m, 5H), 6.13 (m, 2H), 5.65 (m, 4H), 4.6 (s, 2H), 4.25 (d, 2H, J = 6 Hz), 4.15 (m, 3H), 3.95 (d, 1H, J = 6 Hz), 3.88 (d, 2H, J = 6 Hz), 3.72 (d, 2H, J = 6 Hz), 3.6 (m, 4H), 3.45 (m, 4H), 2.95 (s, 3H), 1.85 (m, 4H), 1.55 (m, 2H); MS M − H 484.6; HPLC >73%. Anal. ($C_{27}H_{43}N_5Br_2O_3 \cdot 0.5H_2O$) C, H, Br; N: calcd, 10.97; found, 10.45.

1-[1-[2-[*N*-Methyl-*N*-(4-nitrophenylmethyl)-*N*-(phenylmethyl)ammonio]ethylamino]-*N*-[1-(4-nitrophenylmethyl)piperidinomethylcarbonyl]glycyl]glycine Dibromide (11c). Compound **10** (300 mg, 0.74 mmol) was reacted with 4-nitrobenzyl bromide (321 mg, 1.49 mmol) using conditions similar to those described for the synthesis of **11a**, giving 460 mg of the product (77% yield): ^{1}H NMR (DMSO) δ 8.98 (m, 1H), 8.51 (m, 1H), 8.35 (m, 5H), 7.96 (m, 2H), 7.88 (m, 2H), 7.62 (m, 2H), 7.54 (m, 3H), 5.05 (s, 2H), 5.0−4.5 (m, 4H), 4.05 (s, 2H), 3.95 (d, 2H, J = 4 Hz), 3.75 (m, 4H), 3.58 (m, 4H), 3.28 (m, 2H), 2.98 (s, 3H), 1.9 (m, 4H), 1.61 (m, 2H); MS M − H 674.2; HPLC >97%. Anal. ($C_{35}H_{45}N_7Br_2O_7 \cdot H_2O$) H, N; C: calcd, 49.25; found, 50.63; Br: calcd, 18.72; found, 17.86.

***N*,*N*-(Diphenylmethyl)glycyl-glycine (13a).** *N*,*N*-Dibenzylglycine (**12a**)[20] (2.55 g, 10 mmol) was suspended in DCM (100 mL) then glycine ethyl ester HCl salt (1.39 g, 10 mmol) was added followed by HOBT (1.35 g, 10 mmol), DCC (2 g, 10 mmol) and finally triethylamine (1.39 mL, 10 mmol). The resulting solution was stirred at room temperature for 60 h, the volume was reduced and DCU was removed by filtration. The solvent was evaporated and the residue was dissolved in ethyl acetate then shaken with 5% Na_2CO_3 (3 × 20 mL), dried and evaporated to give 3.2 g of the ester as a gum (94% yield): ^{1}H NMR ($CDCl_3$) δ 7.72 (m, 1H), 7.35 (m, 10H), 4.25 (q, 2H, J = 6 Hz), 4.0 (d, 2H, J = 4 Hz), 3.62 (s, 4H), 3.15 (s, 2H), 1.29 (t, 3H, J = 6 Hz). The ester (3.2 g, 9.4 mmol) was dissolved in MeOH (30 mL) and treated with 4 N NaOH (2.49 mL. 9.9 mmol) for 2 h at room temperature. 5 N HCl (1.99 mL, 9.9 mmol) was added and the solvent was removed under reduced pressure. The residue was extracted with DCM, filtered through Celite and evaporated to give 2.8 g of the product (95% yield): ^{1}H NMR ($CDCl_3$) δ 7.78 (m, 1H), 7.32 (m, 10H), 4.5 (s, 1H), 3.91 (d, 2H, J = 2 Hz), 3.67 (s, 4H), 3.19 (s, 2H).

***N*,*N*-(Diphenylmethyl)glycyl-glycine-2-(1-piperidino)-ethylamide (14a).** Compound **13a** (1 g, 3.2 mmol) was reacted with 2-aminoethylpiperidine (0.45 mL, 3.2 mmol) in the presence of HOBT (430 mg, 3.2 mmol) and DCC (660 mg, 3.2 mmol) in DCM (20 mL) using conditions similar to those described for the synthesis of **10**, giving 1.3 g of the product (98% yield): ^{1}H NMR (as di-HCl salt in D_2O) δ 7.52 (s, 10H), 4.5 (s, 4H), 3.95 (s, 2H), 3.76 (s, 2H), 3.55 (m, 4H), 3.22 (t, 2H, J = 6 Hz), 2.95 (t, 2H, J = 8 Hz), 1.95−1.55 (m, 6H).

