Solid-Phase Synthesis and Biological Evaluation of a Combinatorial Library of Philanthotoxin Analogues

Kristian Strømgaard,[†] Tim J. Brier,[‡] Kim Andersen,[§] Ian R. Mellor,[‡] Alex Saghyan,[‡] Denis Tikhonov,[‡] Peter N. R. Usherwood,[‡] Povl Krogsgaard-Larsen,[†] and Jerzy W. Jaroszewski*,[†]

Department of Medicinal Chemistry and NeuroScience PharmaBiotec Research Center, Royal Danish School of Pharmacy, Universitetsparken 2, DK-2100 Copenhagen, Denmark, Division of Molecular Toxicology, School of Biological Sciences, University of Nottingham, University Park, Nottingham NG7 2RD, United Kingdom, and Department of Combinatorial Chemistry, Medicinal Chemistry Research, H. Lundbeck A/S, Ottiliavej 9, DK-2500 Valby, Denmark

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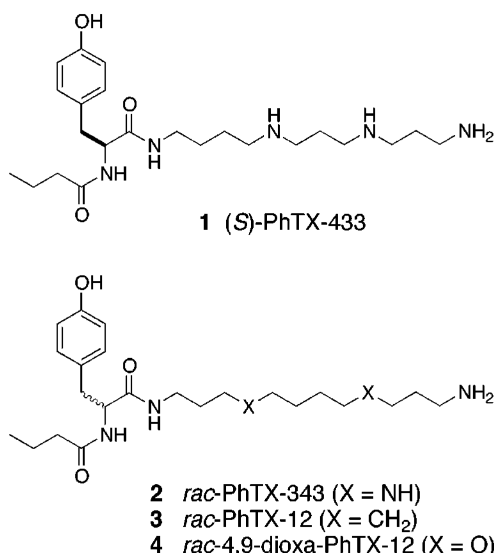
The modular structure of philanthotoxins was exploited for construction of the first combinatorial library of these compounds using solid-phase parallel synthesis. (*S*)-Tyrosine and (*S*)-3-hydroxyphenylalanine were used as amino acid components, spermine, 1,12-dodecanediamine, and 4,9-dioxa-1,12-dodecanediamine as amine components, and butanoyl, phenylacetyl, and cyclohexylacetyl as *N*-acyl groups. Following automated preparative HPLC, the resulting 18 compounds were isolated as the *S*-forms in 40–70% yields. The purity of the products was determined by HPLC with evaporative light scattering detection and by ^{1}H and ^{13}C NMR. The thus obtained philanthotoxins were tested electrophysiologically for their antagonist properties on human muscle-type nicotinic acetylcholine receptors (nAChR) expressed in TE671 cells and on rat brain non-NMDA glutamate receptors (non-NMDAR) expressed in *Xenopus* oocytes. 4-Hydroxy analogues lacking the secondary amino groups (PhTX-12 and 4,9-dioxa-PhTX-12 and their analogues) were inactive on non-NMDAR, whereas the potency of the spermine derivatives (PhTX-343 and its analogues) increased with steric bulk of the *N*-acyl group. The analogue of PhTX-343 in which the *N*-butanoyl group was replaced by phenylacetyl group had IC_{50} of 15 ± 4 nM on non-NMDAR. Increasing the steric bulk of the *N*-acyl group was not advantageous for activity at nAChR, and a sharp decrease in potency with increased steric bulk was observed with the derivatives of PhTX-12. 3-Hydroxy analogues generally exhibited lower activity and different response to alterations of the *N*-acyl groups as compared to the 4-hydroxy analogues. Since the acyl group alterations in PhTX-343 and 4,9-dioxa-PhTX-12 have a similar effect on potency, which is distinctly different from that observed for PhTX-12, the two former compounds may bind to nAChR in a similar fashion but differently from that of PhTX-12. The combinatorial library approach described in this work represents a prototype methodology for future exploration of structure–activity relationships of philanthotoxins.

Introduction

Philanthotoxins, a group of noncompetitive antagonists of ionotropic receptors, are composed of long-chain polyamines connected to a relatively nonpolar head-group via an amide bond.[1–6] The prototype structure is philanthotoxin-433 [PhTX-433 (**1**), Chart 1], a venom constituent of the Egyptian digger wasp *Philanthus triangulum*.[1] The interest in medicinal chemistry and pharmacology of philanthotoxins has recently been highlighted by the observation that specificity of their antagonist action on various classes of ionotropic receptors can be achieved by modification of the polyamine portion of the molecule.[7,8] Thus, the natural toxin PhTX-433 (**1**) and its synthetic analogue PhTX-343 (**2**) antagonize various types of nicotinic acetylcholine receptors (nAChRs)[3,6,9–11] and ionotropic glutamate receptors (iGluRs)[2–5,12–16] with rather similar potency. On the other hand, the analogues **3** (PhTX-12) and **4** (4,9-dioxa-PhTX-12), in which the secondary amino groups are replaced by methylene groups or oxygen atoms, exhibit enhanced antagonist activity at mammalian muscle-type nAChR and *Torpedo* nAChR while being inactive

Chart 1



* To whom correspondence should be addressed. Phone: 45 3530 6372. Fax: 45 3530 6040. E-mail: jj@dfh.dk.
† Royal Danish School of Pharmacy.
‡ University of Nottingham.
§ H. Lundbeck A/S.

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on several types of iGluR.[7,8,17] The latter selectivity is not obtained when the aromatic headgroup portion of the philanthotoxin molecule is modified.[3,4,10]

In previous structure–activity investigations, a considerable number of synthetic analogues of **2** containing the symmetrical spermine moiety or a closely related polyamine have been tested on iGluR and nAChR.[2–6,9,10,15] The results of these investigations emphasize the importance of the hydrophobic character of the headgroup. By contrast, no information about the influence of the structure of the headgroup on the potency of philanthotoxin analogues that lack inner basic sites (such as **3** and **4**) is available. This issue is addressed in the studies described herein. A comparison of structure–activity relationships (SARs) for **2**–**4** might indicate whether the three compounds bind to nAChR in a similar fashion.

Results

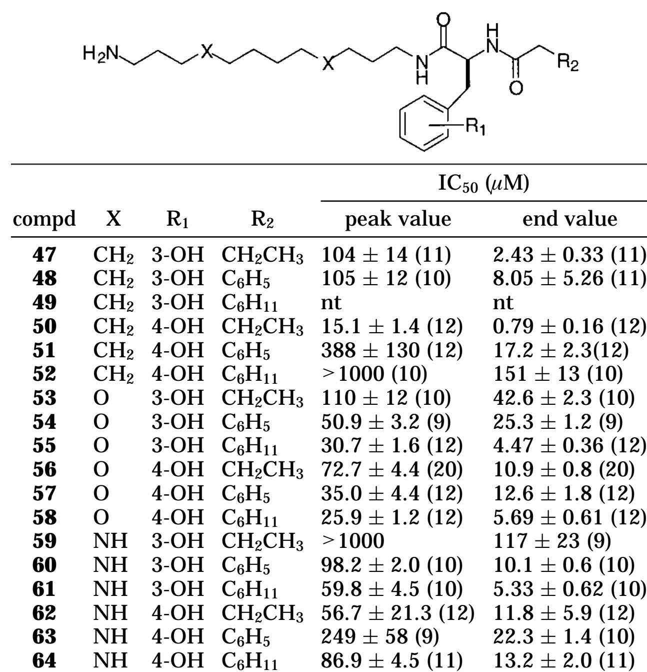
Synthesis. Because of the presence of three distinct parts of the molecule (an amino acid moiety, an acyl group, and a long-chain polyamine moiety) joined by amide bonds, philanthotoxins are particularly suitable for synthesis using solid-phase methodology similar to that used in peptide synthesis. Moreover, variation of the structures of the three building blocks can provide a combinatorial library of philanthotoxins for exploration of SARs. In the work described herein, two different amino acids [(*S*)-tyrosine and (*S*)-3-hydroxyphenylalanine], three carboxylic acids (butanoic, phenylacetic, and cyclohexylacetic acid), and three amines (spermine, 1,12-dodecanediamine, and 4,9-dioxa-1,12-dodecanediamine) were used for parallel synthesis of a library of 18 analogues. Solid-phase methods employed in this work are essentially the same as those recently published by Strømgaard et al. for synthesis of the enantiomers of **2** and **3**.[8]

The synthetic procedure is shown in Scheme 1. The diamines **5**–**7** were attached to trityl chloride resin to afford the resins **8**–**10**, respectively. The required diamines **5** and **6** were commercial products, whereas **7** was synthesized from spermine in three steps as previously described.[8] A 10-fold molar excess of the diamines was used in the resin derivatization step to minimize cross-linking reaction.[8]

The coupling of either (*S*)-*N*-(9-fluorenylmethoxycarbonyl)-3-(*tert*-butoxy)phenylalanine (**11**) or (*S*)-*N*-(9-fluorenylmethoxycarbonyl)-*O*-(*tert*-butyl)tyrosine (**12**) to the free amino groups of the resins **8**–**10** was accomplished with excess of HATU (**13**) and collidine,[18–21] to give the respective resins **14**–**19**. Deprotection of the primary amino group was carried out with piperidine, which gave the resins **20**–**25**. In the subsequent step, the *N*-acyl groups were attached using butanoic acid (**26**), phenylacetic acid (**27**), or cyclohexylacetic acid (**28**), again using HATU (**13**) and collidine. This gave the resins **29**–**46**.

