Recent Advances in the Medicinal Chemistry of Polyamine Toxins

Kristian Strømgaard,[1,*] Kim Andersen,[2] Povl Krogsgaard-Larsen[1] and Jerzy W. Jaroszewski[1]

[1]*Department of Medicinal Chemistry and NeuroScience PharmaBiotec Research Center, Royal Danish School of Pharmacy, Universitetsparken 2, DK-2100 Copenhagen, Denmark, and* [2]*Department of Combinatorial Chemistry, Medicinal Chemistry Research, H. Lundbeck A/S, Ottiliavej 9, DK-2500 Valby, Denmark*

Abstract: This review describes the recent developments in the field of polyamine toxins, with focus on structure activity relationship investigations, including studies of importance of the polyamine moiety for biological activity, photolabeling studies using polyamine toxins as templates, as well as use of solid phase methods for the synthesis of polyamine toxins. The review is mainly concerned with effects of polyamine toxins on nicotinic acetylcholine receptors and ionotropic glutamate receptors.

INTRODUCTION

Polyamine toxins are non-oligomeric, low molecular-weight compounds isolated from the venoms of spiders and wasps. These secondary metabolites, which are only present in the venom glands, seem to have evolved specifically as tools for paralyzing prey [1]. The first reports on polyamine toxins date more than 40 years back, when such toxins were found in the venom of tarantula spiders [2]. Almost 30 years later, in 1986, Volkova and colleagues isolated and characterized the first polyamine toxin from the venom of the orb weaver spider *Argiope lobata*, designated argiotoxin-636[a] (**1**, ArgTX-636, previously named argiopin, Fig. **1**) [3]. Later, other structurally related toxins such as ArgTX-659 (**2**) and ArgTX-673 (**3**) were found in the same spider [4] as well as in other *Argiope* species [5,6].

Subsequently, the structures of Joro spider toxins (JSTX and NSTX) from *Nephilia clavata* and *Nephilia maculata*, like JSTX-3[b] (**4**) and NSTX-3 (**5**) were published [7], followed by *Nephilia* peptide-like spider toxins (NPTX) such as NPTX-8 (**6**) [8] from the same species. From the North American funnel web spider *Ageleonopsis aperta* agatoxins (AGEL) such as AGEL-489 (**7**) and AGEL-505 (**8**) were isolated [9-11]. Polyamine toxins have also been isolated from the fishing spider *Dolomedes okefinokensis* [12], the trap-door spider *Hebestatis theveniti* [13] and the funnel web spider *Hololenta curta* [14]. Structures of several new polyamine toxins have recently been elucidated, when the crude venom of *Araneidae* spiders was analyzed using various mass spectrometric techniques [15-19].

The Egyptian digger wasp *Philanthus triangulum* is known as the bee wolf as it preys solely on worker honeybees. The wasp feeds on the nectar it squeezes out from the crop of the paralyzed bee and uses some bees as a source of live food for its larvae [20]. The wasp stings the bee injecting the venom that paralyses the prey, by affecting its central nervous system (CNS), and by neuromuscular paralysis [21]. Piek and co-workers were the first to show that the venom of *Philanthus triangulum* [22,23] decreases the release of glutamate from presynaptic nerve terminals in a reversible and non-competitive manner [24-26].

Eldefrawi, Nakanishi, Usherwood, and colleagues succeeded in isolating, elucidating the structure, and synthesizing the most active fraction of the venom of *Philanthus triangulum*, originally known as δ-philanthotoxin (δ-PhTX), but now renamed philanthotoxin-433[c] (**9**, PhTX-433, Fig. **2**) [27]. Almost simultaneously, Piek and co-workers announced the structure of δ-PhTX [28].

A high degree of structural similarity of polyamine toxins isolated from the venom of spiders is apparent (Fig. **1**). As the name polyamine toxins imply, the polyamine chain is a common structural feature, with an aromatic moiety in one end, and either a primary amino group (**4**, **6**, **7**, **8**) or a guanidine group (**1**, **2**, **3**, **5**) in the other. The aromatic moiety is either bound directly to the polyamine through an amide bond (**7**, **8**), or indirectly through asparagine residue (**1**-**6**). The aromatic moiety is 2,4-dihydroxyphenylacetyl (**1**, **4**, **5**) or indol-3-acetyl group, with (**2**, **3**, **8**) or without (**6**, **7**) a hydroxyl group in the 4-position. Variations of the polyamine component include differences in the number of methylene groups separating the amino groups, presence of amides (**4**, **5**, **6**), methylation (**3**), or hydroxylation (**7**, **8**) of the secondary amino groups.

Polyamine toxins from spiders and wasps also share pharmacological similarities, which might be expected since these toxins are contrived to block neuromuscular junctions or the CNS in arthropods, primarily targeting ionotropic glutamate receptors (iGluRs). This family of receptors comprises *N*-methyl-D-aspartate (NMDA), 2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)propionic acid (AMPA), and

*Address correspondence to this author at the Department of Medicinal Chemistry, Royal Danish School of Pharmacy, Universitetsparken 2, DK-2100 Copenhagen, Denmark. Phone: 45 3530 6000. Fax: 45 3530 6040. E-mail: krst@dfh.dk. Present address: Columbia University, Department of Chemistry, 3000 Broadway, New York, NY 10027, USA

[a] Numerals denote the molecular weight of the compound.

[b] Numerals denote the HPLC elution order with respect to other components of the venom.

[c] Numerals denote the number of methylene groups separating the amino groups in the polyamine moiety.

1389-5575/01 $20.00+.00

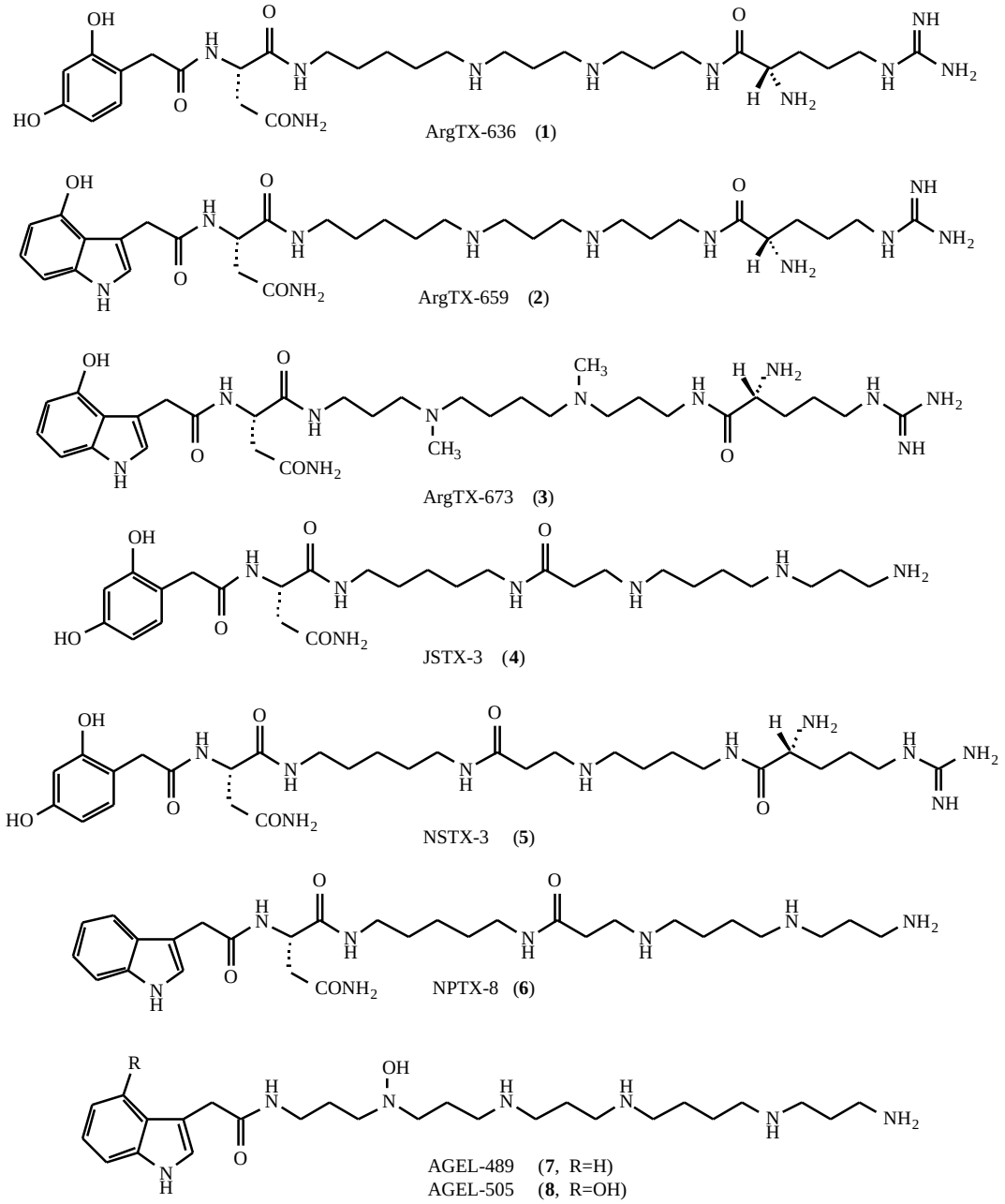


Fig. (1). Examples of polyamine toxins isolated from spiders.

kainate (KA) receptors containing NR1, NR2A-2D and 3A, GluR1-4, and GluR5-7, KA1 and KA2 subunits, respectively.

The pharmacological profile of polyamine toxins is somewhat complex. Contributing to the complexity of the available information, polyamine toxins have been characterized using a variety of different assays comprising different vertebrate as well as invertebrate pharmacological systems. Since polyamine toxins such as ArgTX-636 (**1**), JSTX-3 (**4**), PhTX-433 (**9**) and PhTX-343 (**10**) have become commercially available, an immense number of papers describing various effects of these compounds have been published. However, polyamine toxins generally appear to be non-competitive open-channel blockers of cation-conducting channels, in particular iGluRs. Their action is in most cases use- and voltage-dependent [1,29-34], although exceptions have been reported. Polyamine toxins are believed to antagonize ion channels by binding to an intra-ion-channel binding site, probably at a unique site [1], but no clear consensus on this point has been achieved.

Recently, however, the use of recombinant receptors has revealed valuable information about the otherwise confusing actions of polyamine toxins. The variable potency and selectivity of polyamine toxins reported in the literature

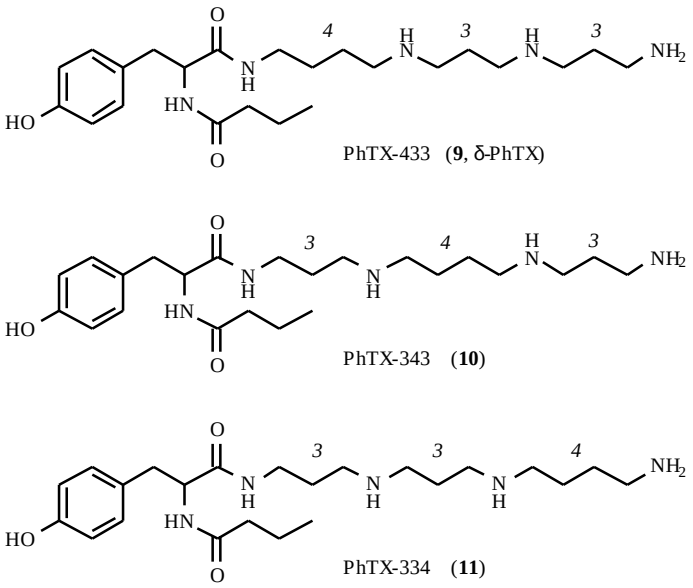


Fig. (2). The native wasp toxin, PhTX-433 (**9**) and its structural analogues PhTX-343 (**10**) and PhTX-334 (**11**).

might reflect variations in the subunit composition of the receptors present in the biological preparations that have been employed in studies of these compounds [1].

A striking feature of polyamine toxins is their ability to selectively antagonize Ca^{2+}-permeable AMPA and KA receptors, which are receptors not containing the edited GluR2 subunit and the GluR5 or GluR6 subunits, for AMPA and KA receptors, respectively [35,36]. The control of Ca^{2+}-permeability has been traced to a single amino acid in the second membrane-associated domain, the so-called "Q/R-site". In the AMPA receptor subunits GluR1, GluR3 and GluR4, glutamine (Q) resides in this position, whereas virtually all GluR2 subunits are edited so that this glutamine is replaced by arginine (R) [37]. Thus, the arginine (R) residue in the Q/R-site prevents the Ca^{2+}-permeability. In the KA receptors, the GluR5 and GluR6 subunits can be edited with replacement of the glutamine (Q) by the arginine (R) residue [38].

Several studies have shown that polyamine toxins such as ArgTX-636 (**1**), JSTX-3 (**4**), PhTX-433 (**9**) and PhTX-343 (**10**) are potent and selective antagonists of Ca^{2+}-permeable AMPA and KA receptors [39-47]. Thus polyamine toxins are advantageous pharmacological tools for determining subunit composition of AMPA and KA receptors [48,49]. This has been used in several studies, e.g. to demonstrate that multiple AMPA receptor populations exist within a single cell [50].

ArgTX-636 (**1**) is also a potent blocker of native NMDA receptors [30,51-56] as well as of recombinant NMDA receptors [46,57,58], but no firm conclusions concerning the relationship of subunit composition and inhibition have been reached. Slightly more controversial is the picture concerning antagonism of NMDA receptors by PhTX-433 (**9**) and PhTX-343 (**10**), as some have claimed that philanthotoxins **9** and **10** have no effect [59-61], whereas others have shown moderate effect [62], or have reported that **9** and **10** are more potent antagonists at NMDA receptors than at KA and AMPA receptors [63]. As previously mentioned, this may reflect the difference in subunit composition of the pharmacological preparations employed. Studies with cloned NMDA receptors show an intricate mode of action involving a polyamine like potentiation of NMDA responses at low concentrations of philanthotoxins **9** and **10** [46,64,65]. Interestingly, philanthotoxins **9** and **10** have also been shown to be relatively potent inhibitors of nicotinic acetylcholine receptors (nAChRs) [62,66], which has not been demonstrated for other polyamine toxins.