[2-(*N*-Methylpiperidino)ethylamino][*N*-methyl-*N*,*N*-bis(phenylmethyl)glycyl]glycine Diiodide (15a). Prepared from compound **14a** using conditions and reagent quantities similar to those described during the synthesis of **11a**, giving 300 mg of the product (60% yield): ^{1}H NMR (DMSO) δ 8.95 (m, 1H), 8.35 (m, 1H), 7.58 (m, 10H), 4.85 (q, 4H, J = 10 Hz), 3.85 (m, 4H), 3.56 (m, 2H), 3.38 (m, 6H), 3.07 (s, 3H), 3.02 (s, 3H), 1.78 (m, 4H), 1.54 (m, 2H); MS M − H 451.0; HPLC >97%. Anal. ($C_{27}H_{40}N_4I_2O_2 \cdot 0.25H_2O$) C, H, N, I.

***N*-(1-Piperidinomethylcarbonyl)-L-phenylalanine (13b).** Compound **12b**[19] (700 mg, 5 mmol) was reacted with L-phenylalanine methyl ester HCl salt (1.08 g, 5 mmol) using conditions and reagent quantities similar to those described for the synthesis of **13a** giving 1.5 g of the ester (99% yield): ^{1}H NMR ($CDCl_3$) δ 7.71 (d, 1H, J = 6 Hz), 7.28 (m, 2H), 7.15 (m, 3H), 4.9 (m, 1H), 3.74 (s, 3H), 3.15 (t, 2H, J = 6 Hz), 2.9 (d, 2H, J = 4 Hz), 1.85 (m, 4H), 1.45 (m, 6H). The ester (1.4 g, 4.6 mmol) was hydrolyzed in dioxane (14 mL) using conditions and reagent quantities similar to those described during the synthesis of **13a**, giving 540 mg of the product (41% yield): ^{1}H NMR ($CDCl_3$) δ 8.45 (d, 1H, J = 4 Hz), 7.25 (m, 5H), 5.0 (s, 1H), 4.69 (m, 1H), 3.55 (d, 1H, J = 8 Hz), 3.29 (m, 2H), 2.95 (m, 1H), 2.46 (m, 4H), 1.66 (m, 4H), 1.46 (m, 2H).

***N*-(1-Piperidinomethylcarbonyl)-L-phenylalanine-2-(4-morpholino)ethylamide (14b).** Compound **13b** (540 mg, 1.9 mmol) was reacted with 4-(2-aminoethyl)morpholine (0.24 mL, 1.9 mmol) using conditions and reagent quantities similar to those described for the synthesis of **14a** giving 700 mg of the

product (93% yield): ^{1}H NMR ($CDCl_3$) δ 7.56 (d, 1H, J = 6 Hz), 7.25 (m, 5H), 6.25 (m, 1H), 4.65 (q, 1H, J = 6 Hz), 3.61 (m, 4H), 3.26 (q, 2H, J = 4 Hz), 3.08 (d, 2H, J = 6 Hz), 2.89 (d, 2H, J = 6 Hz), 2.31 (m, 10H), 1.5 (m, 6H).

2-[*N*-(4-Methylmorpholino)]ethylamino-*N*-(1-methylpiperidinomethylcarbonyl)-L-phenylalanine Diiodide (15b). Prepared from compound **14b** using conditions and reagent quantities similar to those described during the synthesis of **15a**, giving 370 mg of the product (73% yield): ^{1}H NMR (D_2O) δ 7.35 (m, 5H), 4.71 (t, 1H, J = 6 Hz), 4.05 (m, 6H), 3.65 (m, 2H), 3.42 (m, 10H), 3.15 (s, 3H), 3.13 (m, 2H), 3.08 (s. 3H), 1.87 (m, 4H), 1.55 (m, 2H); MS M − H 431.5; HPLC >98%. Anal. ($C_{24}H_{40}N_4I_2O_3\cdot 0.75H_2O$) C, H, N, I.