Finally, cleavage from the resins with simultaneous deprotection of the phenol groups, and in the case of **41**–**46** also deprotection of the secondary amino groups, afforded philanthotoxin analogues **47**–**64**. This step was accomplished with a mixture of trifluoroacetic acid (TFA) and the scavengers triisopropylsilane and H_2O. The crude products were subjected to automated preparative HPLC–MS.[22] The yields of purified **47**–**52** were about 40%, based on **8**, and those of **53**–**58** and **59**–**64** about 60–70%, based on **9** and **10**, respectively. Purity of the final products was in the range of 97–100%, as determined by HPLC with evaporative light scattering (ELS)[23] detection. This method is capable of detection and quantification of amine impurities in the final products. The amines originate from the resin cross-linking reactions and cannot be detected by conventional UV monitoring of the HPLC eluate.

Table 1. Antagonist Effect of Philanthotoxin Analogues on nAChR Expressed in TE671 Cells[a]



compd	X	R1	R2	IC50 (μM) peak value	IC50 (μM) end value
47	CH_2	3-OH	CH_2CH_3	104 ± 14 (11)	2.43 ± 0.33 (11)
48	CH_2	3-OH	C_6H_5	105 ± 12 (10)	8.05 ± 5.26 (11)
49	CH_2	3-OH	C_6H_{11}	nt	nt
50	CH_2	4-OH	CH_2CH_3	15.1 ± 1.4 (12)	0.79 ± 0.16 (12)
51	CH_2	4-OH	C_6H_5	388 ± 130 (12)	17.2 ± 2.3(12)
52	CH_2	4-OH	C_6H_{11}	>1000 (10)	151 ± 13 (10)
53	O	3-OH	CH_2CH_3	110 ± 12 (10)	42.6 ± 2.3 (10)
54	O	3-OH	C_6H_5	50.9 ± 3.2 (9)	25.3 ± 1.2 (9)
55	O	3-OH	C_6H_{11}	30.7 ± 1.6 (12)	4.47 ± 0.36 (12)
56	O	4-OH	CH_2CH_3	72.7 ± 4.4 (20)	10.9 ± 0.8 (20)
57	O	4-OH	C_6H_5	35.0 ± 4.4 (12)	12.6 ± 1.8 (12)
58	O	4-OH	C_6H_{11}	25.9 ± 1.2 (12)	5.69 ± 0.61 (12)
59	NH	3-OH	CH_2CH_3	>1000	117 ± 23 (9)
60	NH	3-OH	C_6H_5	98.2 ± 2.0 (10)	10.1 ± 0.6 (10)
61	NH	3-OH	C_6H_{11}	59.8 ± 4.5 (10)	5.33 ± 0.62 (10)
62	NH	4-OH	CH_2CH_3	56.7 ± 21.3 (12)	11.8 ± 5.9 (12)
63	NH	4-OH	C_6H_5	249 ± 58 (9)	22.3 ± 1.4 (10)
64	NH	4-OH	C_6H_{11}	86.9 ± 4.5 (11)	13.2 ± 2.0 (11)

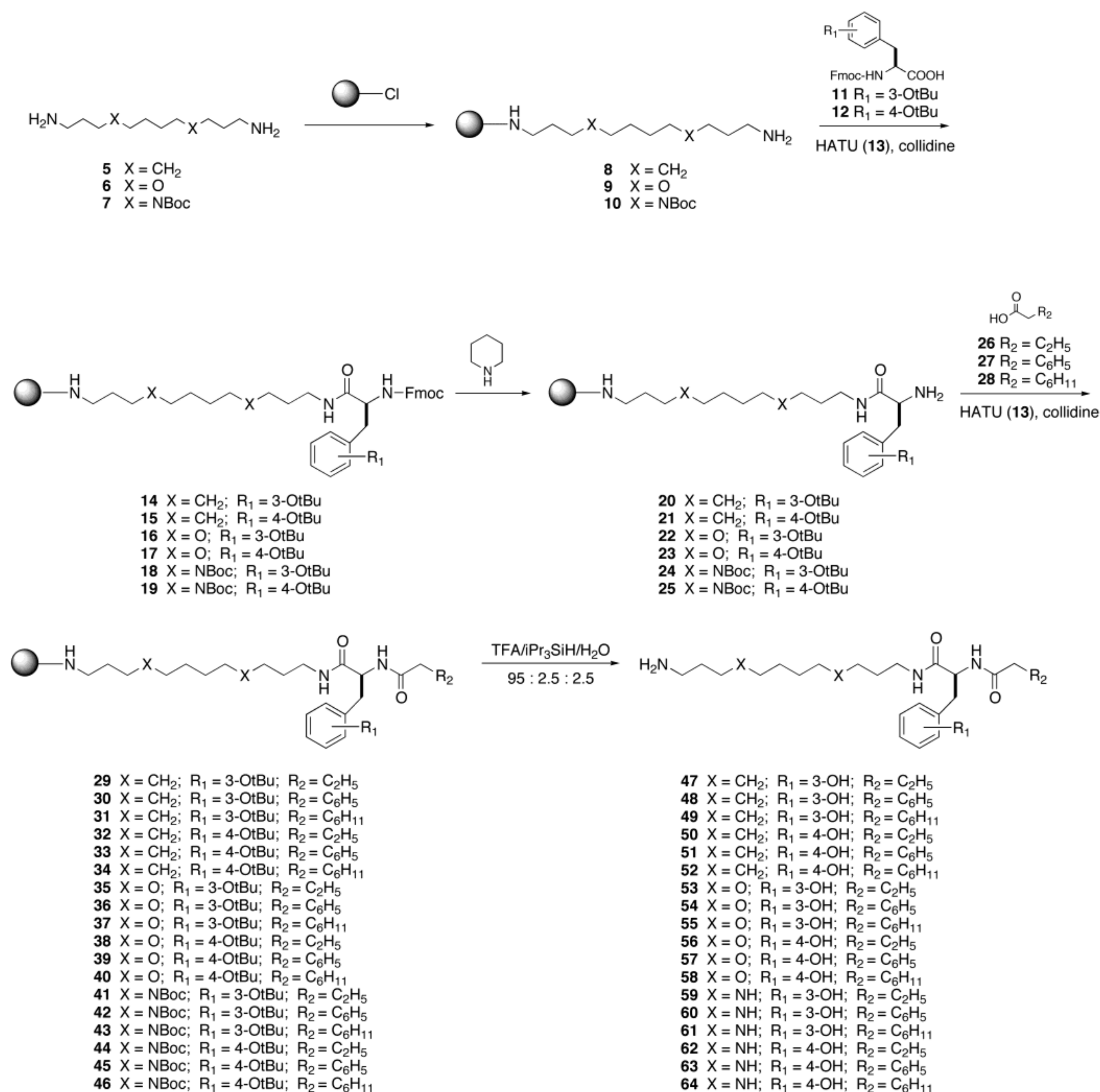
[a] From decrease of the current elicited by 10 μM acetylcholine (holding potential V_H = −100 mV) by simultaneous co-application of the antagonist, at the peak value of the current and at the end of a 1-s application period (number of experiments given in parentheses); nt, not tested.

All final products were characterized by ^1H and ^{13}C NMR spectroscopy as well as mass spectrometry. Since enantiomerically pure amino acids **11** and **12** were used as the starting materials, and the synthesis as described in Scheme 1 was previously demonstrated to take place without racemization,[8] the products **47**–**64** are assumed to be pure *S*-enantiomers.