Altogether, the ability of polyamine toxins to block Ca^{2+}-permeable AMPA and KA receptors selectively, along with their purported NMDA receptor antagonism, could represent therapeutically advantageous characteristics. Thus, polyamine toxins have been suggested for clinical use in the treatment of neurological disorders, as they are effective glutamate antagonists *in vitro*. Moreover, it has been demonstrated that such compounds are active in animal models predictive of anticonvulsant and neuroprotectant effects following systemic administration [1,67-69]. Thus, quite remarkably, polyamine toxins cross the blood-brain barrier (BBB) and produce CNS effects when administered systemically, although it is not known whether this is due to impairment of the BBB or due to active transport *via* polyamine transport mechanisms. Very recently, it was found that polyamine toxins such as **1**, **4**, **7** and **9** do not affect hippocampal long-term potentiation (LTP), contrary to most other NMDA receptor antagonists [70].

With a few exceptions, all structure-activity relationship (SAR) studies on polyamine toxins have been carried out using PhTX-433 (**9**) or PhTX-343 (**10**) as the lead structures. Piek and co-workers have performed SAR studies using **9**, **10**, PhTX-334 (**11**) and nine analogues thereof, which have been tested in approximately 10 different pharmacological

assays comprising iGluRs as well as nAChRs [71-80]. The reported information about the synthesis and characterization of the analogs studied by Piek and co-workers is, however, very limited, and only the synthesis of **10** has been described [81]. The trifluoromethyl analogue (**12**) and the dideaza analogue (**13**, PhTX-12) (Fig. **3**) seem to be the most interesting analogues, as **12** is the most active analogue in insect iGluR and nAChR assays [71,72,77,79]. On the other hand **12**, along with **13**, are potent in blocking presynaptic glutamate uptake in rat hippocampus [73,74,76,79].

Blagbrough and co-workers have, besides developing several synthetic routes to polyamine toxins [82], prepared a few so-called hybrid-analogues (Fig. **3**), that combine the structural features of PhTX-343 (**10**) and ArgTX-636 (**1**) [83-86]. Compounds **14** and **15** turned out to be slightly (4-fold) more potent than PhTX-343 (**10**) on invertebrate iGluRs sensitive to quisqualate (quisR) (locust *Schistocerca gregaria* muscle), whereas **16** and **17** were by the same order of magnitude less potent than PhTX-343 (**10**).

Much of the knowledge generated from SAR studies on philanthotoxins is based on to the synthetic effort by Nakanishi and co-workers, together with the pharmacological characterization performed by the groups of Eldefrawi and Usherwood. In the process of structure elucidation of PhTX-433 (**9**), it was necessary to synthesize PhTX-343 (**10**), containing the symmetrical spermine moiety, together with PhTX-334 (**11**) in order to verify the structure of the polyamine component of **9** [27]. The pharmacological characterization of **9**, **10** and **11** on iGluRs and nAChRs revealed only minor differences in their antagonistic activity [62,87,88]. Thus, PhTX-343 (**10**) has been used as a reference compound for SAR studies, since it is more easily synthesized due to the symmetry of the polyamine moiety.

More than 100 compounds have been synthesized by Nakanishi and co-workers and tested at the invertebrate quisR and nAChR [62,87-97]. The conclusions from these

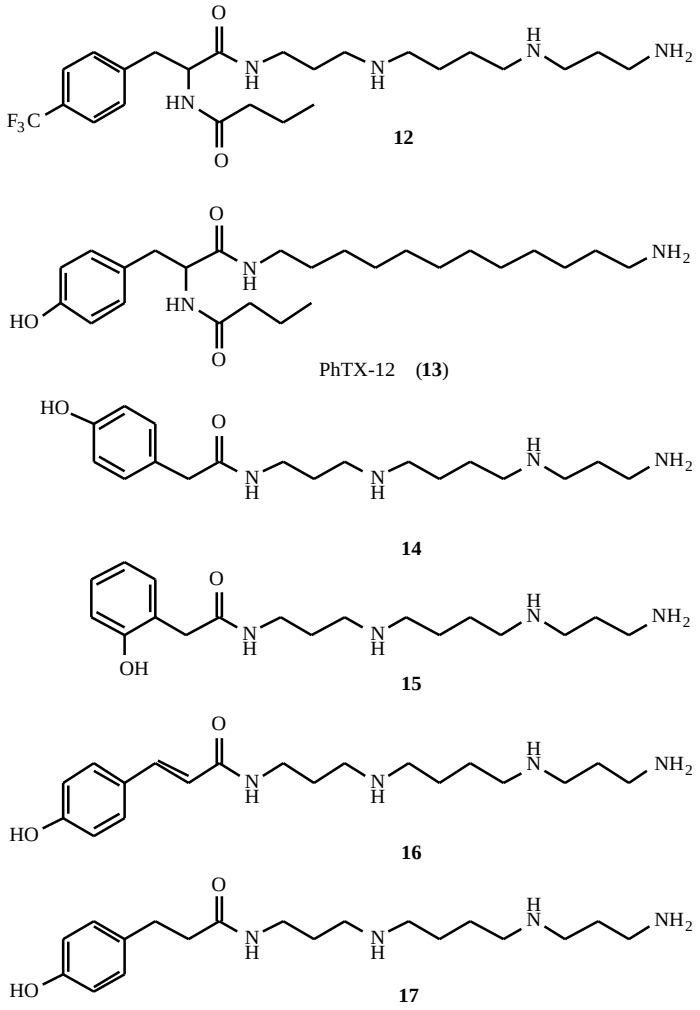


Fig. (3). Componds synthesized by the groups of Piek (**12** and **13**) and Blagbrough (**14**-**17**).

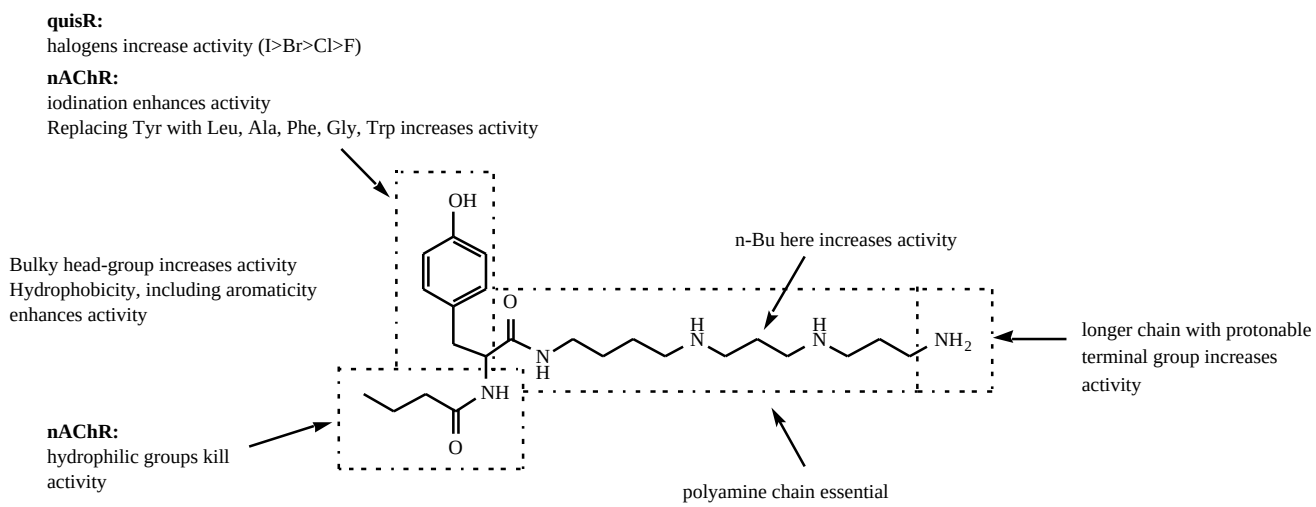


Fig. (4). A summary of the SARs of philanthotoxins performed by the Nakanishi group.

SAR studies have been summarized in Fig. **4**. Generally, it was essentially impossible to differentiate the activities at the quisR from those at the nAChR. The polyamine chain has been regarded as essential, as a shortening of this moiety markedly decreased activity, whereas increasing the length of the polyamine chain, e.g. by a lysine residue, increased the activity. Interestingly, addition of an n-butyl group at the middle carbon atom in between the two secondary amino groups significantly increases the potency, whereas the same group placed between the other secondary amino groups decreases the activity. Replacing the head-group, i.e. the tyrosine and butyric acid moieties, with more lipophilic groups, or substituting the former with halogens increases the activity.

Several reviews of the area of polyamine toxins have been published. Thus, McCormick and Meinwald have reviewed the field of polyamine toxins with emphasis on the structural features of polyamines [29]. In a particularly extensive review, Hesse and co-workers dealt with the synthetic aspects of polyamine toxins [98], while Mueller and co-workers focused on the pharmacological aspects [1]. Very recently, Usherwood gave a short account of general aspects of polyamines and polyamine toxins [99]. Recent reviews have covered the synthesis of polyamines in general [100,101], as well as new developments of the use of mass spectrometry for identification of polyamine toxins [102].

This mini-review will focus on the most recent SAR studies and, in particular, new synthetic developments based on solid phase methodology. Recent applications of photolabile polyamine toxin analogues, primarily covering the literature from 1996 up till today, are also covered.

STUDIES USING ANALOGUES OF NATURAL POLYAMINE TOXINS

This part describes recent results from SAR investigations and receptor photolabeling studies on polyamine toxins.

In a SAR study of Nakanishi and co-workers, effect of elongation of the polyamine moiety, as well as the effect of the amide carbonyls on the potency at the quisR were investigated [103]. Generally, increasing the length of the polyamine moiety increased the activity. However, the PhTX-3343 analogue (**18**) (Fig. **5**) was 5-fold less potent than the corresponding PhTX-3334 analogue (**19**), thus emphasizing the importance of the relative positions of the secondary amino groups. The analogues where the amide bonds were reduced to secondary amines as in **20** were generally equipotent with their amide counterparts [103].

In another study by the same group several new analogues, including analogues labeled with photoactive groups, fluorine-containing analogues, and analogues with large head-groups were described [104,105]. These analogues were synthesized for studies of ligand-receptor interactions by photoaffinity labeling and solid state NMR. The binding assays performed consisted on inhibition of [^{3}H]-dodecahistrionicotoxin ([^{3}H]-H_{12}-HTX) to activated nAChR. However, longer than normal application times were used, leading to a 2.5 fold decrease of the activity of PhTX-343 (**10**), as compared to what has been reported previously [105].

Analogues of philanthotoxins bearing bulky head-group include bis-iminodibenzyl derivatives such as **21** and **22** (Fig. **6**), as well as hydrophilic or hydrophobic porphyrin derivatives such as **23a-d** [104]. These analogues could be advantageous in elucidating the mode of entry of polyamine toxins into the receptor, and their mode of binding to the receptor, as their bulky head-groups constrict the mode of blocking to a "head out" orientation (Fig. **7**). However, it was shown by circular dichroism (CD) spectroscopy that increasing hydrophobicity of the porphyrin group leads to amphiphillic compounds that form micelles in aqueous solution, thus leading to questionable results [104]. Activity of the bis-iminodibenzyl analogues was highly dependent on the nature of the polyamine; thus, analogue **21** having the spermine moiety was 6-fold more potent compared to PhTX-

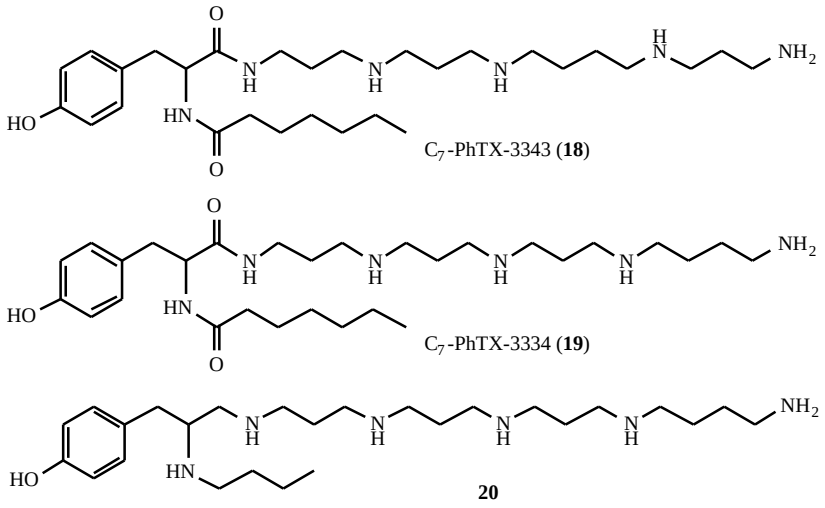


Fig. (5). Long polyamine chain and reduced analogues prepared by Nakanishi and co-workers.