2-*N*-[4-(4-Nitrophenylmethyl)morpholino]ethylamino-*N*-[1-(4-nitrophenylmethyl)piperidinomethylcarbonyl]-L-phenylalanine Dibromide (15c). Prepared from compound **14b** using conditions and reagent quantities similar to those described during the synthesis of **11c**, giving 460 mg of the product (58% yield): ^{1}H NMR (DMSO) δ 9.17 (d, 1H, J = 4 Hz), 8.78 (m, 1H), 8.35 (d, 2H, J = 6 Hz), 8.29 (d, 2H, J = 6 Hz), 7.97 (d, 2H, J = 6 Hz), 7.77 (d, 2H, J = 6 Hz), 7.28 (m, 4H), 7.22 (m, 1H), 5.0 (s, 2H), 4.88 (m, 2H), 4.75 (m, 1H), 3.98 (m, 6H), 3.75 (m, 2H), 3.53 (m, 4H), 3.43 (m, 2H), 3.34 (m, 2H), 3.25 (m, 2H), 3.15 (m, 1H), 2.82 (m, 1H), 1.85 (m, 2H), 1.75 (m, 2H), 1.55 (m, 1H), 1.46 (m, 1H); MS M − H 673.6; HPLC >98%. Anal. ($C_{36}H_{46}N_6Br_2O_7\cdot 0.75H_2O$) C, H, N; Br: calcd, 18.21; found, 17.74.

***N*-Methyl-*N*-(1-piperidinomethylcarbonyl)glycine (17a).** Compound **12b**[19] (1.29 g, 9 mmol) was reacted with sarcosine ethyl ester HCl salt (1.31 g, 9 mmol) using conditions and reagent quantities similar to those described for the synthesis of **13a** giving 1.7 g of the ester (83% yield): ^{1}H NMR ($CDCl_3$) δ 4.22 (m, 4H), 3.19 (s, 1H), 3.17 (s, 1H), 3.08, 2.99 (2s, 3H), 2.44 (m, 4H), 1.5 (m, 6H), 1.3 (m, 3H). The ester (1.7 g, 7 mmol) was hydrolyzed using conditions and reagent quantities similar to those described for the synthesis of **13a**, giving 1.5 g of the product (99% yield): ^{1}H NMR ($CDCl_3$) δ 6.25 (s, 1H), 3.91−3.78 (3s, 4H), 3.35 (m, 3H), 3.21 (m, 1H), 3.06−3.0 (2s, 3H), 1.88 (m, 4H), 1.62 (m, 2H).

***N*-Methyl-*N*-[*N*-methyl-*N*-(1-piperidinomethylcarbonyl)glycyl]glycine (18a).** Compound **17a** (1.5 g, 7 mmol) was reacted with sarcosine ethyl ester HCl salt (1.02 g, 7 mmol) using conditions and reagent quantities similar to those described for the synthesis of **17a** giving 1.4 g of the ester (64% yield): ^{1}H NMR ($CDCl_3$) δ 4.28−4.13 (m, 6H), 3.19 (s, 2H), 3.17 (s, 2H), 3.08 (m, 6H), 2.44 (m, 3H), 2.38 (m, 1H), 1.62 (m, 2H), 1.42 (m, 2H), 1.25 (m, 3H). The ester (1.4 g, 4.5 mmol) was hydrolyzed using conditions and reagent quantities similar to those described for the synthesis of **17a**, giving 1.3 g of the product (99% yield): ^{1}H NMR ($CDCl_3$) δ 6.75 (s, 1H), 4.24 (m, 2H), 3.92 (m, 4H), 3.32 (m, 4H), 2.99 (m, 6H), 1.88 (m, 4H), 1.62 (m, 2H).

***N*-Methyl-*N*-[*N*-methyl-*N*-(1-piperidinomethylcarbonyl)glycyl]glycine-*N*-methyl-2-[(methyl-1-phenylmethyl)amino]ethylamide (19a).** Compound **18a** (3.5 g, 12.27 mmol) was reacted with 2-(methylaminoethyl)benzylmethylamine[18] (1.97 g, 11.05 mmol) in DMF (30 mL) in the presence of DIPEA (4.3 mL, 22.1 mmol) and HBTU (4.65 g, 12.27 mmol), stirring at room temperature for 24 h. The solution was added to water (200 mL) and extracted with DCM (100 mL + 50 mL) and the combined extracts were washed with brine then evaporated to give a gum, which was dried under high vacuum to remove residual DMF. The product was purified using flash chromatography over fine silica (30 g) and gradient elution (DCM−DCM/MeOH 90/10−DCM/MeOH/NH_3 190/10/2) giving 3.5 g (71% yield): ^{1}H NMR ($CDCl_3$) δ 7.28 (m, 5H), 4.2 (m, 2H), 3.52 (m, 3H), 3.3 (m, 1H), 3.18 (m, 1H), 3.15 (m, 2H), 3.05 (m, 3H), 2.96 (m, 4H), 2.8 (m, 1H), 2.5 (m, 6H), 2.24 (m, 3H), 1.85 (s, 2H), 1.58 (m, 4H), 1.44 (m, 2H).