In Vitro Electrophysiology. Whole-cell patch-clamp recordings from TE671 cells, which express embryonic muscle-type nAChR,[24,25] were undertaken as previously described.[7,26] The effects of the test compounds (**47**–**64**) on whole-cell currents elicited by 10 μM acetylcholine, applied as 1-s pulses, were investigated. All data were obtained at a holding potential (V_H) of −100 mV. Because responses of TE671 cells to acetylcholine rapidly desensitize, the effects of the compounds on the maximum amplitude of the acetylcholine-induced current (peak value) and on the current remaining at the end of the 1-s application of agonist (end value) were determined. The resulting IC_{50} values are summarized in Table 1.

Inhibition of non-NMDA receptors (non-NMDAR) by philanthotoxin analogues was determined by measuring the reduction of the response to 100 μM kainic acid of

Scheme 1



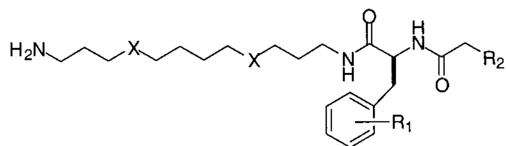
Xenopus laevis oocytes injected with rat brain RNA and voltage-clamped at −80 mV, as previously described.[12] The IC_{50} values are given in Table 2.

Discussion

Solid-phase synthesis of philanthotoxins[8,27] offers distinct advantages as compared to traditional solution-phase chemistry[2,6,7,15,28−33] in terms of speed, yield, and purity of the products. Enantiomerically pure compounds were previously shown to be formed on solid phase from (*S*)-tyrosine by use of HATU (**13**) and collidine as coupling reagents.[8] Moreover, the modular composition of philanthotoxins enables the easy construction of libraries of analogues by parallel synthesis. These advantages were exploited in the present work to obtain a small philanthotoxin library containing compounds **47**−**64** (Scheme 1).

An objective of this work was to investigate whether the structural changes to the headgroup of compounds lacking inner basic sites, such as PhTX-12 (**3**) and 4,9-dioxa-PhTX-12 (**4**), lead to changes in biological activity similar to those obtained following modifications of the headgroup of PhTX-343 (**2**). Previous studies with **2** and its analogues have shown that replacement of the *N*-butanoyl moiety with a longer acyl group invariably leads to increased antagonist potency at nAChR and iGluR. This has been shown by [^{3}H]HTX binding to *Torpedo* electric organ membranes,[3] by [^{3}H]MK-801 binding to rat brain membranes,[3] by antagonism of nAChR of locust neuronal somata[10] and cockroach cholinergic synapse,[10] and by antagonism of iGluR sensitive to quisqualate (qGluR) of locust muscle.[2] Similarly, replacement of the *N*-butanoyl moiety of **2** with an *N*-benzoyl[2,3,10] or *N*-cyclohexylcarbonyl[2,3] group

Table 2. Antagonist Effect of Philanthotoxin Analogues on Non-NMDAR Expressed in *Xenopus* Oocytes from Rat Brain mRNA[a]



compd	X	R_1	R_2	IC_{50} (μM)
50	CH_2	4-OH	CH_2CH_3	>100
56	O	4-OH	CH_2CH_3	>100
62	NH	4-OH	CH_2CH_3	0.75 ± 0.08 (5)
63	NH	4-OH	C_6H_5	0.015 ± 0.004 (10)
64	NH	4-OH	C_6H_{11}	0.18 ± 0.12 (4)

[a] From decrease of the current elicited by 100 μM kainic acid (holding potential $V_H = -80$ mV) by simultaneous co-application of the antagonist (number of experiments given in parentheses).

increased potency in all these assays. In contrast, the presence of the *N*-phenylacetyl group increased potency on nAChR and NMDAR, as reflected by the [^{3}H]HTX and [^{3}H]MK-801 binding assay,[3] respectively, but slightly decreased potency at locust muscle qGluR.[2] Generally, an increase of the hydrophobic character of the *N*-acyl group raises the potency of the analogues of **2**, and steric bulk in the acyl group (such as a phenyl or cyclohexyl group) is tolerated very well.

Previous modifications of the tyrosine phenol function of **2** revealed a somewhat complex pharmacology. Acetylation or benzylation of the phenol group led to unchanged or slightly decreased potency on qGluR.[11] On the other hand, acetylation did not change and benzylation strongly increased the potency on insect nAChR.[10] Removal of the phenol group resulted in a 3-fold increase of the potency on qGluR[2] and a 16-fold increase on locust nAChR.[10] The latter structural change did not affect binding to *Torpedo* electric organ membranes ([^{3}H]HTX binding assay) but abolished binding of the compound to rat brain membranes ([^{3}H]MK-801 binding assay).[3] There have been no studies of the effect on potency of the position of the hydroxy group on the benzene ring of **2**.

On the basis of these previous investigations, a library was constructed using (*S*)-tyrosine and (*S*)-3-hydroxyphenylalanine as the amino acid residues, spermine, 1,12-dodecanediamine (**5**), and 4,9-dioxa-1,12-dodecanediamine (**6**) as the amine moieties, and butanoyl, phenylacetyl, and cyclohexylacetyl as the *N*-acyl groups. Use of the two amino acids addresses the question of the importance of the position of the hydroxy group in the amino acid moiety. The three carboxylic acids were used since considerable amount of information about the effect of a benzene and cyclohexane ring in the spermine-derived philanthotoxins exists, as described above.

The results of the electrophysiological assays with **47**−**64** are summarized in Tables 1 and 2. The nAChR assays show that for all compounds tested, the IC_{50} values for end currents were lower than those for peak currents. The ratio of the IC_{50} for peak current and end current varied between the compounds tested, reflecting the different onset rates for use-dependent antagonism that occurs during co-application of all of the analogues with acetylcholine. In accordance with previous studies with iGluR,[7,8,19] the monoamines **50** and **56** (*S*-enantiomers of **3** and **4**) were inactive when tested on rat brain non-NMDAR (Table 2). Also in accordance with previous studies,[2,3,6,10,11] potency within the polyamine series **62**−**64** increased with the steric bulk of the *N*-acyl groups (Table 2). The analogue **63** is 50 times more potent than the lead structure **62** (*S*-enantiomer of **2**). In the latter series (**62**−**64**), the steric bulk did not cause significant changes of potency when tested at nAChR (Table 1). A similar situation was observed for the 4,9-dioxa-PhTX-12 analogues **56**−**58**. In contrast, a pronounced decrease of potency with increased steric bulk was observed for the derivatives of PhTX-12 (**50**−**52**). It can thus be concluded that in contrast to the action of **2** or **62** on iGluR, introduction of a bulky apolar substituent into the acyl group of **50** and **56** does not increase antagonist potency at nAChR (Table 1). Since the acyl group alterations in **56** and **62** have a similar effect on potency, which is distinctly different from that for **50** (Table 1), the two compounds containing heteroatoms within the polyamine chain may bind to nAChR in a similar fashion but differently from that of **50**.

In the case of antagonist action of 3-hydroxy analogues of **2** (compounds **59**−**61**) on nAChR, an increase of the potency with the steric bulk of the acyl group was observed (Table 1). Interestingly, the 3-hydroxy analogue **59** is about 10-fold less potent than the 4-hydroxy analogue **62**, but this difference is largely removed by the presence of a benzene (compounds **60** and **63**) or cyclohexane (compounds **61** and **64**) ring. A similar trend is observed with the dioxa analogues **53**−**55**. There is a general trend that the 4-hydroxy analogues are more potent than the 3-hydroxy analogues, although some exceptions exist (Table 1).

In conclusion, *N*-acyl group modifications of the lead structure **3** (compounds **47**−**52**) did not improve the potency at nAChR, contrary to what has been observed with analogues of **2** at iGluR. The SARs described in this work contribute to our understanding of the antagonism of nAChR by philanthotoxin analogues lacking the inner basic sites. Further electrophysiological studies are necessary to delineate potential differences in the interaction of these analogues with nAChR.

Our previous work has demonstrated that solid-phase synthesis is an efficient source of enantiomerically pure philanthotoxin analogues.[8] We believe that the combinatorial library approach described in the present work will be of crucial importance in future studies of SARs in the field of neuroactive polyamine toxins and in further optimization of the structure of PhTX-12 (**3**).