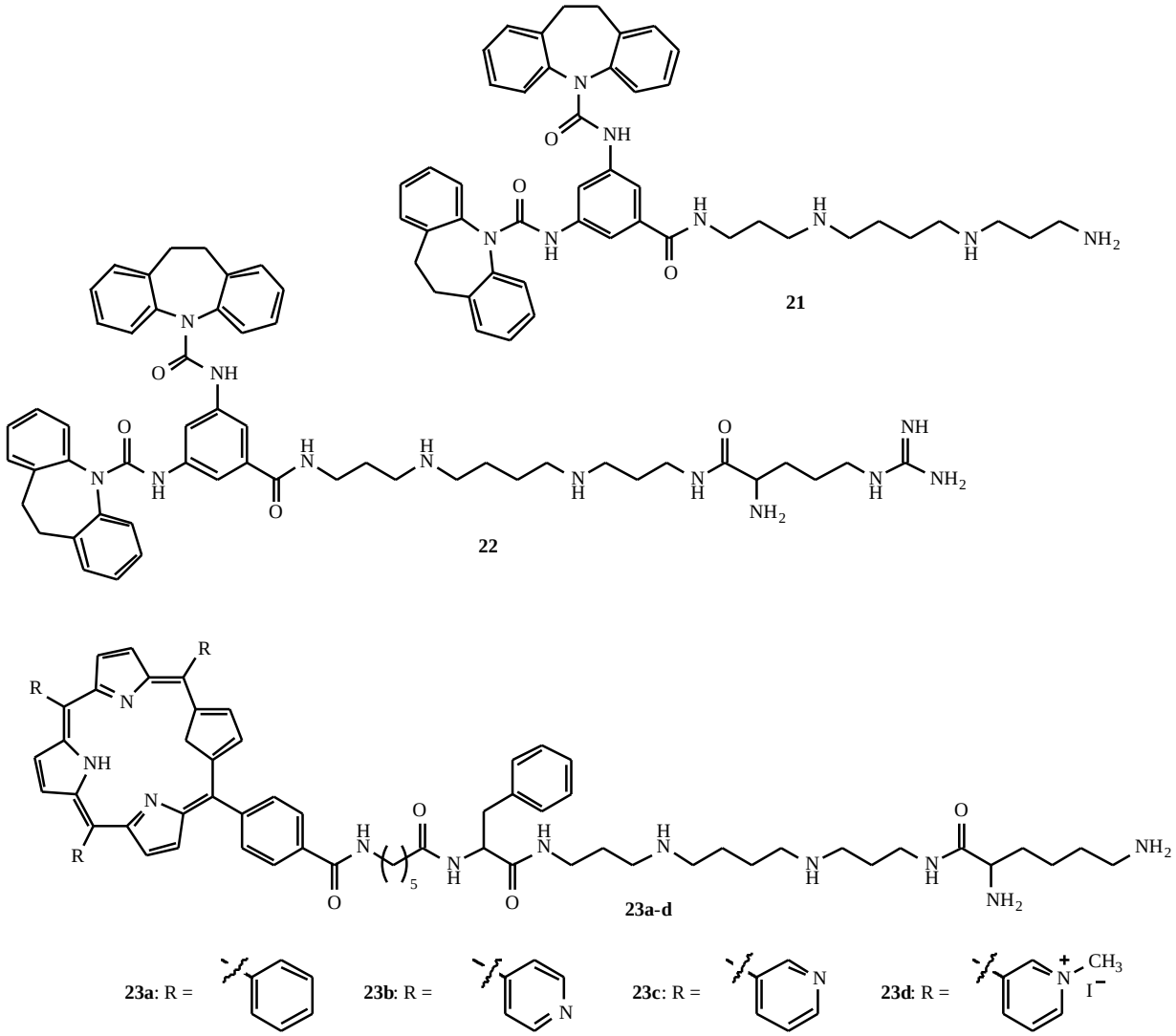


Fig. (**6**). Philanthotoxin analogues with large head-groups.

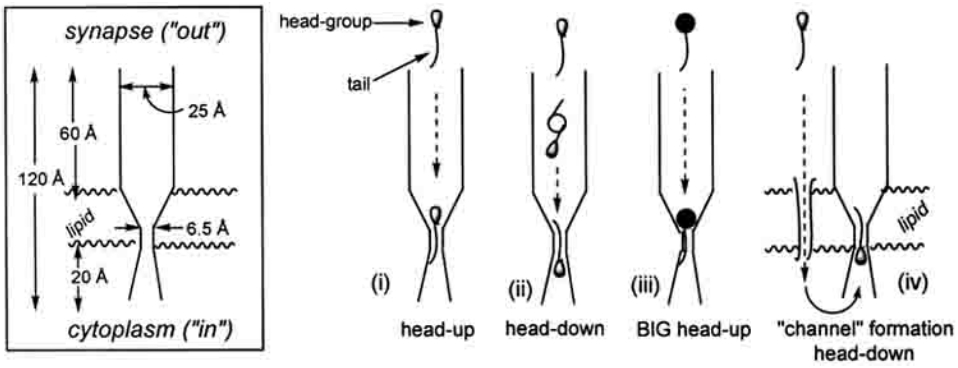


Fig. (7). Schematic illustration of modes of interaction of polyamine toxins with membrane-bound ion channels (reprinted from ref [105] with permission from Elsevier Science).

343 (**10**), whereas arginine substitution as in **22** increased the activity 16-fold. Further increase of the length of the polyamine only led to a 2-fold improvement of the potency on nAChR [105].

In the most recent SAR study by the Nakanishi group, eight biotinylated philanthotoxin derivatives were synthesized (Fig. **8**) and tested for inhibition of the binding of [^{3}H]-thienylcyclohexylpiperidine ([^{3}H]-TCP) to nAChR [106]. All of the biotinylated analogues were more potent than the native PhTX-433 (**9**) or PhTX-343 (**10**), the latter two being equipotent in the assay used. Compounds such as **24** and **25** (Fig. **8**) were respectively 37 and 50 times more potent than **9** and **10**. Interestingly, the biotinylated PhTX-343 analogue having a bifunctional photoaffinity probe (BPP, see later) attached at the end of the polyamine chain (**26**) (Fig. **8**) was 30-fold more potent than **9** and **10**, which makes this analogue a very promising candidate for future photolabeling studies. Preliminary electrophysiological studies on nAChR in the TE671 cells confirmed these activities [106].

A recent article describes the synthesis of analogues of methoctramine (**27**), philanthotoxin-343 (**10**) and hybrid analogues hereof [107]. Methoctramine is a tetramine with nitrogen functionalities separated by 6, 8 and 6 methylene groups and symmetrically disubstituted with *o*-methoxybenzyl groups (Fig. **9**). The analogues were tested in various assays including a muscle-type nAChR from frog rectus abdominis muscle. In addition to the symmetrically substituted analogues of methoctramine (**27**) with aromatic groups in both ends, a range of asymmetrically substituted

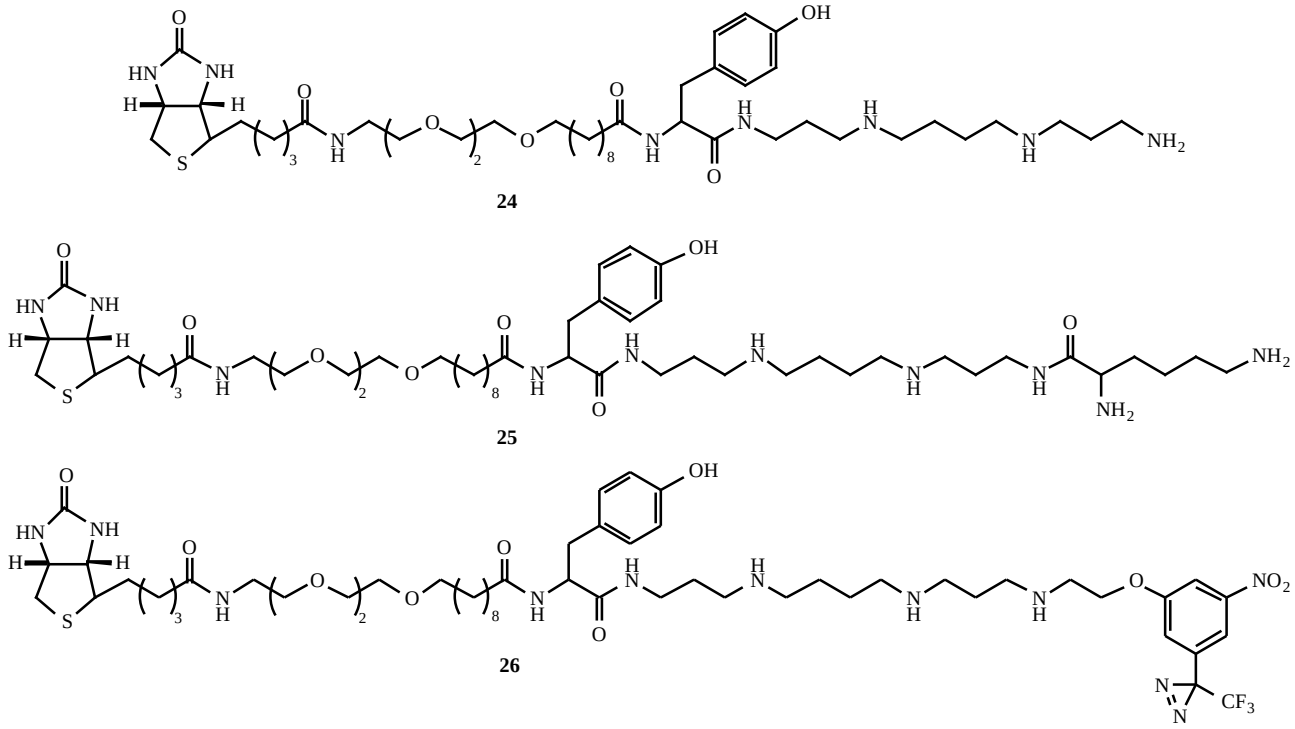


Fig. (8). Biotinylated analogues of PhTX-343 (**10**).

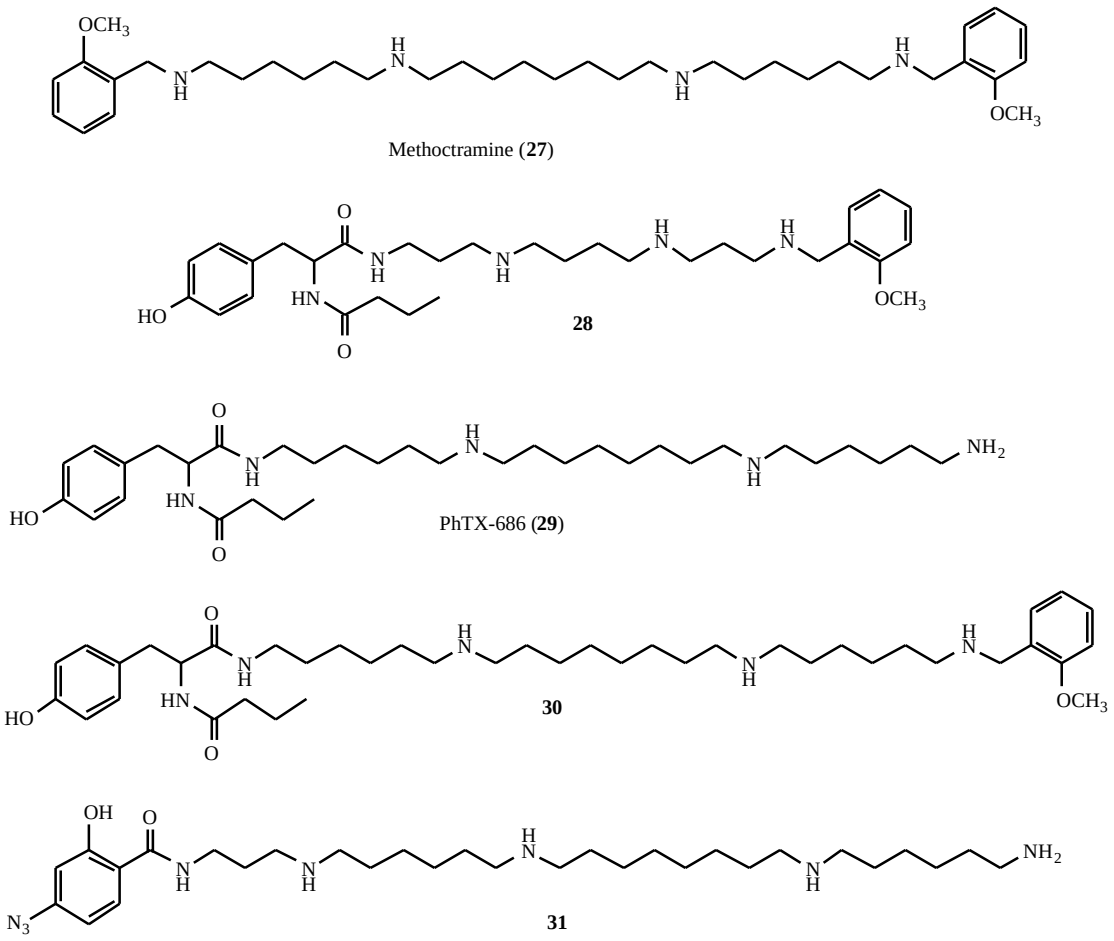


Fig. (9). Hybrid analogues of methoctramine (**27**) and PhTX-343 (**10**).

analogues has been synthesized. Somewhat surprisingly, substituting the primary amino group of PhTX-343 (**10**) with the *o*-methoxybenzyl group as in **28** increased the inhibitory activity at nAChR. Likewise, substituting the primary amino group of PhTX-686 (**29**), which itself is slightly more potent than PhTX-343 (**10**), as in **30** also increased the potency [107]. Interestingly, compound **31**, which is a photolabile analogue bearing the azido group, is 15 times more potent than PhTX-343 (**10**) on nAChR, thus serving as a promising candidate for photolabeling of nAChR [107].

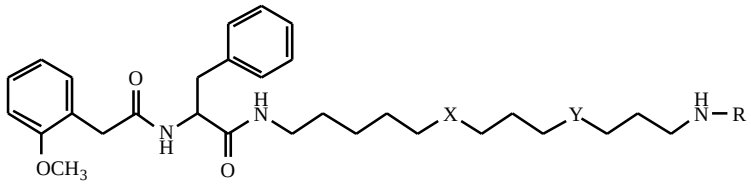
Bixel *et al.* recently published a study in which 11 known philanthotoxin analogues together with three known polymethylene tetramines, were investigated at the nAChR [108]. A fluorescence titration assay was used, based on ethidium, a fluorescent, non-competitive inhibitor of nAChR. Since the compounds compete with bound ethidium, it was assumed that they act as high-affinity non-competitive inhibitors at the nAChR. This SAR study confirms previous findings, i.e., that increased hydrophobicity of the head-group increases affinity and that polarity decreases the activity, whereas no firm conclusions were reached with respect to the effects on SARs of the polyamine moiety [108].

The major dogma within the field of polyamine toxins has been the importance of the polyamine moiety for the ability of the polyamine toxin to inhibit ionotropic receptors. Thus, the protonated amino groups were believed to interact with polar or negatively charged amino acid residues in the interior of the ion channel. Two recent articles address the importance of the inner basic sites of analogues of ArgTX-636 (**1**) [109] and PhTX-343 (**10**) [110], respectively.

Mueller and co-workers replaced the amino groups of the analogue **32** with methylene groups or oxygen atoms (Table **1**) [109]. The rationale was to decrease the polarity of the spider toxins, thereby increasing the likelihood of penetrating the BBB, and to minimize their associated cardiovascular side effects. Furthermore, compounds with truncated polyamines moieties were synthesized, as well as compounds with varying distances between the secondary amino groups, while keeping the total polyamine length constant.