1-[1-[2-[*N*,*N*-Dimethyl-*N*-(phenylmethyl)ammonio]ethyl-*N*-methylamino]-*N*-(1-methylpiperidinomethylcarbonyl)-*N*-methylglycyl]-*N*-methylglycine Diiodide (20a). Prepared from compound **19a** (300 mg, 0.67 mmol) using conditions and reagent quantities similar to those described during the synthesis of **11a**, giving 450 mg of the product (92% yield): ^{1}H NMR (DMSO) δ 7.56 (m, 5H), 4.68 (m, 2H), 4.52 (m, 2H), 4.32 (m, 2H), 4.22 (m, 2H), 3.85 (m, 2H), 3.72 (m, 2H), 3.48 (m, 4H), 3.25 (m, 2H), 3.02 (m, 14H), 2.85 (m, 2H), 1.85 (m, 4H), 1.55 (m, 2H); MS M + I 602.2 (M − H not found); HPLC >99%. Anal. ($C_{26}H_{45}N_5I_2O_3\cdot H_2O$) C, H, N; I: calcd, 33.95; found, 36.50.

1-[1-[2-[*N*-Methyl-*N*-(phenylmethyl)-*N*-(2-propenyl)ammonio]ethyl-*N*-methylamino]-*N*-[1-(2-propenyl)piperidinomethylcarbonyl]-*N*-methylglycyl]-*N*-methylglycine Dibromide (20b). Prepared from compound **19a** (300 mg, 0.67 mmol) using conditions and reagent quantities similar to those described during the synthesis of **11b**, giving 340 mg of the product (73% yield): ^{1}H NMR ($CDCl_3$) δ 7.28 (m, 2H), 7.47 (m, 3H), 6.05 (m, 2H), 5.75 (m, 4H), 5.18 (m, 2H), 4.85 (m, 2H), 4.65 (m, 2H), 4.45 (m, 4H), 4.28 (m, 2H), 4.15 (m, 6H), 3.52 (m, 2H), 3.25 (m, 6H), 2.95 (m, 6H), 1.95 (m, 6H); MS M − H 526.7; HPLC >98%. Anal. ($C_{30}H_{49}N_5Br_2O_3\cdot 2H_2O$) H, N, Br; C: calcd, 49.80; found, 50.50.

1-[1-[2-[*N*-Methyl-*N*-(4-nitrophenylmethyl)-*N*-(phenylmethyl)ammonio]ethyl-*N*-methylamino]-*N*-[1-(4-nitrophenylmethyl)piperidinomethylcarbonyl]-*N*-methylglycyl]-*N*-methylglycine Dibromide (20c). Prepared from compound **19a** (300 mg, 0.67 mmol) using conditions and reagent quantities similar to those described during the synthesis of **11c**, giving 450 mg of the product (76% yield): ^{1}H NMR (D_2O) δ 8.38 (m, 1H), 8.25 (m, 3H), 7.65 (m, 9H), 4.97 (s, 2H), 4.69 (m, 4H), 4.53 (s, 2H), 4.25 (m, 2H), 4.22 (s, 2H), 4.11 (m, 2H), 3.88 (m, 2H), 3.68 (m, 2H), 3.54 (m, 2H), 3.15 (m, 4H), 3.05 (m, 4H), 2.95 (m, 4H), 2.05 (m, 4H), 1.78 (m, 2H); HPLC >97%. Anal. ($C_{38}H_{51}N_7Br_2O_7\cdot 3.75H_2O$) C, H, N, Br.