Experimental Section

Chemistry. General Procedures. Unless otherwise stated, starting materials were obtained from commercial suppliers and were used without further purification. Polystyrene-based trityl chloride resin (1% divinylbenzene, 200−400 mesh) and (*S*)-*N*-Fmoc-*O*-(*tert*-butyl)tyrosine were obtained from Novabiochem (Läufelingen, Switzerland). (*S*)-*N*-Fmoc-3-(*tert*-butoxy)phenylalanine was obtained from RSP Amino Acid Analogues, Inc. (Worchester, MA). N^4,N^9-Bis(*tert*-butoxycarbonyl)spermine (**7**) was synthesized as previously described.[8] Tetrahydrofuran (THF) was distilled under N_2 from sodium/benzophenone immediately prior to use. ^{1}H and ^{13}C NMR spectra were recorded at 500.13 and 125.67 MHz, respectively, on a Bruker Avance DRX 500 spectrometer, using CD_3OD as solvent and tetramethylsilane (TMS) as internal reference. Coupling constants (^nJ values) are in Hz. Multiplicities of ^{1}H

NMR signals are given as follows: s, singlet; d, doublet; t, triplet; q, quartet; p, pentet; s, sextet; m, multiplet.

Analytical and preparative high-performance liquid chromatography−mass spectrometry (HPLC−MS) were performed on a Perkin-Elmer API 150EX instrument equipped with a turbo ionspray (electrospray ionization) source. The HPLC system consisted of two Shimadzu LC8A pumps. UV trace was obtained with a Shimadzu SPD10A detector operating at 274 nm. Evaporating light scattering (ELS) trace was obtained with an Eurosep DDL 31 light scattering detector and was used for estimation of purity of final products. Analytical HPLC−MS was performed on a 50 × 4.6-mm YMC RP18 column, using 2 mL/min of water/acetonitrile/TFA 90:10:0.05 raising to 10:90:0.05 during 7 min, with 10-μL injections. Preparative HPLC−MS (split-flow MS detection) was run with 190-μL injections (50-mg samples in 1.0 mL of MeOH) to a 50 × 20-mm YMC RP18 column eluted with the same solvent gradient at 22.7 mL/min.

Attachment of Amines 5−7 to Trityl Chloride Resin. 1,12-Dodecanediamine (**5**; 1.00 g, 5.00 mmol) was attached to the resin (0.50 mmol) as previously described.[8] The loading of the resulting resin **8** was 1.53 mmol/g under the assumption that the reaction had gone to completion. 4,9-Dioxa-1,12-dodecanediamine (**6**; 1.02 g, 5.00 mmol) was dissolved in THF (10 mL), and trityl chloride resin (0.50 mmol) was added to the solution in five portions. The suspension was stirred at room temperature for 2 h. The solvent was removed by filtration and the resin was treated with a 10% solution of diisopropylethylamine (DIEA) in MeOH (10 mL) for 5 min to cap any remaining chlorine substituents. The resin was washed with DMF (3 × 5 mL), CH_2Cl_2 (3 × 5 mL), MeOH (3 × 5 mL) and CH_2Cl_2 (3 × 5 mL), and dried in vacuo. The loading of the resulting resin **9** was 1.52 mmol/g under the assumption that the reaction had gone to completion. Reaction of N^4,N^9-bis(*tert*-butoxycarbonyl)spermine (**7**; 2.01 g, 5.00 mmol) with trityl chloride resin (0.50 mmol) was carried out as previously described.[8] The loading of the resulting resin **10** was 1.17 mmol/g under the assumption that the reaction had gone to completion.

General Synthetic Procedure. A solution of either (*S*)-*N*-Fmoc-3-(*tert*-butoxy)phenylalanine (**11**) or (*S*)-*N*-Fmoc-*O*-(*tert*-butyl)tyrosine (**12**) (1.54 mmol) and HATU (**13**; 1.54 mmol) in DMF (3 mL), and of collidine (2.32 mmol) in DMF (2 mL), was added to each of the resins **8−10** (0.39 mmol) placed in 10-mL syringes equipped with a polypropylene filter and a stopper. The syringes were agitated for 2 h at room temperature, the solvent removed, and the resins washed with DMF (3 × 5 mL), CH_2Cl_2 (3 × 5 mL), MeOH (3 × 5 mL) and CH_2Cl_2 (3 × 5 mL), to give the resins **14−19**.

A solution of 20% piperidine in DMF (v/v, 5 mL) was added to each of the resins **14−19**, and the mixtures agitated for 3 min at room temperature. The resins were subsequently washed with DMF (3 × 5 mL), treated with 20% piperidine in DMF (5 mL) for further 20 min, and washed with DMF (3 × 5 mL), CH_2Cl_2 (3 × 5 mL), MeOH (3 × 5 mL) and CH_2Cl_2 (3 × 5 mL), to give the resins **20−25**.

A solution of butanoic acid (**26**), phenylacetic acid (**27**) or cyclohexylacetic acid (**28**) (1.54 mmol) and HATU (**1;** 1.54 mmol) in DMF (3 mL) was added to each of the resins **20−25**, followed by addition of collidine (2.32 mmol) in DMF (2 mL). The mixtures were agitated for 2 h at room temperature, and the resins washed with DMF (3 × 5 mL), CH_2Cl_2 (3 × 5 mL), MeOH (3 × 5 mL) and CH_2Cl_2 (3 × 5 mL), to give resins **29−34**, **35−40** and **41−46**, respectively.

TFA/^{i}Pr$_3$SiH/H_2O (95:2.5:2.5) (5 mL) was added to each of the above resins, and the mixtures were agitated for 2 h at room temperature. The solvent was removed, and the resins washed with MeOH (2 × 5 mL) and CH_2Cl_2 (2 × 5 mL). The combined solvents were concentrated in vacuo and triturated with Et_2O to give the crude products **47−64**, which were purified using automated preparative HPLC using split-flow MS detection for identification of peaks, giving the products as trifluoroacetates in yields from 39−43%, 63−69% and 53−59% for compounds **47−52**, **53−58** and **59−64**, respectively.

(*S*)-*N*-(12-Aminododecyl)-3-hydroxy-α-[(1-oxobutyl)-amino]benzenepropanamide Trifluoroacetate (47). Yield: 39%. ^{1}H NMR: δ 0.84 (t), 1.54 (s), 2.15 (m) (respectively 4-CH_3, 3-CH_2 and 2-CH_2 of the 1-oxobutyl moiety, both ^3J = 7.5), 2.80 and 2.98 (each dd, $^2J_{AB}$ = 13.7, $^3J_{AX}$ = 8.5, $^3J_{BX}$ = 7.1, β-CH_2) 4.16 (dd, α-CH), 6.61−6.64 (m, 1 H), 6.65−6.66 (m, 1 H), 6.68−6.71 (m, 1 H) and 7.07 (m, 1 H) (aromatic H), 2.90 (t, ^3J = 7.4, 12-CH_2), 3.03−3.17 (m, 1-CH_2), 1.60−1.67 (m, 2H) and 1.19−1.42 (m, 18 H) (the remaining CH_2 of the diamine moiety). ^{13}C NMR: δ 13.9, 20.3, 27.5, 27.9, 28.7, 30.3, 30.4, 30.5, 30.7 (3 C), 38.8, 39.2, 40.4, 40.8, 56.2, 114.7, 117.2, 121.5, 130.4, 139.9, 158.6, 173.5, 175.9. HPLC-ELS: 97.7%. MS (ES): 434.4 (M + 1).

(*S*)-*N*-(12-Aminododecyl)-3-hydroxy-α-[(1-oxo-2-phenylethyl)amino]benzenepropanamide Trifluoroacetate (48). Yield: 40%. ^{1}H NMR: δ 2.82 and 2.98 (each dd, $^2J_{AB}$ = 13.7, $^3J_{AX}$ = 8.4, $^3J_{BX}$ = 6.9, β-CH_2), 3.47 (d) and 3.52 (d) (^2J = 14.5, benzylic H), 4.52 (dd, α-CH), 6.63−6.65 (m, 3 H), 7.03−7.06 (m, 1 H), 7.13−7.15 (m, 2 H) and 7.18−7.31 (m, 3 H) (aromatic H), 2.90 (t, ^3J = 7.4, 12-CH_2), 3.05−3.14 (m, 1-CH_2), 1.61−1.67 (m, 2H) and 1.19−1.40 (m, 18 H) (the remaining CH_2 of the diamine moiety). ^{13}C NMR: δ 27.5, 27.9, 28.6, 30.3, 30.4, 30.5, 30.6, 30.7, 39.2, 40.4, 40.8, 43.6, 44.0, 56.3, 114.8, 117.2, 121.5, 127.9, 129.6 (2 C), 130.1, 130.2, 130.5, 136.7, 139.7, 158.6, 173.3, 173.8. HPLC-ELS: 98.1%. MS (ES): 482.4 (M + 1).