The compounds were tested in a functional NMDA receptor assay, measuring the inhibition of NMDA/glycine-induced increase of intracellular Ca^{2+} concentration. Compounds having three basic nitrogen atoms in the polyamine moiety, such as **32** and **33** (Table **1**), all had IC_{50}

Table 1. Effect of Substituting the Secondary Amino Groups of 32, an ArgTX-636 (1) Analogue, on NMDA Responses



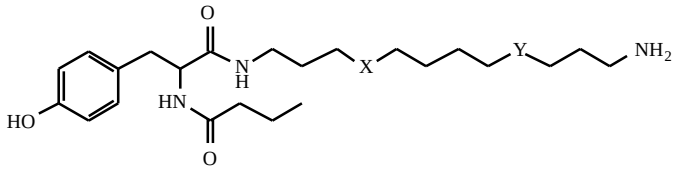
compound	X	Y	R	IC_{50} (μM)[a]
32	NH	NH	H	1.5
33	NH	NH	Gly	0.69
34	NH	CH_2	Gly	71
35	CH_2	NH	Gly	19
36	NH	O	H	> 100
37	O	NH	H	53

[a]Inhibition of increases in intracellular Ca^{2+} concentration in rat cerebellar granule cell cultures in the prescence of NMDA (50 μM) and Gly (1 μM).

values around 1 μM, whereas truncated analogues and the analogues **34**-**37** (Table **1**) were significantly less potent. This highlights the importance of the three basic sites present in **32** and **33** for the ability to inhibit NMDA receptors. Moreover, the most potent analogue exhibited significant cardiovascular effects in rats, more pronounced than those observed with ArgTX-636 (**1**). The authors suggest that secondary amino groups are necessary, not for ionic interactions with the receptor, but for assuming a bioactive conformation by intramolecular hydrogen bonds [109].

Strømgaard *et al.* carried out a systematic investigation of the importance of the inner basic sites of PhTX-343 (**10**) by replacement of the secondary amino groups with either methylene groups or an oxygen atom (Table **2**) [110]. The compounds were assayed in an electrophysiological nAChR

Table 2. Effect of Analogues of PhTX-343 (10) Lacking Inner Basic Sites on quisR and nAChR



			qGluR[a]	nAChR[b]
Compound	**X**	**Y**	IC_{50} (μM)	
10	NH	NH	23	16.6 ± 0.24
13	CH_2	CH_2	>> 100	0.9 ± 0.09
38	O	O	>> 100	10.7 ± 0.5
39	NH	CH_2	>> 100	12.3 ± 2.3
40	CH_2	NH	>> 100	4.1 ± 0.42
41	NH	O	>> 100	9.0 ± 0.87
42	O	NH	>> 100	2.6 ± 1.05

[a]Inhibition of contraction of *Locust* muscle preparation. [b]From a decrease of the current elicited by acetylcholine, at the end of a 1 second application period of the antagonist.

assay using TE671 cells, as well as the conventional quisR assay (Table **2**). In the latter assay, compounds lacking the secondary amino groups such as **13** and **38**-**42** (Table **2**) were completely devoid of activity, underlining the importance of three protonable groups for the activity at quisR (Table **2**). On the other hand, removal of one of the secondary amino groups, as in **39**-**42** (Table **2**), led to retained or even increased activity at the nAChR, and replacement of both secondary amino groups with methylene groups, as in PhTX-12 (**13**), increased the potency 50-fold (Table **2**). This is the first example of philanthotoxin analogues being able to discriminate between the two types of ionotropic receptors, i.e. nAChR and iGluR [110].

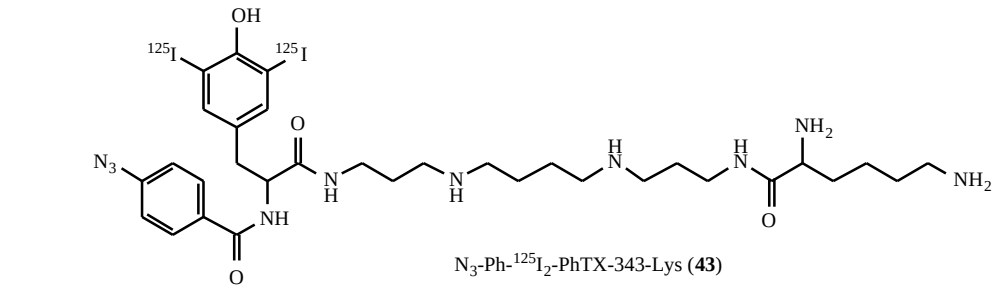
Investigations have then been made in order to determine whether PhTX-12 (**13**) and PhTX-343 (**10**) bind to the nAChR in a similar manner. A recent SAR study has shown that the same modifications of the butyric acid moiety of PhTX-343 (**10**) and PhTX-12 (**13**) led to opposite changes of the potency on nAChR [111]. Furthermore, recent pharmacological studies by Usherwood and co-workers have revealed that PhTX-12 (**13**) has a higher affinity for the closed channel conformation, in contrast to PhTX-343 (**10**), which primarily binds to the open channel conformation of nAChR (unpublished results).

A large amount of work has been devoted to the synthesis of photolabile analogues for use of receptor structure studies. Synthesis of photolabile analogues has been described and used in traditional SAR studies [92,95,97,105,107,108,112]. However, Nakanishi and co-workers were the first to use a radiolabeled, photolabile philanthotoxin analogue, namely N_3-Ph-$^{125}I_2$-PhTX-343-Lys (**43**, Fig. **10**), in a photo-linking study at the nAChR [113]. The study showed labeling of all five subunits in the absence of the 43 kDa protein, whereas the α-subunit was preferentially labeled in the presence of this protein. The binding domain was constricted to be in the cytoplasmic loops Thr237-Met243 and Thr298-Glu338, which led to a proposed model of binding in a "head-down" fashion (Fig. **7**) in the gate of the channel, close to the cytoplasmic face. Other wasp and spider toxins competed with the binding of analogue **43**, indicating that all of these compounds bind approximately at the same site. A disadvantage of this study was that the analogues were applied in a manner that allowed access to both the extracellular and cytoplasmic side of the receptor [113].

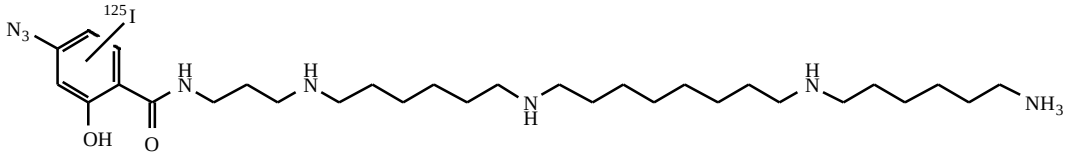
Bixel *et al.* performed another photolabeling experiment using N_3-Ph-$^{125}I_2$-PhTX-343-Lys (**43**) and nAChR [108], similarly as in the previous study [113]. It was found that **43** binds to all five receptor subunits. However, contrary to the findings of Nakanishi *et al*. [113], it was concluded that the ligand is likely to enter from the extracellular side of the ion channel, with the hydrophobic head-group binding in the upper part of the channel (to the high-affinity non-competitive inhibitor site) and with the positively charged polyamine moiety interacting with the negatively charged selectivity filter located deep within the channel pore, where it is in close contact with all five receptor subunits. Furthermore, increased affinity of **43** for the closed channel state, rather than to the open channel state, was observed [108].

In another photolabeling study performed by Bixel *et al.* the ^{125}I-iodinated **44** (Fig. **10**), was incorporated in the *Torpedo* nAChR [114]. The labeled subunit of the receptor was isolated, and contrary to the results of the previous study [108], it was shown to bind to the α-subunit only. Proteolytic mapping and subsequent sequencing of the proteolytic fragments of the α-subunit narrowed the hydrophobic binding site to the area constituted by αAsp-180 to αLeu-199. This confirms the findings of the previous study, namely that polyamine toxins bind to the nAChR with the aromatic head-group located in the upper vestibule of the ion channel, and with the polyamine moiety located within the narrow part of the ion channel close to the selectivity filter [114].

This study also demonstrated that two molecules of **31** bind to each receptor, contrary to other non-competitive inhibitors, which show a binding stoichiometry of 1:1.



N_3-Ph-$^{125}I_2$-PhTX-343-Lys (**43**)



44

Fig. (10). Photolabile analogues of polyamine toxins used in labeling studies.

Moreover, electrophysiological studies showed that the binding of **31** was voltage-independent, and that the presence of the agonist carbachol increased the affinity of **31** for nAChR. This indicates binding to the closed desensitized state of the receptor rather than to an open-channel block [114].

Recently, the Nakanishi group has developed a particularly elegant approach to the photoaffinity labeling by the introduction of a bifunctional photoaffinity probe (BPP) [115,116]. This probe consists of two different photolabile groups, linked to the primary amino group of the PhTX-343 (**10**) molecule giving analogue **26** (Fig. **8**) [115]. By irradiation of the receptor incubated with ligand, a carbene is generated from the trifluoromethyldiazirine moiety, which links the ligand to the receptor. The biotin moiety (which is linked to the philanthotoxin molecule through a polyether linker) is used for separation of the labeled and non-labeled peptide fragments from proteolyzed receptor by binding to an avidin column. The separated peptide fragments are irradiated again under mildly basic conditions leaving only the nitrophenolic marker linked to the peptide, which then can be sequenced by mass spectrometry (MS). The major advantage of this approach is the elimination of the need of radioactive labeling. To date, however, no photolabeling experiments using this BPP analogue **26** have been reported.

In conclusion, the two most recent photolabeling studies [108,114] both suggest channel block by polyamine toxins entering from the extracellular side binding in a "head up" fashion (Fig. **7**). This contradicts the first study by Nakanishi *et al*. [113], that suggested binding to the intracellular site of the receptor. Thus, although information from photolabeling studies is mounting, these studies nevertheless suffer from certain limitations, the major one being the position of the photolabile group, which in all cases is present in the aromatic head-group. The shortcoming comes from the fact that the aromatic head-group presumably binds in a rather non-specific fashion to a hydrophobic pocket. Thus, placing a photolabile group in the polyamine moiety, which is believed to bind more closely to the interior of the ion channel, would be of major interest. The group of Nakanishi has synthesized compounds with photolabile groups in proximity to the polyamine moiety [97], but unfortunately these compounds are only very weak antagonists as demonstrated with quisR, thus severely limiting their potential as photoaffinity probes. Another limitation has been the choice of the photolabile group, which in most cases has been the azide group. Use of other photolabile groups, which act through different mechanisms, would be of major interest.

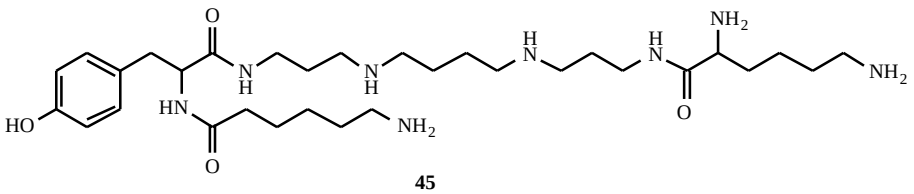
STUDIES USING PARENT POLYAMINE TOXINS

The number of studies using polyamine toxins is immense. However, in the vast majority of these studies polyamine toxins are used as tools, in particular to distinguish different types of AMPA and KA receptors. In the following, use of parent polyamine toxins such as ArgTX-636 (**1**), JSTX-3 (**4**), PhTX-433 (**9**) and PhTX-343 (**10**) for the study of the mechanism of their interaction with the receptor will be discussed.

Nakanishi and co-workers showed that philanthotoxins could mediate ion transport themselves. Using reconstituted vesicle membranes made from egg yolk phosphatidylcholine employing ^{23}Na NMR and fluorescence spectroscopy [117], it was shown that **9** and **45** (Fig. **11**) were able to conduct Na^+ ions across the membrane. Interestingly, spermine did not conduct Na^+ ions, emphasizing the importance of the lipophilic head-group. It is suggested that the head-group is providing an anchor for the philanthotoxins to enter the membrane. Basically, two possibilities for conducting ions by philanthotoxins exist, namely by forming a channel, or as an ion carrier. From the study it is not clear which of these mechanisms is operating when philanthotoxins **9** and **45** are conducting Na^+ ions across the membrane. It is concluded that the observed agonist activity of polyamine toxins at very low concentrations may be due, indirectly, by nonspecific binding to the lipid bilayer [117].

A study of the protolytic properties and proton distribution in PhTX-343 (**10**) by ^{13}C NMR suggested that the charge at the secondary amino group closest to the primary amino group in the polyamine moiety may not be essential for the pharmacological activity of philanthotoxins [118]. This hypothesis was based on the fact that in the case of PhTX-433 (**9**) the triprotonated form was the predominant species at the physiological pH, whereas PhTX-343 (**10**) exists mainly in the fully protonated form, and that these differences may reflect small differences in pharmacological activity [118]; similar differences were observed with other philanthotoxin analogues. This study eventually led to the synthesis of analogues of PhTX-343 (**10**) lacking inner basic sites, the protolytic properties of which were likewise analyzed by ^{13}C NMR titrations [110].

Jayaraman *et al.* have performed fast kinetic studies of the inhibition of the nAChR of BC_3H1 muscle cells by PhTX-343 (**10**) using a laser pulse technique [119]. The kinetic measurements indicate a 5-fold higher affinity of PhTX-343 (**10**) for the closed-channel form of nAChR than for the open-channel form. Single-channel current measurements indicate an inhibitory site on the open-channel



45

Fig. (11). A philanthotoxin analogue showing ionophoric properties.

form of nAChR. However, the dissociation constant is considerably higher than that of the closed channel form, ca. 500 and 50 μM, respectively. PhTX-343 (**10**) had no effect when applied to inside-out patches, indicating that the binding site is on the extracellular side of the nAChR of BC_3H1 cells [119]. The extent of inhibition is voltage-dependent, which can be attributed to a large extent to a decreasing effect of the channel-opening equilibrium constant. Thus, the voltage dependence of the inhibition cannot be used as an indicator for an inhibitory site on the open-channel form.