***N*-Methyl-*N*-(1-piperidinoethylcarbonyl)glycine (17b).** 1-Piperidinepropionic acid (**16**) (7.68 g, 50 mmol) was reacted with sarcosine ethyl ester HCl salt (7.3 g, 47.5 mmol) and DIPEA (8.28 mL, 47.5 mmol) using conditions and reagent quantities similar to those described for the synthesis of **13a** giving 4.35 g of the ester (36% yield): ^{1}H NMR ($CDCl_3$) δ 4.22 (m, 4H), 3.09, 2.98 (2s, 3H), 2.68 (m, 4H), 2.45 (m, 4H), 1.61 (m, 4H), 1.45 (m, 2H), 1.29 (m, 3H). The ester (4.35 g, 17 mmol) was hydrolyzed using conditions and reagent quantities similar to those described for the synthesis of **13a**, giving 3 g of the product (67% yield), as the HCl salt: ^{1}H NMR (D_2O) δ 4.38 (m, 1H), 3.95 (m, 1H), 3.71 (m, 4H), 3.18 (m, 7H), 2.11 (m, 4H), 1.81 (m, 2H).

***N*-Methyl-*N*-[*N*-methyl-*N*-(1-piperidinoethylcarbonyl)glycyl]glycine (18b).** Compound (**17b**) (3 g, 11.3 mmol) was reacted with sarcosine ethyl ester HCl salt (1.77 g, 11.5 mmol) and DIPEA (4.2 mL, 22 mmol) using conditions and reagent quantities similar to those described for the synthesis of **17a** giving 3.7 g of the ester, which was purified by chromatography over silica (40 g), using DCM/MeOH/NH_3 (500/10/1) as eluant, which gave 2.3 g (62% yield): ^{1}H NMR ($CDCl_3$) δ 4.17 (m, 6H), 3.10, 3.07, 2.96, 2.95 (4s, 6H), 2.56 (m, 4H), 2.45 (m, 4H), 1.58 (m, 4H), 1.45 (m, 2H), 1.28 (m, 3H). The ester (2.3 g, 7 mmol) was hydrolyzed in dioxane (20 mL), using conditions and reagent quantities similar to those described for the synthesis of **17a**, giving 2.15 g of the product (89% yield), as the HCl salt: ^{1}H NMR (D_2O) δ 4.45 (m, 4H), 3.92 (2s, 1H), 3.68 (m, 2H), 3.55 (m, 2H), 3.15 (m, 9H), 2.05 (m, 4H), 1.85 (m, 2H).

***N*-Methyl-*N*-[*N*-methyl-*N*-(1-piperidinoethylcarbonyl)glycyl]glycine-*N*-methyl-2-[(methyl-1-phenylmethyl)amino]ethylamide (19b).** Compound **18b** (2.15 g, 6.4 mmol) was reacted with 2-(methylaminoethyl)benzylmethylamine[18] (1.08 g, 6.1 mmol) using conditions and reagent quantities similar to those described for the synthesis of **19a**, giving on extraction 2.8 g of the product, which was chromatographed using similar elution techniques giving 1.55 g (55% yield): ^{1}H NMR ($CDCl_3$) δ 7.28 (s, 5H), 4.17 (m, 3H), 3.51 (m, 3H), 3.28 (m, 1H), 3.05 (m, 10H), 2.81 (m, 3H), 2.53 (m, 7H), 2.24 (m, 3H), 1.62 (m, 4H), 1.49 (m, 2H).

1-[1-[2-[*N*-Methyl-*N*-(4-nitrophenylmethyl)-*N*-(phenylmethyl)ammonio]ethyl-*N*-methylamino]-*N*-[1-(4-nitrophenylmethyl)piperidinoethylcarbonyl]-*N*-methylglycyl]-*N*-methylglycine Dibromide (20d). Compound **19b** (600 mg,

1.31 mmol) was reacted with 4-nitrobenzyl bromide (680 mg, 3.1 mmol) in acetonitrile (8 mL) for 24 h at room temperature. The solvent was removed and the residue was chromatographed over alumina (18 g) using DCM/2-propanol (5/1) as eluant, followed by precipitation of the product from acetone and ether, giving 200 mg (17% yield): ^{1}H NMR (DMSO) δ 8.31 (m, 4H), 7.95 (m, 2H), 7.81 (m, 2H), 7.64 (m, 2H), 7.51 (m, 3H), 4.8 (m, 4H), 4.55 (m, 2H), 4.29 (m, 3H), 3.93 (m, 2H), 3.48 (m, 4H), 3.32 (m, 4H), 2.99 (m, 11H), 2.81 (m, 4H), 1.86 (m, 4H), 1.55 (m, 2H); MS M − H 730.8; HPLC >91%. Anal. ($C_{39}H_{53}N_7Br_2O_7 \cdot 4H_2O$) C, H, N, Br.