(*S*)-*N*-(12-Aminododecyl)-3-hydroxy-α-[(1-oxo-2-cyclohexylethyl)amino]benzenepropanamide Trifluoroacetate (49). Yield: 39%. ^{1}H NMR: δ 2.77 and 2.99 (each dd, $^2J_{AB}$ = 13.7, $^3J_{AX}$ = 8.4, $^3J_{BX}$ = 6.9, β-CH_2), 4.54 (dd, α-CH), 6.61−6.61 (m, 1 H), 6.67 (m, 1 H), 6.69−6.71 (m, 1 H) and 7.05−7.09 (m, 1 H) (aromatic H), 2.03 (d, 2-CH_2 of the cyclohexylacetyl moiety), 2.90 (t, ^3J = 7.5, 12-CH_2), 3.09−3.14 (m, 1-CH_2), 1.61−1.67 (m, 8 H), 1.19−1.40 (m, 22 H) and 0.77−0.91 (m, 1 H) (the remaining CH and CH_2 of the diamine and the cyclohexylacetyl moiety).^{13}C NMR: δ 27.3, 27.4, 27.5, 27.9, 28.7, 30.3, 30.4, 30.5, 30.7, 30.7, 34.0, 34.1, 34.2, 36.8, 39.1, 40.4, 40.8, 45.0, 56.2, 114.7, 117.2, 121.4, 130.4, 140.0, 158.6, 173.6, 175.3. HPLC-ELS: 98.6%. MS (ES): 488.7 (M + 1).

(*S*)-*N*-(12-Aminododecyl)-4-hydroxy-α-[(1-oxobutyl)-amino]benzenepropanamide Trifluoroacetate (50).[7,9] Yield: 41%. ^{1}H NMR: δ 0.84 (t), 1.54 (s), 2.15 (m) (respectively 4-CH_3, 3-CH_2 and 2-CH_2 of the 1-oxobutyl moiety, both ^3J = 7.5), 2.77 and 2.95 (each dd, $^2J_{AB}$ = 13.7, $^3J_{AX}$ = 8.5, $^3J_{BX}$ = 7.1, β-CH_2) 4.48 (dd, α-CH), 6.68 and 7.03 (each 2H, AA′BB′ system, aromatic H), 2.90 (t, ^3J = 7.4, 12-CH_2), 3.02−3.16 (m, 1-CH_2), 1.64 (m, 2H) and 1.19−1.42 (m, 18 H) (the remaining CH_2 of the diamine moiety). ^{13}C NMR: δ 13.9, 20.3, 27.5, 27.9, 28.6, 30.2, 30.3, 30.4, 30.5, 30.66, 30.67, 30.71, 38.5, 38.8, 40.4, 40.8, 56.5, 116.2 (2 C), 129.1, 131.3 (2 C), 157.3, 173.7, 175.9. HPLC-ELS: 98.1%. MS (ES): 434.6 (M + 1).

(*S*)-*N*-(12-Aminododecyl)-4-hydroxy-α-[(1-oxo-2-phenylethyl)amino]benzenepropanamide Trifluoroacetate (51). Yield: 43%. ^{1}H NMR: δ 2.78 and 2.96 (each dd, $^2J_{AB}$ = 13.7, $^3J_{AX}$ = 8.5, $^3J_{BX}$ = 6.6, β-CH_2), 3.46 (d) and 3.52 (d) (^2J = 14.1, benzylic H), 4.48 (dd, α-CH), 6.65 (m, 2 H), 6.97 (m, 2 H), 7.12 (m, 2 H) and 7.19−7.29 (m, 3 H) (aromatic H), 2.90 (t, ^3J = 7.4, 12-CH_2), 3.03−3.17 (m, 1-CH_2), 1.60−1.66 (m, 2H) and 1.18−1.41 (m, 18 H) (the remaining CH_2 of the diamine moiety). ^{13}C NMR: δ 27.5, 27.9, 28.6, 30.3, 30.3, 30.4, 30.5, 30.6, 30.7, 38.4, 40.4, 40.8, 43.7, 44.0, 47.9, 56.6, 116.3, 127.9, 128.9, 129.6 (2 C), 130.1, 130.1, 131.3, 136.7, 157.4, 173.5, 173.8. HPLC-ELS: 98.7%. MS (ES): 482.2 (M + 1).

(*S*)-*N*-(12-Aminododecyl)-4-hydroxy-α-[(1-oxo-2-cyclohexylethyl)amino]benzenepropanamide Trifluoroacetate (52). Yield: 43%. ^{1}H NMR: δ 2.75 and 2.97 (each dd, $^2J_{AB}$ = 13.7, $^3J_{AX}$ = 8.4, $^3J_{BX}$ = 6.9, β-CH_2), 4.50 (dd, α-CH), 6.69 (m, 2 H) and 7.04 (m, 2 H) (aromatic H), 2.02 (d, 2-CH_2 of the cyclohexylacetyl moiety), 2.90 (t, ^3J = 7.5, 12-CH_2), 3.10−3.14 (m, 1-CH_2), 1.61−1.67 (m, 8 H), 1.15−1.42 (m, 22 H) and 0.77−0.91 (m, 1 H) (the remaining CH and CH_2 of the diamine and the cyclohexylacetyl moiety). ^{13}C NMR: δ 27.3, 27.4, 27.5, 27.9, 28.7, 30.3, 30.4, 30.5, 30.7, 30.7, 34.0, 34.1, 34.2, 36.8,

39.1, 40.4, 40.8, 45.0, 56.2, 114.7, 117.2, 121.4, 130.4, 140.0, 158.6, 173.6, 175.3. HPLC-ELS: 96.8%. MS (ES): 488.5 (M + 1).

(*S*)-*N*-(4,9-Dioxa-12-aminododecyl)-3-hydroxy-α-[(1-oxobutyl)amino]benzenepropanamide Trifluoroacetate (53). Yield: 66%. ^{1}H NMR: δ 0.85 (t), 1.55 (s), 2.16 (t) (respectively 4-CH_3, 3-CH_3 and 2-CH_3 of the 1-oxobutyl moiety, both ^3J = 7.5), 2.81 and 2.99 (each dd, $^2J_{AB}$ = 13.7, $^3J_{AX}$ = 8.0, $^3J_{BX}$ = 7.1, β-CH_2), 4.50 (t, α-CH), 6.62−6.70 (m, 3 H) and 7.07 (m, 1 H) (aromatic H), 1.58−1.65 (m, 6 H) and 1.91 (p, 2 H) (2-, 6-, 7- and 11-CH_2), 3.05 (t, 2 H, ^3J = 7.1), 3.13−3.27 (m, 2 H), 3.32−3.37 (m, 2 H), 3.41 (t, 2 H, ^3J = 5.7), 3.47 (t, 2 H, ^3J = 6.1) and 3.56 (t, 2 H, ^3J = 5.7) (1-, 3-, 5-, 8-, 10- and 12-CH_2). ^{13}C NMR: δ 14.0, 20.3, 27.5, 27.6, 28.6, 30.4, 37.9, 38.8, 39.2, 39.5, 56.3, 69.4, 69.4, 71.8, 72.1, 114.8, 117.3, 121.5, 130.5, 139.9, 158.6, 173.6, 176.0. HPLC-ELS: 98.3%. MS (ES): 438.4 (M + 1).

(*S*)-*N*-(4,9-Dioxa-12-aminododecyl)-3-hydroxy-α-[(1-oxo-2-phenylethyl)amino]benzenepropanamide Trifluoroacetate (54). Yield: 65%. ^{1}H NMR: δ 2.81 and 2.99 (each dd, $^2J_{AB}$ = 13.7, $^3J_{AX}$ = 8.0, $^3J_{BX}$ = 7.1, β-CH_2), 4.50 (t, α-CH), 6.62−6.70 (m, 3 H) and 7.07 (m, 1 H) (aromatic H), 1.58−1.65 (m, 6 H) and 1.91 (p, 2 H) (2-, 6-, 7- and 11-CH_2), 3.05 (t, 2 H, ^3J = 7.1), 3.13−3.27 (m, 2 H), 3.32−3.37 (m, 2 H), 3.41 (t, 2 H, ^3J = 5.7), 3.47 (t, 2 H, ^3J = 6.1) and 3.56 (t, 2 H, ^3J = 5.7) (1-, 3-, 5-, 8-, 10- and 12-CH_2). ^{13}C NMR: δ 27.5, 27.5, 28.6, 30.3, 37.8, 39.1, 39.5, 43.6, 56.4, 69.3, 69.3, 71.8, 72.1, 114.8, 117.2, 121.5, 127.9, 129.6 (2 C), 130.2 (2 C), 130.5, 136.7, 139.7, 158.7, 173.4, 173.8. HPLC-ELS: 99.8%. MS (ES): 486.4 (M + 1).