Moreover, it is concluded that PhTX-343 (**10**) acts predominantly by a regulatory mechanism, in which it binds to a regulatory site of the closed-channel form of the nAChR, and allosterically inhibits signal transmission by decreasing the channel-opening equilibrium constant. At very high concentrations, however, an additional inhibitory process is observed, in which the complex of the inhibitor with the open-channel form isomerizes to a non-conducting form [119].

The group of Mayer has recently reported the results of mechanistic studies of inhibition of various iGluRs by a range of antagonists including PhTX-343 (**10**). These studies have emphasized the importance of hydrophobicity when polyamines interact with receptors, such as the recombinant KA receptor consisting of homomeric edited GluR6(Q). Moreover, they have shown that the characteristic double rectification caused by polyamines by penetration of the receptors is dependent of the cross-sectional width of the molecule [120,121].

In a thorough study of the block of recombinant KA receptors, GluR6(Q), by PhTX-343 (**10**) Mayer *et al.* have showed that the ion channel must be open for **10** to bind, as no inhibitory action of **10** on the closed channel was observed. It is shown that the antagonism by **10** includes a two component block, a fast component probably representing the binding to the open channel, and a slow component probably representing trapping of **10** due to channel closure *via* an allosteric mechanism. Furthermore, it was shown that externally applied **10** results in permeation at negative membrane potentials, but does not involve binding to a high-affinity site at the internal face of the membrane. These observations led the authors to conclude that **10** acts as an open-channel blocker, and that if **10** remains bound to the channel, an allosteric mechanism destabilizes the open channel state, leading to channel closing and trapping [122,123].

The above studies suggest an open-channel block by PhTX-343 (**10**) of KA receptors, whereas photolabeling studies suggest a closed channel block by **44** on nAChR [114]. These results might account for basically different modes of interaction with iGluRs and nAChR by polyamine toxins. Furthermore, polyamine toxin analogues such as PhTX-343 (**10**) and PhTX-12 (**13**) seem to antagonize the same receptor, nAChR, by different modes of interaction, the former through open-channel block, and the latter by a closed-channel block.

Recent studies have addressed the possibility of an intracellular block by polyamine toxins, using ArgTX-636 (**1**) [124,125] and PhTX-343 (**10**) [126], as has previously been suggested by Usherwood and co-workers [127] and also proposed from the photolabeling study performed by the group of Nakanishi [113]. Scott *et al.* have investigated the ability of extra- and intracellular application of ArgTX-636 (**1**) on excitability in cultured rat dorsal root ganglion neurons. In this study, intracellular application of ArgTX-636 (**1**) altered the properties of evoked action potentials, as well as voltage-activated Na^+ and K^+ currents. The latter, in particular, were more sensitive to ArgTX-636 (**1**) applied to the intracellular environment [124,125]. Usherwood and co-workers investigated the effect on quisR of intracellularly applied PhTX-343 (**10**), which antagonized the receptor in a use-independent and voltage-independent manner, which suggest that intracellularly-injected PhTX-343 (**10**) does not bind in the quisR ion channel. Thus, clearly PhTX-343 (**10**) binds to an intracellular domain of the quisR [126]. It is envisaged that polyamine toxins **1** and **10** gain access to an intracellular site of action *via* endogenous polyamine transporters, or by passing through ion channels [124-126].

SOLID PHASE SYNTHESIS (SPS)

The use of solid phase chemistry in the synthesis of polyamines and polyamine derivatives has recently gained substantial interest [100,101]. The advantages of using solid phase synthesis (SPS) for the synthesis of polyamines in general and polyamine toxins in particular are obvious. One of the major obstacles in the synthesis of polyamine toxins is the purification of these highly polar species, particularly separation from unwanted polyamines. Using solid phase methodology these purification steps are simple washings, thus, in principle leading to more pure compounds. A second challenge in the synthesis of polyamine toxins in solution is the need of using a range of protecting groups, which makes synthesis of polyamine toxins a laborious and uneconomical task. The use of SPS could provide an advantage also in this respect. Moreover, SPS could be an effective tool for preparing combinatorial libraries, allowing more comprehensive SAR studies in a shorter time.

Bycroft and co-workers were the first to use SPS in the synthesis of polyamine toxins, as illustrated by the synthesis of nephilatoxin-9 (**46**) and -11 (**47**) (Fig. **12**) [128]. This was achieved by use of established solid-phase peptide synthesis procedures, using a newly developed bis-*N*-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl] (Dde) protecting group [129]. In 1996, the same group published a solid phase synthesis of PhTX-343 (**10**) [130], which was accomplished as outlined in Scheme **1**. Spermine (**48**) was attached to a 2-chlorotrityl chloride linker, followed by a selective Dde protection of the primary amino group of the linked spermine, using 2-acetyldimedone, to give **49**. The secondary amino groups were protected using di(*tert*-butyl) dicarbonate [$(Boc)_2O$], and the Dde group was removed to give **50**. This was followed by sequential coupling of protected tyrosine, removal of the 9-fluorenylmethoxy-carbonyl (Fmoc) group and coupling of butyric acid, before cleavage of the product from the resin with concomitant deprotection to give PhTX-343 (**10**) (Scheme **1**).

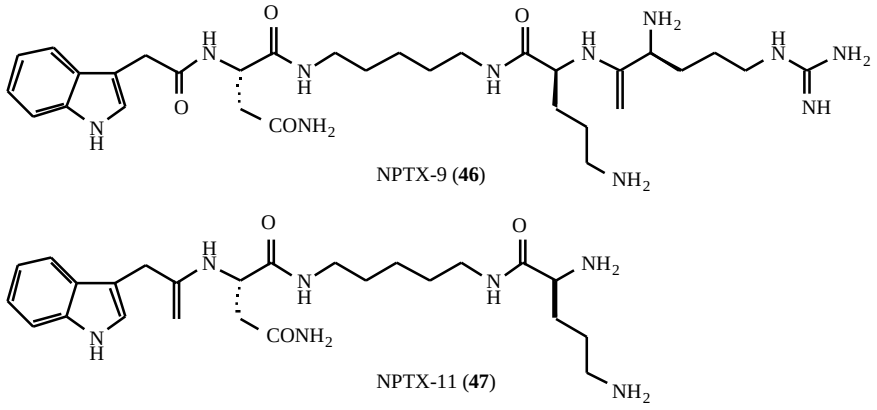
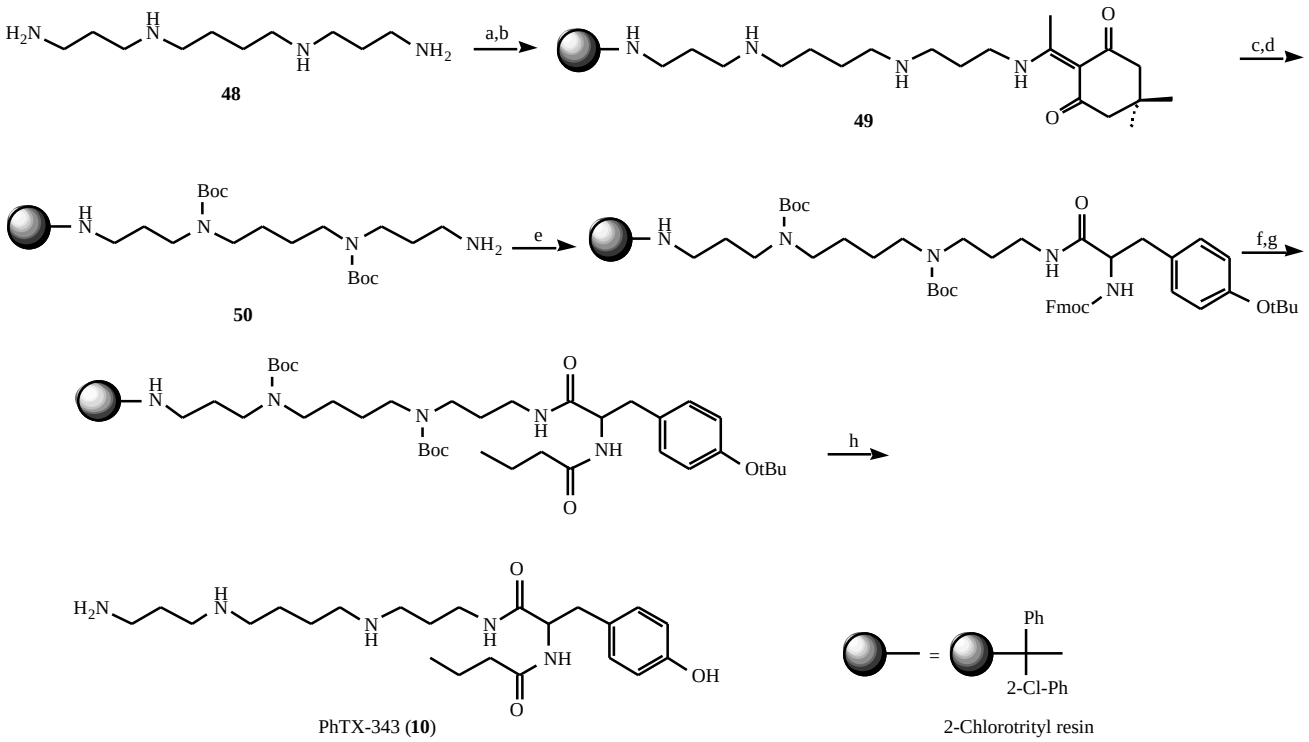


Fig. (12). Structures of the polyamine toxins first synthesized by SPS.



Scheme **1**. (**a**) 2-chlorotrityl chloride resin; (**b**) 2-acetyldimedone; (**c**) $(Boc)_2O$; (**d**) H_2NNH_2; (**e**) *N*-Fmoc-O-*tert*-butyl-(*S*)-tyrosine, HBTU, HOBt; (**f**) piperidine; (**g**) butyric acid, HBTU, HOBt; (**h**) TFA/iPr_3SiH/H_2O.

A slightly modified procedure was used in the synthesis of the two enantiomers of PhTX-343 (**10**) and PhTX-12 (**13**), respectively [131]. N^4,N^9-Di-Boc-spermine or 1,12-dodecanediamine was attached to a trityl resin, the former giving the resin corresponding to **50** (Scheme **1**). The primary amino group could then be derivatized directly employing appropriately protected (*R*)- or (*S*)-tyrosine. This peptide-type coupling was carried out using *N*-[(dimethylamino)-1*H*-1,2,3-triazol[4,5-*b*]pyridin-1-yl-methylene]-*N*-methyl-methanaminium hexafluorophosphate *N*-oxide (HATU) and 2,4,6-trimethylpyridine (collidine) as coupling reagents, which are known to cause little or no racemization [132,133]. The Fmoc group was removed, followed by another coupling with butyric acid. Finally, cleavage from the resin, with simultaneous deprotection led to the enantiomers of **10** and **13**. Chiral capillary

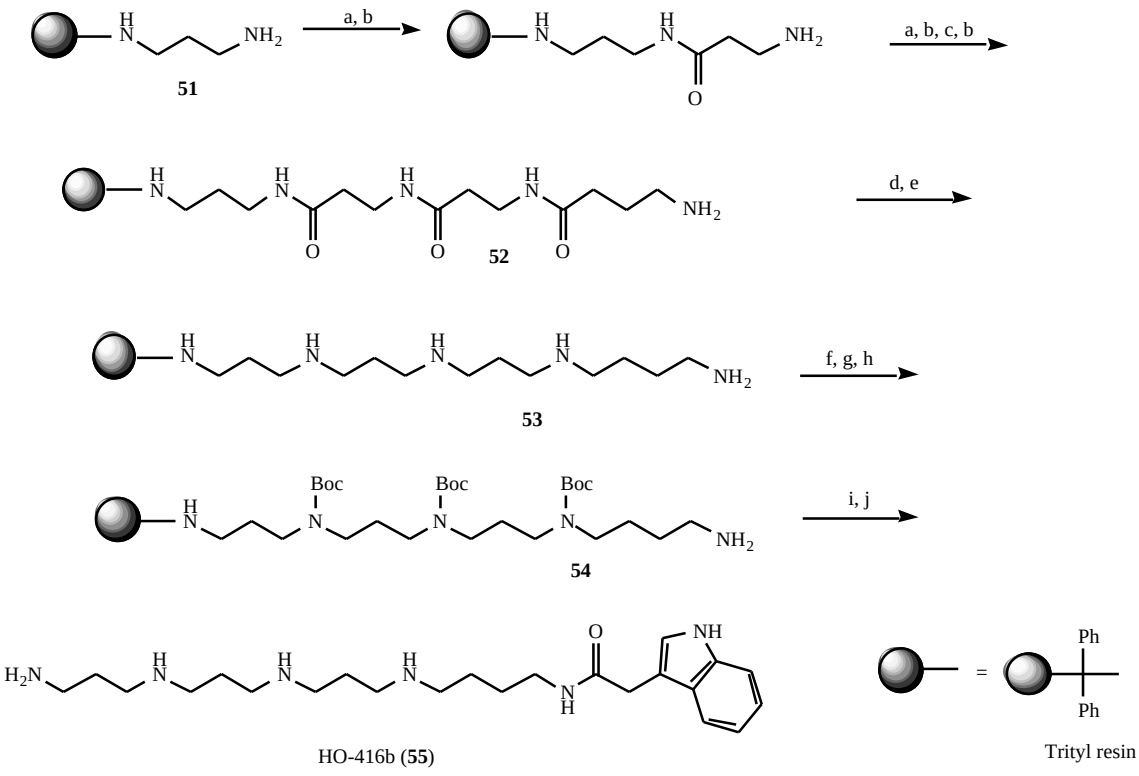
electrophoretic analysis proved that the enantiomers had an enantiomeric excess (ee) of more than 99% [131].

The above synthetic principle was used in the synthesis of the first combinatorial library of polyamine toxins [111]. Generally, a philanthotoxin molecule consists of three distinct parts: (1) an amino acid; (2) an acyl group and (3) a polyamine moiety joined by amide bonds. Thus, the molecule is particularly suitable for the generation of a combinatorial library using established solid-phase peptide synthetic procedures. The combinatorial library was generated by attachment of three different poly- or diamines to a trityl resin, and each of the resins reacted with two different tyrosine analogues. The Fmoc groups were removed prior to coupling with three different acids, leading to protected, resin-bound philanthotoxin analogues. Cleavage from the resin and deprotection led to 18 philanthotoxin analogues [111], which were subsequently characterized pharmacologically, using electrophysiological methods to determine antagonism of nAChR, and non-NMDA receptors [111].