***N*-Methyl-*N*-[*N*-methyl-*N*-(1-piperidinomethylcarbonyl)glycyl]glycine-1-(4-phenylmethyl)piperazine Amide (19c).** Compound **18a** (3 g, 10.5 mmol) was reacted with 1-benzylpiperazine (1.66 g, 9.5 mmol) using conditions and reagent quantities as described for the synthesis of **19a**, giving 3.05 g of the product after chromatography (65% yield): ^{1}H NMR ($CDCl_3$) δ 7.31 (s, 5H), 4.21 (m, 4H), 3.51 (m, 8H), 3.15 (m, 4H), 3.07 (m, 4H), 2.45 (m, 6H), 1.55 (m, 4H), 1.42 (m, 2H).

1-[[*N*-(4-Nitrophenylmethyl)-*N*-(phenylmethyl)piperazin-4-yl]-*N*-[1-(4-nitrophenylmethyl)piperidinomethylcarbonyl]-*N*-methylglycyl]-*N*-methylglycine Dibromide (20e). Compound **19c** (1.5 g, 3.38 mmol) was reacted with 4-nitrobenzyl bromide (1.9 g, 8.79 mmol) using conditions and reagent quantities as described for the synthesis of **20d**, giving after chromatography 520 mg of the product (18% yield): ^{1}H NMR (D_2O) δ 8.36 (m, 4H), 7.75 (m, 4H), 7.56 (m, 5H), 4.86 (m, 6H), 4.38 (m, 4H), 4.15 (m, 6H), 3.85 (m, 2H), 3.62 (m, 6H), 3.08, 2.98, 2.93, 2.89 (4s, 6H), 2.02 (m, 4H), 1.75 (m, 2H); MS M − H 714.7; HPLC >95%. Anal. ($C_{38}H_{49}N_7Br_2O_7 \cdot 4H_2O$) C, H, N; Br: calcd, 16.86; found, 16.41.

Molecular Modeling − Experimental. X-ray data: Compounds were extracted from the Cambridge Crystallographic Structure Database[29] as Fdat files and read into Chem-X,[30] where the interquaternary nitrogen distances were measured.

Conformational analyses: Crude range-finding conformational analyses were performed using Chem-X. For the tripeptide derivative **11a**, a grid search through all the nonterminal freely rotatable torsion angles was carried out. Amide bonds were excluded and remained trans throughout. All non-amide bonds between the two keto groups nearest the two quaternary nitrogens were rotated through 360° in 60° degree increments. All other non-amide bonds between the two quaternary nitrogens were rotated through 360° in 120° increments. The default Chem-X parameter files, Gasteiger charges, and molecular mechanics force field were used throughout. The minimum energy conformation had an interquaternary distance of 1.43 nm. This distance varied between 1.15 and 1.49 nm in conformations within 10 kcal/mol of the search minimum.

For the dipeptide derivative **9a**, a similar crude conformational analysis was performed. Again all amide bonds were held trans throughout. All other nonterminal bonds between the two quaternary nitrogens were rotated through 360° in 60° degree increments, with the exception of the only CH_2-CH_2 bond, where 120° increments were used. The conformational search energy minimum conformation had an interquaternary distance of 1.08 nm. This distance varied between 0.91 and 1.13 nm in conformations within 10 kcal/mol of the search minimum. Clog *P* values were calculated using PCModels. [28] Plots were produced using Microsoft Excel.[31]

Pharmacological − Experimental. Neuromuscular Studies. a. Isolated chick biventer cervicis muscle: Biventer cervicis nerve muscle preparations from young chickens (3−12 day old) were dissected and mounted in Krebs Henseleit solution of the following composition: NaCl 118, KCl 5, KH_2PO_4 1, $MgSO_4$ 1, $NaHCO_3$ 30, $CaCl_2$ 2.5, and glucose 20 mmol/L. The bathing fluid was bubbled with 5% carbon dioxide in oxygen to maintain a pH of 7.4. The methodology for setting up this preparation was essentially similar to that described by Ginsborg and Warriner.[32] The motor nerves were stimulated at 10-s intervals (0.1 Hz) with rectangular pulses of 0.2-ms duration and at a supramaximal voltage. Recordings of twitch tension were made on an ink writing chart recorder, via Grass FT03 force displacement transducers. After an initial 30-min stabilization period, drugs were added cumulatively to the tissue bath (normally 4−5 concentrations) to produce 85%−95% twitch block. Concentration−twitch inhibition response lines were constructed using the method of least squares, and concentrations of the compounds producing 50% twitch block were determined.