(*S*)-*N*-(4,9-Dioxa-12-aminododecyl)-3-hydroxy-α-[(1-oxo-2-cyclohexylethyl)amino]benzenepropanamide Trifluoroacetate (55). Yield: 63%. ^{1}H NMR: δ 2.78 and 3.01 (each dd, $^2J_{AB}$ = 13.7, $^3J_{AX}$ = 8.5, $^3J_{BX}$ = 7.1, β-CH_2), 4.52 (dd, α-CH), 6.62−6.71 (m, 3 H) and 7.07 (m, 1 H) (aromatic H), 0.79−0.92 (m, 2 H), 1.08−1.25 (m, 3 H), 1.47 (d, 1 H), 1.56−1.69 (m, 6 H from polyamine moiety and 5 H from the cyclohexylacetyl moiety), 1.91 (p, 2 H) (2-, 6-, 7- and 11-CH_2), 3.05 (t, 2 H, ^3J = 7.1), 3.15−3.27 (m, 2 H), 3.34−3.39 (m, 2 H), 3.42 (t, 2 H, ^3J = 5.7), 3.48 (t, 2 H, ^3J = 6.1) and 3.56 (t, 2 H, ^3J = 5.7) (1-, 3-, 5-, 8-, 10- and 12-CH_2). ^{13}C NMR: δ 27.3, 27.3, 27.4, 27.5, 27.5, 28.6, 30.4, 34.0, 34.2, 36.8, 37.9, 39.1, 39.5, 45.0, 56.3, 69.4, 69.4, 71.8, 72.1, 114.7, 117.2, 121.5, 130.5, 140.0, 158.6, 1743.7, 175.3. HPLC-ELS: 99.8%. MS (ES): 492.3 (M + 1).

(*S*)-*N*-(4,9-Dioxa-12-aminododecyl)-4-hydroxy-α-[(1-oxobutyl)amino]benzenepropanamide Trifluoroacetate (56).[7] Yield: 69%. ^{1}H NMR: δ 0.85 (t), 1.55 (s), 2.15 (t) (respectively 4-CH_3, 3-CH_3 and 2-CH_3 of the 1-oxobutyl moiety, both ^3J = 7.5), 2.79 and 2.96 (each dd, $^2J_{AB}$ = 13.7, $^3J_{AX}$ = 8.0, $^3J_{BX}$ = 7.1, β-CH_2), 4.52 (t, α-CH_2), 6.69 and 7.03 (each 2 H, ArH), 1.58−1.68 (m, 6 H) and 1.91 (p, 2 H) (2-, 6-, 7- and 11-CH_2), 3.05 (t, 2 H, ^3J = 6.6), 3.13−3.26 (m, 2 H), 3.31−3.36 (m, 2 H), 3.41 (t, 2 H, ^3J = 5.7), 3.48 (t, 2 H, ^3J = 6.1) and 3.56 (t, 2 H, ^3J = 5.7) (2-, 6-, 7- and 11-CH_2). ^{13}C NMR: δ 14.0, 20.3, 27.5, 27.5, 28.6, 30.4, 37.8, 38.5, 38.8, 39.5, 56.6, 69.4, 71.8, 72.1, 116.3 (2 C), 129.1, 131.3 (2 C), 157.4, 173.8, 175.9. HPLC-ELS: 99.7%. MS (ES): 438.4 (M + 1).

(*S*)-*N*-(4,9-Dioxa-12-aminododecyl)-4-hydroxy-α-[(1-oxo-2-phenylethyl)amino]benzenepropanamide Trifluoroacetate (57). Yield: 67%. ^{1}H NMR: δ 2.80 and 2.97 (each dd, $^2J_{AB}$ = 13.7, $^3J_{AX}$ = 8.5, $^3J_{BX}$ = 7.1, β-CH_2), 3.50 (two d, benzylic H), 4.46 (t, α-CH), 6.66 (m, 2 H), 6.98 (m, 2 H), 7.14 (m, 2 H), 7.20−7.22 (m, 1 H) and 7.23−7.27 (m, 1 H) (aromatic H), 1.59−1.66 (m, 6 H) and 1.89 (p, 2 H) (2-, 6-, 7- and 11-CH_2), 3.03 (t, 2 H, ^3J = 6.6), 3.13−3.25 (m, 2 H), 3.30−3.35 (m, 2 H), 3.40 (t, 2 H, ^3J = 6.1), 3.47 (t, 2 H, ^3J = 6.1) and 3.56 (t, 2 H, ^3J = 6.1) (1-, 3-, 5-, 8-, 10- and 12-CH_2). ^{13}C NMR: δ 27.5, 27.5, 28.6, 30.4, 37.9, 38.3, 39.5, 43.7, 56.7, 69.4, 71.8, 72.1, 116.3, 127.9, 128.9, 129.6, 130.2, 131.3, 136.7, 157.4, 173.5, 173.8. HPLC-ELS: 99.8%. MS (ES): 486.4 (M + 1).

(*S*)-*N*-(4,9-Dioxa-12-aminododecyl)-4-hydroxy-α-[(1-oxo-2-cyclohexylethyl)amino]benzenepropanamide Trifluoroacetate (58). Yield: 69%. ^{1}H NMR: δ 2.76 and 2.98 (each dd, $^2J_{AB}$ = 13.7, $^3J_{AX}$ = 9.0, $^3J_{BX}$ = 6.6, β-CH_2), 4.48 (dd, α-CH), 6.69 (m, 2 H) and 7.04 (m, 2 H) (aromatic H), 0.78−0.93 (m, 2 H), 1.09−1.25 (m, 3 H), 1.46 (d, 1 H), 1.56−1.69 (m, 6 H from polyamine moiety and 5 H from the cyclohexylacetyl moiety), 1.89 (p, 2 H) (2-, 6-, 7- and 11-CH_2), 3.05 (t, 2 H, ^3J = 7.1), 3.15−3.26 (m, 2 H), 3.33−3.38 (m, 2 H), 3.41 (t, 2 H, ^3J = 6.1), 3.48 (t, 2 H, ^3J = 6.1) and 3.56 (t, 2 H, ^3J = 5.7) (1-, 3-, 5-, 8-, 10- and 12-CH_2). ^{13}C NMR: δ 27.2, 27.3, 27.3, 27.5, 27.5, 28.6, 30.4, 34.0, 34.1, 36.9, 37.8, 38.4, 39.5, 45.0, 56.5, 69.4, 69.4, 71.8, 72.1, 116.3 (2 C), 129.1, 131.3 (2 C), 157.4, 173.8, 175.3. HPLC-ELS: 99.9%. MS (ES): 492.5 (M + 1).

(*S*)-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-3-hydroxy-α-[(1-oxobutyl)amino]benzenepropanamide Tris(trifluoroacetate) (59). Yield: 57%. ^{1}H NMR: δ 0.87 (t), 1.56 (s), 2.18 (t) (respectively 4-CH_3, 3-CH_3 and 2-CH_3 of the 1-oxobutyl moiety, both ^3J = 7.5), 4.42 (t, α-CH), 6.66 (m, 2 H), 6.71 (m, 1 H) and 7.10 (m, 1 H) (aromatic H), 1.78−1.81 (m, 6 H) and 2.08 (m, 2 H) (2-, 6-, 7- and 11-CH_2), 2.84−2.94 (m, 3 H), 2.97−3.02 (m, 3 H), 3.03−3.09 (m, 4 H), 3.13 (t, 2 H, ^3J = 8.0) and 3.18−3.30 (m, 2 H) (β-CH_2 and remaining CH_2 of the polyamine moiety). ^{13}C NMR: δ 14.0, 20.3, 24.4, 24.5, 25.6, 27.5, 36.8, 38.0, 38.6, 38.7, 46.0, 46.2, 48.2, 48.4, 49.7, 56.8, 114.9, 117.2, 121.4, 130.6, 139.8, 158.7, 175.2, 176.3. HPLC-ELS: 100%. MS (ES): 436.6 (M + 1).