The above described synthetic procedure were based on established solid-phase peptide synthetic procedures, using symmetrical poly- or diamines attached to the solid phase. This limits the scope of the method. In order to be able to synthesize polyamine toxins containing non-symmetric polyamines, a different synthetic approach has to be developed. Several groups have recently addressed this point.

Hall and co-workers synthesized HO-416b (**55**) and PhTX-433 (**9**) [134] using a polyamide reduction strategy as outlined in Scheme **2**. The strategy was based on their previous development of an efficient amide reduction method on solid phase [135]. The starting point was commercially available 1,3-diaminopropane bound to a trityl resin (**51**), and Fmoc-protected amino acids were used in sequential peptide coupling steps. Fmoc removal gave resin-bound polyamide **52**, which was reduced by treatment with borane followed by decomplexation with iodine in a mixture of THF, acetic acid and diisopropylethylethylamine (DIEA), to give resin-bound polyamine **53**. Similarly to the synthesis of **50** (Scheme **1**), the primary amino group was selectively protected using 2-acetyldimedone, followed by Boc protection of the secondary amino groups, and the liberation of the primary amino group to yield polyamine **54** (Scheme **2**). The latter was acylated with indole-3-acetic acid completing the synthesis of HO-416b (**55**) (Scheme **2**). For the synthesis of PhTX-433 (**9**), resin-bound, protected polyamine with the nitrogen functionalities separated by 4, 3, and 3 methylene groups, corresponding to **54**, was acylated with tyrosine, and butyric acid to give PhTX-433 (**9**). This approach also offers access to branched polyamine toxin analogues. Very recently, Hall *et al*. used this strategy to produce a large polyamine library [136].

Polyamine toxin HO-416b (**55**) was likewise synthesized by Fukuyama and co-workers in a semi-SPS, that is 2-nitrobenzenesulfonamide (NS) protected HO-416b was loaded onto a trityl resin, the NS protection groups were removed by treatment of the resin with mercaptoethanol and

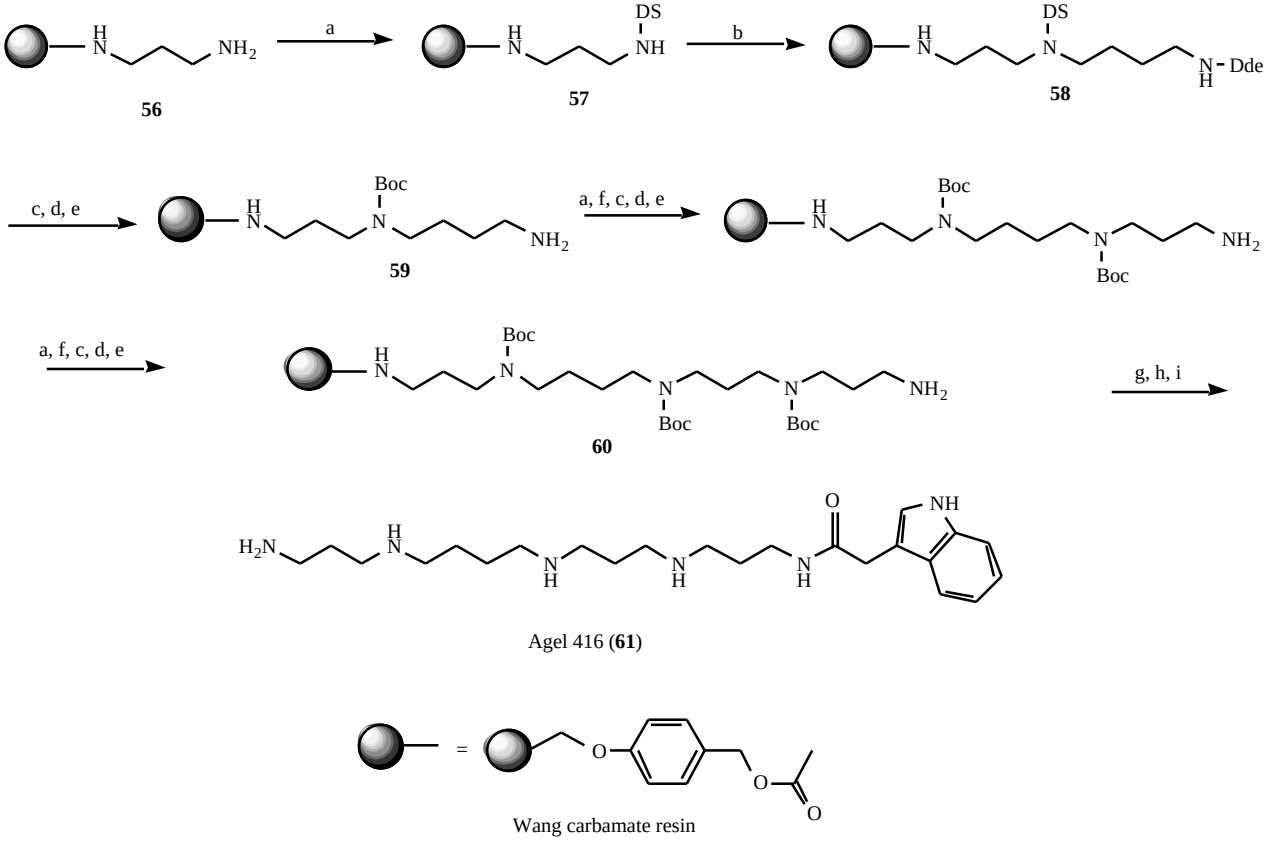


Scheme **2**. (**a**) $HO_2C(CH_2)_2NHFmoc$, HBTU, HOBt; (**b**) piperidine; (**c**) $HO_2C(CH_2)_3NHFmoc$, HBTU, HOBt; (**d**) BH_3; (**e**) I_2, THF, DIEA, AcOH; (**f**) 2-acetyldimedone; (**g**) $(Boc)_2O$; (**h**) H_2NNH_2; (**i**) indole-3-acetic acid *tert.* butyl anhydride; (**j**) $TFA/H_2O/iPr_3SiH$.

DBU, and the resulting resin-bound HO-416b cleaved from the resin [137,138]. In the solution phase synthesis, the NS group was used for protection and activation in the N-alkylation, a procedure that has been named the Fukuyama amination [139,140]. The latter reaction has likewise been used for the SPS of polyamine toxins (see below).

Hone and Payne have synthesized Agel-416 (**61**) containing a polyamine with the nitrogen functionalities separated by 3, 3, 3, and 4 methylene groups (Scheme **3**) [141]. 1,3-Diaminopropane was immobilized onto Wang carbamate resin giving **56**, and the resin-bound amine was derivatized with 2,4-dinitrobenzenesulfonyl chloride giving the corresponding 2,4-dinitrobenzenesulfonamide (**57**). The use of the dinitrobenzenesulfonamide is rationalized by a higher rate of sulfonamide formation and, in particular, a higher rate of the subsequent Mitsunobu reaction, due to the lower pKa value of the amide proton, as compared to mononitrobenzenesulfonamides. The disadvantage is a higher reactivity towards bases, leading to deprotetion of the tertiary sulfonamide. The next step is the alkylation of the sulfonamide by a Dde-protected amino alcohol, using Ph_3P and diethylazadicarboxylate (DEAD) as redox reagents, giving **58**. The three following steps are protecting group manipulation. Thus, firstly the 2,4-dinitrobenzene-sulfonamide is removed, and the secondary amine is then re-protected by $(Boc)_2O$, followed by removal of Dde-group using hydrazine to afford **59** (Scheme **3**). The reason for this rather laborious protection-deprotection strategy is most likely due to the lability of the 2,4-dinitrobenzene-sulfonamide protecting group, which is unstable during the cleavage conditions of the Dde group with hydrazine. The above described steps are repeated until the required Boc-protected resin-bound polyamine (**60**) of the proper length is obtained. The synthesis is completed by acylation with *N*-Boc indole-3-acetic acid, the fully protected Agel-416 is cleaved from the resin using 5 % trifluoroacetic acid (TFA) in CH_2Cl_2, which leaves the Boc groups intact, allowing the compound to be purified by column chromatography before deprotection of the Boc groups using 4 M HCl [141].

In 1998, Bycroft and co-workers presented a linker based on the Dde-protecting group in the synthesis of pseudoargiopine III. Standard solid-phase peptide synthetic procedures were employed [142]. The linker was recently used for the synthesis of PhTX-343 (**10**) and PhTX-334 (**11**) [143], as well as of PhTX-433 (**9**) [144]. PhTX-343 (**10**) was synthesized by the use of the same principles as those employed by Strømgaard *et al*. [111,131] but using the Dde-linker instead of the trityl linker. PhTX-334 (**11**) was synthesized by attachment of 1,4-diaminobutane to the Dde-linker (**62**), which was reacted with 3-Fmoc-aminopropanal in a reductive alkylation reaction, reducing the generated imine by $NaBH_3CN$, followed by Boc-protection of the



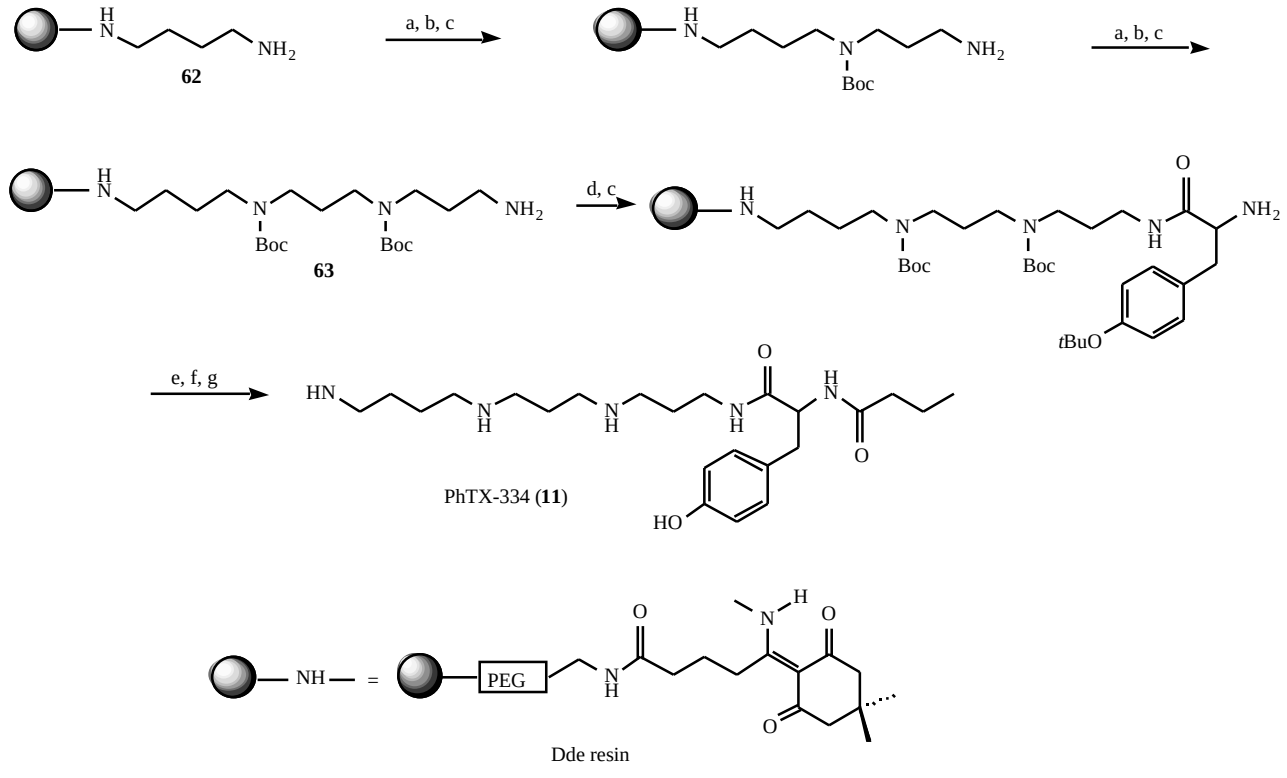
Scheme **3**. (**a**) 2,4-Dinitrobenzenesulfonyl chloride; (**b**) *N*-Dde-butanolamine, PPh_3, DEAD; (**c**) mercaptoacetic acid, DIEA; (**d**) $(Boc)_2O$; (**e**) H_2NNH_2; (**f**) *N*-Dde-propanolamine, DEAD, PPh_3; (**g**) *N*-Boc-indole-3-acetic acid, HOBt, DIC; (**h**) TFA; (**i**) HCl.

secondary amino group (Scheme **4**). The Fmoc group was removed, and the above three steps were repeated. The protected resin-bound polyamine **63** was derivatized, and reaction with appropriately protected tyrosine and butyric acid gave the fully protected product bound to the solid phase. The protecting groups were removed by treatment with TFA, before the product was cleaved from the resin using propylamine to give PhTX-334 (**11**) (Scheme **4**) [143].