b. Anesthetized cat: Neuromuscular blocking experiments in cats were carried out using similar methodology to that described previously.[12] Cats of either sex (1.9−2.6 kg) were anesthetized with a mixture of α-chloralose (80 mg/kg) and sodium pentobarbitone (5 mg/kg) injected intraperitoneally. The lungs were ventilated with room air at a rate of 28 breaths min^{-1} and at a stroke volume (12−15 mL/kg) to maintain arterial blood pH between 7.3 and 7.4. Twitch responses of the tibialis anterior muscle were elicited every 10 s (0.1 Hz), by stimulating the sciatic nerve with rectangular pulses of 0.25-ms duration and at supramaximal voltage, and quantitated using a Grass FT03 force displacement transducer, and recorded on a Grass chart recorder. Arterial pressure was measured via a catheter placed in the right carotid artery and connected to a Gould-Statham pressure transducer. Heart rate was monitored continuously using the arterial pressure pulse to trigger a Grass cardiotachometer. Signals were amplified and recorded on a Grass model 7 chart-recorder. Rectal temperature was monitored and maintained at 37 ± 1 °C. Drugs were administered through a catheter in the right external jugular vein.

Effects on autonomic mechanisms were measured simultaneously every 100 s, by recording the effect of the drugs on decreases in heart rate, produced by stimulating the vagus nerve for 10 s at 2−5 Hz, and on nictitating membrane contractions, induced by stimulating the cervical sympathetic nerve. Toward the end of some experiments, logarithmically increasing doses of the compounds were injected at 100-s intervals to obtain blocks of the vagus nerve-induced bradycardia and cervical sympathetic nerve-induced contractions of the nictitating membrane.

c. Anesthetized monkeys: Experiments were carried out in fasted Rhesus monkeys (5.0−6.5 kg) premedicated with intramuscular atropine sulfate (0.25 mg) to prevent excessive salivation. Anesthesia was induced with 10 mg/kg ketamine intramuscular. The trachea was intubated without the use of a muscle relaxant. Anesthesia was maintained using a loading iv bolus dose of pentobarbitone sodium (15−25 mg/kg) followed by an infusion, which was adjusted to produce a clinically steady depth of anesthesia. Artificial ventilation was applied (60% N_2O in oxygen) with an Ohmeda ventilator and end-tidal carbon dioxide concentration was measured and maintained at 4−4.5%. Heart rate was measured continuously from the ECG signal (automatic counting of QRS-complexes). A catheter was introduced percutaneously into a superficial vein in one of the hind limbs for drug administrations. Arterial blood pressure was recorded continuously using a Finapress noninvasive automated blood pressure monitor. The ulnar nerve was stimulated with rectangular electrical pulses (0.1 Hz) via bipolar subcutaneous needle electrodes. The resulting twitches of the adductor pollicis muscle were recorded using a Statham UC3 transducer, attached by a wire to a U-clamp, which was fixed to the basal phalanx of the thumb. All parameters were recorded on a Nihon-Kohden chart recorder and on a computer hard-disk for further analysis.

Protocol. The effects of the drugs on neuromuscular transmission were determined in cats and monkeys, by injecting increasing doses of the neuromuscular blocking drugs at 30−60-min intervals to produce a range of levels of neuromuscular block, i.e. between 10% and 95% inhibition of twitch tension. Dose−response lines for the data were plotted from regression analysis of points lying between 15% and 85% of maximum block, to confine the analysis to the linear portion of the log dose−inhibition line. Doses producing 50% inhibition of electrically induced contractions of the muscles were calculated by linear interpolation of these lines. Doses of the

neuromuscular blocking compounds producing 50% block of the induced bradycardia and nictitating membrane contractions were calculated similarly.

Time course measurements were made using doses of the drugs producing 80−95% block (cats) and also at 3 × 90% blocking doses (monkeys). Onset time was measured from drug injection to the first maximally depressed twitch, recovery$_{25-75\%}$ time was time taken for the twitches to recover from 25−75% of control, and duration times were from injection to 90% spontaneous recovery compared to predrug twitches.

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