(*S*)-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-3-hydroxy-α-[(1-oxo-2-phenylethyl)amino]benzenepropanamide Tris(trifluoroacetate) (60). Yield: 53%. ^{1}H NMR δ 3.01 (dd, $^2J_{AB}$ = 13.7, $^3J_{BX}$ = 7.1, H_B proton of β-CH_2), 3.51 (d) and 3.55 (d) (^2J = 14.1, benzylic H), 4.42 (t, α-CH), 6.65−6.68 (m, 3 H), 7.06−7.10 (m, 1 H), 7.16−7.19 (m, 2 H), 7.20−7.24 (m, 1 H) and 7.25−7.29 (m, 2 H) (aromatic H), 1.70−1.81 (m, 6 H) and 2.07 (p, 2 H) (2-, 6-, 7- and 11-CH_2), 2.80−2.92 (m, 5 H), 3.05 (apparent t, 4 H), 3.11 (t, 2 H, ^3J = 7.5) and 3.16 (m, 2 H) (H_A proton of β-CH_2 and remaining CH_2 of the polyamine moiety). ^{13}C NMR: δ 24.4, 24.4, 25.6, 27.5, 36.8, 38.0, 38.5, 43.5, 46.0, 46.1, 48.2, 48.4, 57.1, 115.0, 117.2, 121.4, 128.0, 129.7 (2 C), 130.3 (2 C), 130.7, 136.7, 139.6, 158.7, 174.2, 175.0. HPLC-ELS: 99.0%. MS (ES): 484.4 (M + 1).

(*S*)-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-3-hydroxy-α-[(1-oxo-2-phenylethyl)amino]benzenepropanamide Tris(trifluoroacetate) (61). Yield: 54%. ^{1}H NMR: δ 4.45 (t, ^3J = 7.8, α-CH), 6.64−6.72 (m, 3 H) and 7.10 (m, 1 H) (aromatic H), 0.86−0.89 (m, 2 H), 1.16−1.20 (m, 3 H), 1.47 (m, 1 H) and 1.57−1.65 (m, 5 H) (CH and CH_2 of the diamine and the cyclohexylacetyl moiety),1.80 (m, 2-, 6- and 8-CH_2), 2.08 (m, 11-CH_2 and 2-CH_2 of the cyclohexylacetyl moiety), 2.80−3.27 (m, 14H, β-CH_2 and the remaining CH_2 of the polyamine moiety). ^{13}C NMR: δ 24.4, 25.5, 27.2, 27.3 (2 C), 27.5, 33.9, 34.2, 36.7, 36.8, 37.9, 38.5, 44.9, 45.9, 46.1, 48.2, 56.7, 114.8, 117.2, 121.3, 130.6, 139.8, 158.6, 175.2, 175.7. HPLC-ELS: 98.7%. MS (ES): 490.5 (M + 1).

(*S*)-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-4-hydroxy-α-[(1-oxobutyl)amino]benzenepropanamide Tris(trifluoroacetate) (62).[8] Yield: 59%. ^{1}H NMR: δ 0.87 (t), 1.55 (s), 2.17 (m) (respectively 4-CH_3, 3-CH_2 and 2-CH_2 of the 1-oxobutyl moiety, both ^3J = 7.4), 4.37 (t, ^3J = 7.8, α-CH), 6.71 and 7.05 (each 2H, aromatic H), 1.78 (m, 2-, 6- and 8-CH_2), 2.08 (p, 11-CH_2), 2.79−3.28 (m, 14H, β-CH_2 and the remaining CH_2 of the polyamine moiety). ^{13}C NMR: δ 14.0, 20.3, 24.3, 24.4, 25.5, 27.4, 36.8, 37.9, 37.9, 38.7, 45.9, 46.2, 48.2, 48.3, 57.1, 116.3 (2 C), 128.9, 131.3 (2 C), 157.5, 175.2, 176.3. HPLC-ELS: 99.5%. MS (ES): 435.5 (M + 1).

(*S*)-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-4-hydroxy-α-[(1-oxo-2-phenylethyl)amino]benzenepropanamide Tris(trifluoroacetate) (63).[3] Yield: 59%. ^{1}H NMR: δ 4.36 (t, ^3J = 7.8, α-CH), 6.69 (m, 2 H), 7.01 (m, 2 H), 7.17 (m, 2 H) and 7.22−7.28 (m, 3 H) (aromatic H), 1.77 (m, 2-, 6- and 8-CH_2), 2.08 (p, 11-CH_2), 2.79−3.28 (m, 14H, β-CH_2 and the remaining CH_2 of the polyamine moiety), 3.49 (d) and 3.54 (d) (^2J = 14.5, benzylic H). ^{13}C NMR: δ 24.3 (2 C), 25.4, 27.4, 36.8, 37.8, 37.8, 43.5, 45.9, 46.2, 48.1, 48.3, 57.3, 116.4 (2 C), 128.0, 128.7, 129.7 (2 C), 130.2 (2 C), 131.3 (2 C), 136.7, 157.5, 174.2, 175.0. HPLC-ELS: 99.4%. MS (ES): 484.4 (M + 1).

(*S*)-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-4-hydroxy-α-[(1-oxo-2-cyclohexylethyl)amino]benzenepropanamide Tris(trifluoroacetate) (64). Yield: 57%. ^{1}H NMR: δ

4.41 (t, ^3J = 7.8, α-CH), 6.71 (m, 2 H) and 7.06 (m, 2 H) (aromatic H), 0.80−0.92 (m, 2 H), 1.12−1.23 (m, 3 H), 1.48 (m, 1 H) and 1.57−1.68 (m, 5 H) (CH and CH_2 of the diamine and the cyclohexylacetyl moiety),1.80 (m, 2-, 6- and 8-CH_2), 2.08 (m, 11-CH_2 and 2-CH_2 of the cyclohexylacetyl moiety), 2.79−3.28 (m, 14H, β-CH_2 and the remaining CH_2 of the polyamine moiety). ^{13}C NMR: δ 24.3, 24.4, 25.4, 27.2, 27.3, 27.3, 27.4, 34.0, 34.2, 36.8, 37.8, 37.9, 44.9, 45.9, 46.3, 48.2, 48.3, 57.0, 116.3 (2 C), 129.0, 131.3 (2 C), 157.5, 175.2, 175.7. HPLC-ELS: 99.3%. MS (ES): 490.4 (M + 1).

Electrophysiology. nAChR Assay. The nAChR assay was performed essentially as previously described.[7,26] TE671 cells were grown in Petri dishes and transferred to a perfusion bath mounted on the stage of an inverted microscope. Patch pipets filled with 140 mM CsCl, 1 mM $CaCl_2$, 11 mM EDTA and 5 mM HEPES (pH adjusted to 7.2 with 1 M CsOH) were used for whole-cell recording. Cells were constantly perfused at ca. 5 mL/min with saline containing 135 mM NaCl, 5.4 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM HEPES (pH adjusted to 7.4 with 3 M NaOH). Ligands (10 μM acetylcholine alone or 10 μM acetylcholine plus test compound) were applied as 1-s pulses at intervals of at least 30 s to allow the nAChR to recover from the desensitizing effect of agonist and the blocking effect of the antagonist. Whole-cell currents were monitored using an Axopatch 200 patch-clamp amplifier (Axon Instruments) and the output was recorded on the hard disk of a PC using pClamp 5.7.2 software (Axon Instruments). Whole-cell recordings were performed at ambient laboratory temperature (17−23 °C).

Non-NMDAR Assay. *X. laevis* oocytes were injected with rat brain RNA and incubated at 18 °C for at least 3 days.[12] Single oocytes were transferred to a perfusion bath and continuously perfused with saline containing 120 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 10 mM HEPES (pH adjusted to 7.5 with NaOH). The oocytes were voltage-clamped at −80 mV using an Axoclamp 2A (Axon Instruments) and current output was digitized with a Sony PCM and recorded to videotape with a Sony VCR. Responses of non-NMDAR were elicited by perfusion of 100 μM kainic acid for 120 s. PhTX-343 analogues were co-applied from 40−80 s of this kainic acid application.

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References

(1) 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.