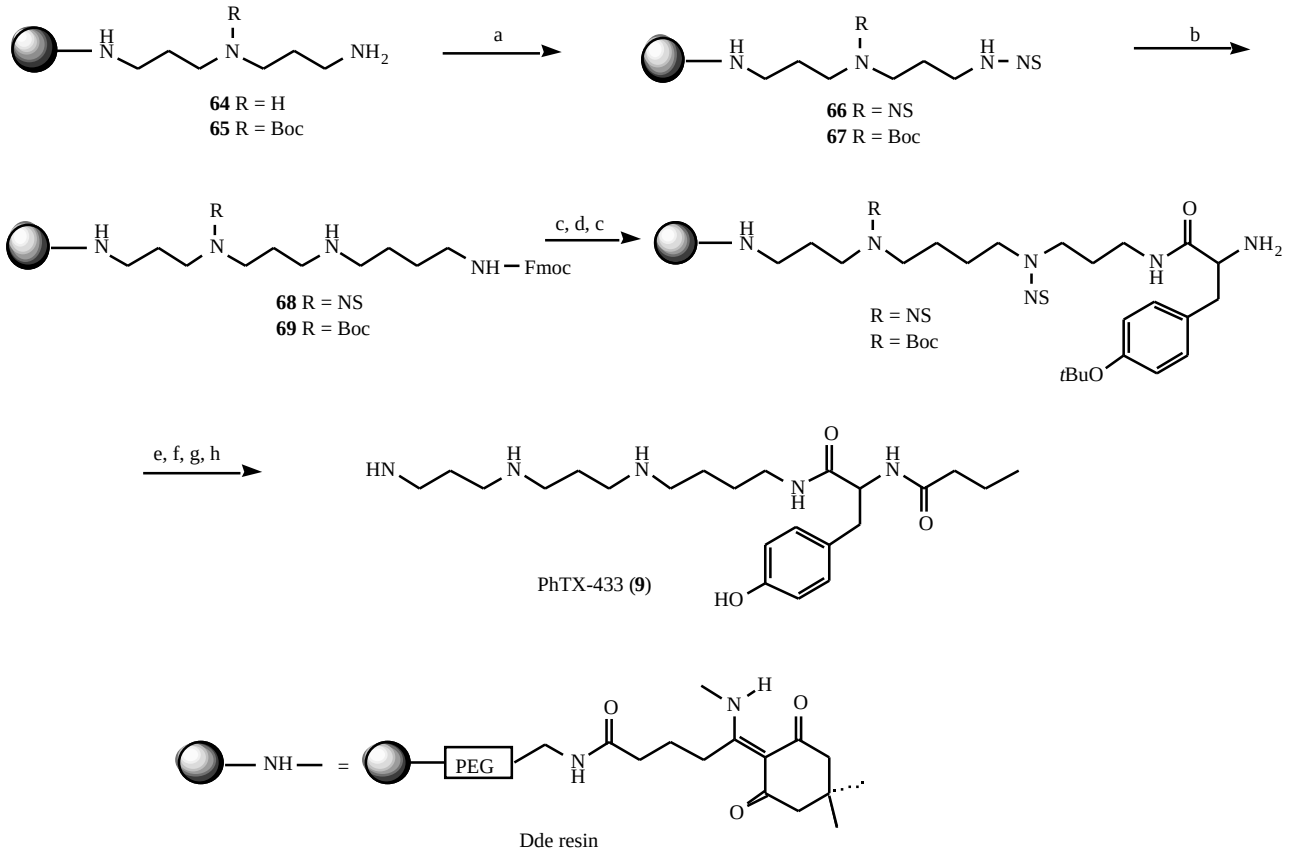
The above synthetic procedure could not be used for the synthesis of PhTX-433 (**9**), because of the inability to achieve a convenient synthesis of *N*-Fmoc-4-aminobutanal. Also, the corresponding C_5-analogue, *N*-Fmoc-5-aminopentanal, could not be obtained, thus severely limiting the scope of the methodology described. For the synthesis of PhTX-433 (**9**) [144], another approach was followed. The symmetrical triamine, 3,3'-diaminopropylamine, or its N^4-Boc derivative was attached to the Dde-linker resulting in **64** and **65**, respectively (Scheme **5**). The primary amino group was reacted with 2-nitrobenzenesulfonyl chloride, to give either **66** or **67**. It is not clear which of the two solutions is the most advantageous. The secondary NS is reacted with *N*-Fmoc-4-aminobutanol in a Mitsunobu reaction using Ph_3P and DEAD as redox reagents to give **68** and **69**, respectively. Standard steps, as those previously described, completed the synthesis of PhTX-433 (**9**) [144].

Strømgaard *et al.* very recently described a general approach to the solid phase synthesis of polyamines, and exemplified it by the synthesis of eight philanthotoxin analogues [145], with systematic variation of the inter-amine distances (Scheme **6**). As in two previously described approaches to SPS of polyamine toxins [141,144], this approach is based on the Fukuyama amination [139,140]. Commercially available 1,3-diaminopropane and 1,4-diaminobutane bound to a trityl resin, respectively **51** and **70**, were used as starting materials, and were derivatized with 2-nitrobenzenesulfonyl chloride to give the corresponding NS derivatives **71** and **72**. These were reacted with various *N*-2-(trimethylsilyl)ethyloxycarbonyl (Teoc) protected amino alcohols in a Mitsunobu reaction using Tsunoda conditions, i.e., using Bu_3P and 1,1'-(azadicarbonyl)dipiperidine (ADDP) [146] as redox reagents to give **74**-**77**. The reaction had to be repeated three times in order to get practically quantitative conversion in the Mitsunobu reaction. Interestingly, use of the standard Mitsunobu reagents, Ph_3P and DEAD, as employed in the two previously described syntheses using the Fukuyama amination [141,144], did not lead to the required products. The importance of the quantitative alkylation reaction was apparent, as even a 95% conversion in the alkylation reaction led to a significant amount of the truncated polyamine toxins, e.g. PhTX-34 or PhTX-43, as observed by ^{13}C NMR.

With the Mitsunobu reaction optimized, a crucial feature was to be able to cleave the protecting group of the amino alcohol, in this case the Teoc group, without cleaving the NS groups. This was achieved by treating the resin with



Scheme **4**. (**a**) (*i*) Fmoc-NH$(CH_2)_2$CHO, (*ii*) $NaBH_3CN$; (**b**) $(Boc)_2O$; (**c**) piperidine; (**d**) *N*-Fmoc-O-*tert*-butyl-(*S*)-tyrosine, TBTU, HOBt; (**e**) butyric acid, TBTU, HOBt ; (**f**) TFA/H_2O/*i*Pr_3SiH; (**g**) n-propylamine.



Scheme **5**. (**a**) 2-nitrobenzenesulfonyl chloride; (**b**) FmocNH$(CH_2)_4$OH, Ph_3P, DEAD; (**c**) piperidine; (**d**) *N*-Fmoc-O-*tert*-butyl-(*S*)-tyrosine, TBTU, HOBt, DIEA; (**e**) butyric acid, TBTU, HOBt ; (**f**) TFA/H_2O/*i*Pr_3SiH; (**g**) NaSPh; (**h**) n-propylamine.

tetrabutylammonium fluoride (TBAF) for 30 min. Thus, by repeating the above steps, i.e. the reaction with 2-nitrobenzenesulfonyl chloride, Mitsunobu reaction, and deprotection of the Teoc group, it was possible to prepare resin-bound, properly protected polyamines **78**-**85** (Scheme **6**), ready for further derivatizarion. The latter was exemplified by the synthesis of philanthotoxins **9**-**11** and **86**-**90** [145] (Scheme **6**) performing steps identical to those previously described [111, 131].

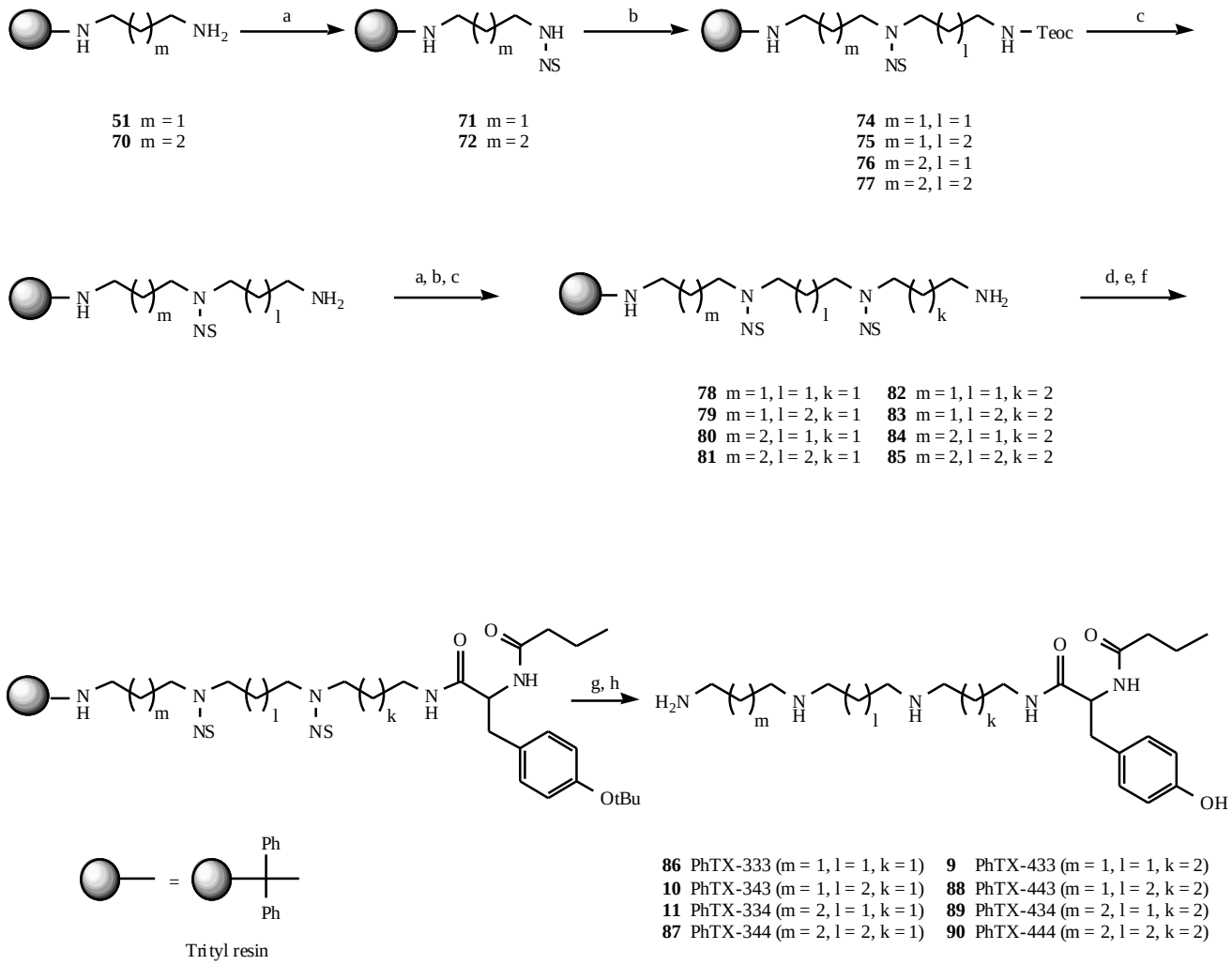
Although the purification of intermediates is avoided using SPS, crude polyamines toxins cleaved from the resin require further purification, in particular to avoid polyamine impurities and truncated toxins. The former is important not only from a chemical point of view, but is crucial from a pharmacological perspective, as polyamine species themselves possess the ability to influence receptors, such as iGluRs [48,49] and nAChRs [147]. Automated, preparative high performance liquid chromatography (HPLC), combined with MS detection [148] has proven to be a particularly efficient tool in the purification of polyamine toxins. Purification is accomplished by an automated injection, and, using the so-called "intelligent" fraction collection only one fraction is collected per injection. The fraction collection is triggered by the mass spectrometer in real-time.

Analytical HPLC is effective in assessing purity of polyamine toxins, using either mass spectrometric total ion current (TIC) or evaporative light scattering (ELS) [149] detection. These two detection systems are vital, given that the major impurities are polyamine species, which lack UV absorption. These procedures have been successfully applied to support the SPS of polyamine toxins [111,131,145].

In conclusion, SPS has proven to be a very efficient tool for the synthesis of polyamine toxins, in particular when combined with automated purification and purity assessment procedures. As mentioned in the introduction, SPS has been successfully applied for the synthesis of other polyamine-containing natural products, as well as polyamines and polyamine analogues [150-160]. This emphasizes the potential of SPS in further investigations of pharmacology and medicinal chemistry of polyamine toxins.

CONCLUSION

Since the first polyamine toxins were isolated and identified in the mid 1980's, a substantial amount of knowledge on these otherwise enigmatic compounds has emerged. As described in this review there is now accumulating structural understanding of how polyamine toxins interact with ionotropic receptors. The unique ability of polyamine toxins to selectively antagonize Ca^{2+}-permeable AMPA and KA receptors may serve as basis for future development of potential drugs for the treatment of



Scheme **6**. (**a**) 2-nitrobenzenesulfonyl chloride; (**b**) *N*-Teoc-3-aminopropanol (m = 1) or *N*-4-Teoc-aminobutanol (m = 2), ADDP, TBP; (**c**) TBAF; (**d**) *N*-Fmoc-O-*tert*-butyl-(*S*)-tyrosine, HATU, collidine; (**e**) piperidine; (**f**) butyric acid, HATU, collidine; (**g**) 2-mercaptoethanol, DBU; (**h**) CH_2Cl_2/TFA/*i*Pr_3SiH/H_2O.

various neurological disorders, given the evidence about the involvement of these receptors in these diseases. Moreover, a large array of synthetic methods for preparation of polyamine toxins and their analogues in a highly efficient manner is now available.

ACKNOWLEDGEMENTS

We thank Dr. Gabi Bixel, Freie Universität Berlin, and Dr. Dennis Hall, University of Alberta, for making their results available to us prior to publication. K. S. thanks the Carlsberg Foundation for financial support.

REFERENCES

[1] Mueller, A. L.; Roeloffs, R.; Jackson, H. In *The Alkaloids. Chemistry and Pharmacology*; Cordell, G. A., Ed.; Academic Press: San Diego, **1995**; *Vol. 46*; pp 63-94.

[2] Fisher, F. G.; Bohn, H. *Liebigs Ann. Chem.* **1957**, *603*, 232.

[3] Grishin, E. V.; Volkova, T. M.; Arseniev, A. S.; Reshetova, O. S.; Onoprienko, V. V. *Bioorg. Khim.* **1986**, *12*, 1121.

[4] Grishin, E. V.; Volkova, T. M.; Arseniev, A. S. *Toxicon* **1989**, *27*, 541.

[5] Adams, M. E.; Carney, R. L.; Enderlin, F. E.; Fu, E. T.; Jarema, M. A.; Li, J. P.; Miller, C. A.; Schooley, D. A.; Shapiro, M. J.; Venema, V. J. *Biochem. Biophys. Res. Commun.* **1987**, *148*, 678.

[6] Budd, T.; Clinton, P.; Dell, A.; Duce, I. R.; Johnson, S. J.; Quicke, D. L. J.; Taylor, G. W.; Usherwood, P. N. R.; Usoh, G. *Brain Res.* **1988**, *448*, 30.