(2) Bruce, M.; Bukownik, R.; Eldefrawi, A. T.; Eldefrawi, M. E.; Goodnow, R., Jr.; Kallimopoulos, T.; Konno, K.; Nakanishi, K.; Niwa, M. Usherwood, P. N. R. Structure−activity relationships of analogues of the wasp toxin philanthotoxin: noncompetitive antagonists of quisqualate receptors. *Toxicon* **1990**, *28*, 1333−1346.

(3) Anis, N.; Sherby, S.; Goodnow, R., Jr.; Niwa, M.; Konno, K.; Kallimopoulos, T.; Bukownik, R.; Nakanishi, K.; Usherwood, P.; Eldefrawi, A.; Eldefrawi, M. Structure−activity relationships of philanthotoxin analogues and polyamines on *N*-methyl-D-aspartate and nicotinic acetylcholine receptors. *J. Pharmacol. Exp. Ther.* **1990**, *254*, 764−773.

(4) Karst, H.; Piek, T.; Van Marle, J.; Lind, A.; Van Weeren-Kramer, J. Structure−activity relationship of philanthotoxins. I. Pre- and postsynaptic inhibition of the locust neuromuscular transmission. *Comp. Biochem. Physiol.* **1991**, *98C*, 471−477.

(5) Karst, H.; Piek, T. Structure−activity relationship of philanthotoxins. II. Effects on the glutamate gated ion channels of the locust muscle fiber membrane. *Comp. Biochem. Physiol.* **1991**, *98C*, 479−489.

(6) 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. *Bioorg. Med. Chem.* **1997**, *5*, 1969−1988.

(7) Strømgaard, K.; Brierley, M. J.; Andersen, K.; Sløk, F. A.; Mellor, I. R.; Usherwood, P. N. R.; Krogsgaard-Larsen, P.; Jaroszewski, J. W. Analogues of neuroactive polyamine wasp toxins that lack inner basic sites exhibit enhanced antagonism toward a muscle-type mammalian nicotinic acetylcholine receptor, *J. Med. Chem.* **1999**, *42*, 5224−5234.

(8) Strømgaard, K.; Bjørnsdottir, I.; J.; Andersen, K.; Brierley, M. J.; Rizoli, S.; Eldursi, N.; Mellor, I. R.; Usherwood, P. N. R.; Hansen, S. H.; Krogsgaard-Larsen, P.; Jaroszewski, J. W. Solid phase synthesis and biological evaluation of enantiomerically pure wasp toxin analogues PhTX-343 and PhTX-12. *Chirality* **2000**, *12*, 93−102.

(9) Piek, T.; Hue, B. Philanthotoxins, a new class of neuroactive polyamines, block nicotinic transmission in the insect CNS. *Comp. Biochem. Physiol.* **1989**, *93C*, 403−306.

(10) Benson, J. A.; Kaufmann, L.; Hue, B.; Pelhate, M.; Schuermann, F.; Gsell, L.; Piek, T. The physiological action of analogues of philanthotoxin-4.3.3 at insect nicotinic acetylcholine receptors. *Comp. Biochem. Physiol.* **1993**, *105C*, 303−310.

(11) Liu, M.; Nakazawa, K.; Inoue, K.; Ohno, Y. Potent and voltage-dependent block by philanthotoxin-343 of neuronal nicotinic receptor channels in PC12 cells. *Br. J. Pharmacol.* **1997**, *122*, 379−385.

(12) Brackley, P. T. H.; Bell, D. R.; Choi, S. K.; Nakanishi, K.; Usherwood, P. N. R. Selective antagonism of native and cloned kainate and NMDA receptors by polyamine-containing toxins. *J. Pharmacol. Exp. Ther.* **1993**, *266*, 1573−1580.

(13) Washburn, M. S.; Dingledine, R. Block of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors by polyamines and polyamine toxins. *J. Pharmacol. Exp. Ther.* **1996**, *278*, 669−678.

(14) Donevan, S. D.; Rogawski, M. A. Multiple actions of arylalkylamine arthropod toxins on the *N*-methyl-D-aspartate receptor. *Neuroscience* **1996**, *70*, 361−375.

(15) Huang, D.; Jiang, H.; Nakanishi, K.; Usherwood, P. N. R. Synthesis and pharmacological activity of philanthotoxin-343 analogues: antagonists of ionotropic glutamate receptors. *Tetrahedron* **1997**, *53*, 12391−12404.

(16) Bähring, R.; Mayer, M. L. An analysis of philanthotoxin block for recombinant rat GluR6(Q) glutamate receptor channels. *J. Physiol.* **1998**, *509*, 635−650.

(17) Unpublished results.

(18) Carpino, L. A. 1-Hydroxy-7-azabenzotriazole. An efficient peptide coupling additive. *J. Am. Chem. Soc.* **1993**, *115*, 4397−4398.

(19) Carpino, L. A.; El-Faham, A. Effect of tertiary bases on O-benzotriazolyluronium salt-induced peptide segment coupling. *J. Org. Chem.* **1994**, *59*, 695−698.

(20) Carpino, L. A.; El-Faham, A. Efficiency in peptide coupling: 1-hydroxy-7-azabenzotriazole *vs.* 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine. *J. Org. Chem.* **1995**, *60*, 3561−3564.

(21) Carpino, L. A.; Ionescu, D.; El-Faham, A. Peptide coupling in the presence of highly hindered tertiary amines. *J. Org. Chem.* **1996**, *61*, 2460−2465.

(22) Zeng, Z.; Burton, L.; Yung, K.; Shushan, B.; Kassel, D. B. Automated analytical/preparative high-performance liquid chromatography−mass spectrometry system for the rapid characterization and purification of compound libraries. *J. Chromatogr. A* **1998**, *794*, 3−13.

(23) Hsu, B. H.; Orton, E.; Tang, S.-Y.; Carlton, R. A. Application of evaporative light scattering detection to the characterization of combinatorial and parallel synthesis libraries for pharmaceutical drug discovery. *J. Chromatogr. B* **1999**, *725*, 103−112.

(24) Luther, M. A.; Schoepfer, R.; Whiting, P.; Casey, B.; Blatt, Y.; Montal, M. S.; Montal, M.; Lindstrom, J. A muscle acetylcholine receptor is expressed in the human cerebellar medulloblastoma cell line TE761. *J. Neurosci.* **1989**, *9*, 1082−109.

(25) Shoepfer, R.; Luther, M.; Lindstrom, J. The human medulloblastoma cell line TE671 expresses a muscle like acetylcholine receptor − cloning of the δ-subunit c-DNA. *FEBS Lett.* **1988**, *226*, 235−240.

(26) Shao, Z.; Mellor, I. R.; Brierley, M. J.; Harris, J.; Usherwood, P. N. R. Potentiation and inhibition of nicotinic acetylcholine receptors by spermine in the TE671 human muscle cell line. *J. Pharmacol. Exp. Ther.* **1998**, *286*, 1269−1276.

(27) Nash, I. A.; Bycroft, B. W.; Chan, W. C. Dde − a selective primary amine protecting group: a facile solid-phase synthetic approach to polyamine conjugates. *Tetrahedron Lett.* **1996**, *37*, 2625−2628.

(28) Tong, Y. C. Synthesis of δ-philanthotoxin. *Pesticide Sci.* **1988**, *24*, 340−343.

(29) Blagbrough, I. S.; Bycroft, B. W.; Mather, A. J.; Usherwood, P. N. R. Philanthotoxin-343 − an analogue of a toxin from *Philanthus triangulum*: a practical synthesis of a potent glutamate antagonist. *J. Pharm. Pharmacol.* **1989**, *41* (Suppl.), 95P.

(30) Goodnow, R., Jr.; Konno, K.; Niwa, M.; Kallimopoulos, T.; Bukownik, R.; Lenares, D.; Nakanishi, K. Synthesis of glutamate receptor antagonist philanthotoxin-433 (PhTX-433) and its analogues. *Tetrahedron* **1990**, *46*, 3267−3286.

(31) 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.
(32) Choi, S.-K.; Nakanishi, K.; Usherwood, P. N. R. Synthesis and assay of hybrid analogues of argiotoxin-636 and philanthotoxin-433: glutamate receptor antagonists. *Tetrahedron* **1993**, *49*, 5777–5790.
(33) Moya, E.; Blagbrough, I. S. Syntheses and neuropharmacological properties of arthropod polyamine amide toxins. In *The Neuropharmacology of Polyamines*; Carter, C., Ed.; Academic Press: New York, 1994; pp 167–184.

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