[7] Aramaki, Y.; Yasuhara, T.; Higashijima, T.; Yoshioka, M.; Miwa, A.; Kawai, N.; Nakajima, T. *Proc. Japan Acad. Ser. B* **1986**, *62*, 359.

[8] Toki, T.; Jasuhara, T.; Aramaki, Y.; Kawai, N.; Nakajima, T. *Biomed. Res.* **1988**, *9*, 75.

[9] Quistad, G. B.; Suwanrumpha, S.; Jarema, M. A.; Shapiro, M. J.; Skinner, W. S.; Jamieson, G. C.; Lui, A.; Fu, E. W. *Biochem. Biophys. Res. Commun.* **1990**, *169*, 51.

[10] Jasys, V. J.; Kelbaugh, P. R.; Nason, D. M.; Phillips, D.; Rosnack, K. J.; Saccomano, N. A.; Stroh, J. G.; Volkmann, R. A. *J. Am. Chem. Soc.* **1990**, *112*, 6696.

[11] Jasys, V. J.; Kelbaugh, P. R.; Nason, D. M.; Phillips, D.; Rosnack, K. J.; Forman, J. T.; Saccomano, N. A.; Stroh, J. G.; Volkmann, R. A. *J. Org. Chem.* **1992**, *57*, 1814.

[12] McCormick, K. D.; Kobayashi, K.; Goldin, S. M.; Reddy, N. L.; Meinwald, J. *Tetrahedron* **1993**, *49*, 11155.

[13] Skinner, W. S.; Dennis, P. A.; Lui, A.; Carney, R. L.; Quistad, G. B. *Toxicon* **1990**, *28*, 541.

[14] Quistad, G. B.; Reuter, C. C.; Skinner, W. S.; Dennis, P. A.; Suwanrumpha, S.; Fu, E. W. *Toxicon* **1991**, *29*, 329.

[15] Itagaki, Y.; Fujita, Y.; Naoki, H.; Yasuhara, T.; Andriantsiferana, M.; Nakajima, T. *Natural Toxins*, **1997**, *5*, 1.

[16] Palma, M. S.; Itagaki, Y.; Fujita, T.; Hisada, M.; Naoki, H., Nakajima, T. *Natural Toxins*, **1997**, *5*, 47.

[17] Fujita, T.; Itagaki, Y.; Hisaka, M.; Nakajima, T.; Andriantsiferana, M. *Rapid. Commun. Mass Spectrom.* **1997**, *11*, 1115.

[18] Hisada, M.; Fujita, T.; Naoki, H.; Itagaki, Y.; Irie, H.; Miyashita, M.; Nakajima, T. *Toxicon*, **1998**, *36*, 1115.

[19] Palma, M. S.; Itagaki, Y.; Fujita, T.; Naoki, H.; Nakajima, T. *Toxicon*, **1998**, *36*, 485.

[20] Evans, H. E.; O'Neill, K. M. *Sci. Am.* **1991**, 56.

[21] Rathmayer, W. *Nature* **1962**, *196*, 1148.

[22] Piek, T. *Experientia* **1966**, *12*, 462.

[23] Piek, T. *Toxicon* **1966**, *4*, 191.

[24] Piek, T.; Mantel, P.; Engels, E. *Comp. Gen. Pharmacol.* **1971**, *2*, 317.

[25] Piek, T.; Nijo, K. D. *Toxicon* **1975**, *13*, 199.

[26] May, T. E.; Piek, T. *J. Insect. Physiol.* **1979**, *25*, 685.

[27] Eldefrawi, A. T.; Eldefrawi, M. E.; Konno, K.; Mansour, N. A.; Nakanishi, K.; Oltz, E.; Usherwood, P. N. R. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 4910.

[28] Piek, T.; Fokkens, R. H.; Karst, H.; Kruk, C.; Lind, A.; van Marle, J.; Nakajima, T.; Nibbering, N. M. M.; Shinozaki, H.; Spanjer, W.; Tong, Y. C. In *Neurotox '88: molecular basis of drug and pesticide action*; Lunt, G. G., Ed.; Elsevier: Amsterdam, **1988**, pp. 61-76.

[29] McCormick, K. D.; Meinwald, J. *J. Chem. Ecol.*, **1993**, *19*, 2411.

[30] Mueller, A. L.; Albensi, B. C.; Ganong, A. H.; Reynolds, L. S.; Jackson, H. *Synapse* **1991**, *9*, 244.

[31] Usherwood, P. N. R.; Blagbrough, I. S. *Pharmac. Ther.* **1991**, *52*, 245.

[32] Usherwood, P. N. R.; Blagbrough, I. S. In *The Neuropharmacology. of Polyamines*. Carter, C., Ed.; Academic Press: London, **1994**, pp. 185-204.

[33] Usherwood, P. N. R.; Blagbrough, I. S. In *Insecticide action. From molecule to organism*; Narahashi, T., Chambers, J. E., Eds.; Plenum: New York, **1989**, pp. 13-31.

[34] Blagbrough, I. S.; Usherwood, P. N. R. *Proc. Royal Soc. Edinburgh* **1992**, *99B*, 67.

[35] Hume, R. I.; Dingledine, R.; Heinemann, S. F. *Science*, **1991**, *253*, 1028.

[36] Hollmann, M.; Hartley, M.; Heinemann, S. F. *Science*, **1991**, *252*, 851.

[37] Higuchi, M.; Single, F. N.; Kohler, M. sommer, B.; Sprengel, R.; Seeburg, P. H. *Cell*, **1993**, *75*, 1361.

[38] Sommer, B.; Kohler, M.; Sprengel, R., Seeburg, P. H. *Cell*, **1991**, *67*, 11.

[39] Meucci, O.; Fatatis, A.; Holzwarth, J. A.; Miller, R. J. *J. Neurosci.* **1996**, *16*, 519.

[40] Washburn, M. S.; Numberger, M.; Zhang, S.; Dingledine, R. *J. Neurosci.* **1997**, *17*, 9393.

[41] Savidge, J. R.; Bristow, D. R. *Eur. J. Pharmacol.* **1998**, *351*, 131.

[42] Tsubokawa, H.; Oguro, K.; Masuzawa, T.; Nakaima, T.; Kawai, N. *J. Neurophys.* **1995**, *74*, 218.

[43] Iino, M.; Koike, M.; Isa, T.; Ozawa, S. *J. Physiol. (Lond.)* **1996**, *496*, 431.

[44] Blaschke, M.; Keller, B. U.; Rivosecchi, R.; Hollmann, M.; Heinemann, S.; Konnerth, A. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 6528.

[45] Herlitze, S.; Raditsch, M.; Ruppersberg, J. P.; Jahn, W.; Monyer, H.; Schoepfer, R.; Witzemann, V. *Neuron* **1993**, *10*, 1131.

[46] Brackley, P. T. H.; Bell, D. R.; Choi, S.-K.; Nakanishi, K.; Usherwood, P. N. R. *J. Pharmacol. Exp. Ther.* **1993**, *266*, 1573.

[47] Washburn, M. S.; Dingledine, R. *J. Pharmacol. Exp. Ther.* **1996**, *278*, 669.

[48] Dingledine, R.; Borges, K.; Bowie, D.; Traynelis, S. *Pharmacol. Rev.* **1999**, *51*, 7.

[49] Ozawa, S.; Kamiya, H.; Tsuzuki, K. *Prog. Neurobiol*. **1998**, *54*, 581.

[50] Tóth, K.; McBain, C. J. *Nature Neurosci.* **1998**, *1*, 572.

[51] Fagni, L.; Bockaert, J. *Neuroreport* **1995**, *6*, 1037.

[52] Guth, S. L.; Scapini, D. A.; Drescher, M. J.; Drescher, D. G. *Life Sci.* **1990**, *47*, 1437.

[53] Reynolds, I. J. *Br. J. Pharmacol.* **1991**, *103*, 1373.

[54] Williams, K. *J. Pharmacol. Exp. Ther.* **1993**, *266*, 231.

[55] Parks, T. N.; Mueller, A. L.; Artman, L. D.; Albensi, B. C.; Nemeth, E. F.; Jackson, H.; Jasys, J.; Saccomano, N. A.; Volkmann, R. A. *J. Biol. Chem.* **1991**, *266*, 21523.

[56] Priestley, T.; Woodruff, G. N.; Kemp, J. A. *Br. J. Pharmacol.* **1998**, *97*, 1315.

[57] Raditsch, M.; Geyer, M.; Kalbitzer, H. R.; Jahn, W.; Ruppersberg, J. P.; Witzemann, V. *Eur. J. Biochem.* **1996**, *240*, 416.

[58] Raditsh, M.; Ruppersberg, J. P.; Kuner, T.; Günther, W.; Schoepfer, R.; Seeburg, P. H.; Jahn, W.; Witzemann, V. *FEBS Lett.* **1993**, *324*, 63.

[59] Jones, M. G.; Anis, N. A.; Lodge, D. *Br. J. Pharmacol.* **1990**, *101*, 968.

[60] Lodge, D.; Jones, M. G. *Adv. Exp. Med. Biol.* **1990**, *268*, 101.

[61] Lodge, D.; Jones, M. G.; Palmer, A. J. *Can. J. Physiol. Pharmacol.* **1991**, *69*, 1123.

[62] Anis, N. A.; Sherby, S.; Goodnow, R. A.; Niwa, M.; Konno, K.; Kallimopoulos, T.; Bukownik, R.; Nakanishi, K.; Usherwood, P. N. R.; Eldefrawi, A. T.; Eldefrawi, M. E. *J. Pharmacol. Exp. Ther.* **1990**, *254*, 764.

[63] Ragsdale, D.; Gant, D. B.; Anis, N. A.; Eldefrawi, A. T.; Konno, K.; Miledi, R. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 156.

[64] Donevan, S. D.; Rogawski, M. A. *Neuroscience* **1996**, *70*, 361.

[65] Brackley, P. T. H.; Goodnow, R. A.; Nakanishi, K.; Sudan, H. L.; Usherwood, P. N. R. *Neurosci. Lett*, **1990**, *114*, 51.

[66] Liu, M.; Nakazawa, K.; Inoue, K.; Ohno, Y. *Br. J. Pharmacol.* **1997**, *122*, 379.

[67] Green, A. C.; Nakanishi, K.; Usherwood, P. N. R. *Brain Res.* **1996**, *717*, 135.

[68] Mueller, A. L.; Van Wagenen, B. C.; Delmar, E. G.; Balandrin, M. F.; Moe, S. T.; Artman, L. D. PCT Intl. Appl. WO 95/21612, August 17, **1995**.

[69] Mueller, A. L.; Artman, L. D.; Chien, Y.; Raszkiewicz, J. L.; Alasti, N.; DelMar, E. G.; Van Wagenen, B. C.; Moe, S. T.; Balandrin, M. F.; Smith, D. L.; Shimizu, S. M.; Rhodes, J. L.; Trovato, R.; Barmore, R. M. *Keystone symposia on molecular and cellular biology: ion channels as therapeutic targets*, Tamarron, Colorado, USA, February 4-10, **1996**.

[70] Albensi, B. C.; Alasti, N.; Mueller, A. L. *J. Neuroscience Res.*, **2000**, *62*, 177-185.

[71] Karst, H.; Piek, T. *Comp. Biochem. Physiol.* **1991**, *98C*, 479.

[72] Karst, H.; Piek, T.; van Marle, J.; Lind, A.; van Weeren-Kramer, J. *Comp. Biochem. Physiol.* **1991**, *98C*, 471.

[73] Schluter, N. C. M.; Piek, T.; Lopes da Silva, F. H. *Comp. Biochem. Physiol.* **1992**, *101C*, 41.

[74] Karst, H.; Joëls, M.; Wadman, W. J.; Piek, T. *Eur. J. Pharmacol.* **1994**, *270*, 357.

[75] Benson, J. A.; Schürmann, F.; Kaufmann, L.; Gsell, L.; Piek, T. *Comp. Biochem. Physiol.* **1992**, *102C*, 267.

[76] Schluter, N. C. M.; van Weeren-Kramer, J.; Piek, T.; van Marle, J. *Comp. Biochem. Physiol.* **1992**, *101*, 35.

[77] Piek, T.; Hue, B. *Comp. Biochem. Physiol.* **1989**, *93C*, 403.

[78] Benson, J. A.; Kaufmann, L.; Hue, B.; Pelhate, M.; Schürmann, F.; Gsell, L.; Piek, T. *Comp. Biochem. Physiol.* **1993**, *105C*, 303.

[79] Piek, T.; Schluter, N. C. M.; Karst, H. *Pol. J. Pharmacol. Pharm.* **1990**, *42*, 573.

[80] Karst, H.; Piek, T.; Van Marle, J. *Neuro. Res. Commun.* **1990**, *7*, 69-74.

[81] Tong, Y. C. *Pestic. Sci.* **1988**, *24*, 340.

[82] Moya, E.; Blagbrough, I. S. In *The Neuropharmacology of Polyamines*; Carter, C., Ed.; Academic Press: London, **1994**; pp 167-184.

[83] Blagbrough, I. S.; Brackley, P. T. H.; Bruce, M.; Bycroft, B. W.; Mather, A. J.; Millington, S.; Sudan, H. L.; Usherwood, P. N. R. *Toxicon* **1992**, *30*, 303.

[84] Blagbrough, I. S.; Bruce, M.; Bycroft, B. W.; Mather, A. J.; Usherwood, P. N. R. *Pestic. Sci.* **1990**, *30*, 397.

[85] Blagbrough, I. S.; Bycroft, B. W.; Mather, A. J.; Usherwood, P. N. R. *Pestic. Sci.* **1990**, *30*, 446.

[86] Blagbrough, I. S.; Bruce, M.; Bycroft, B. W.; Mather, A. J.; Usherwood, P. N. R. *Pestic. Sci.* **1990**, *30*, 448.

[87] Nakanishi, K.; Goodnow, R. A.; Konno, K.; Niwa, M.; Bukownik, R.; Kallimopoulos, T.; Usherwood, P. N. R.; Eldefrawi, A. T.; Eldefrawi, M. E. *Pure Appl. Chem.* **1990**, *62*, 1223.

[88] Bruce, M.; Bukownik, R.; Eldefrawi, A. T.; Eldefrawi, M. E.; Goodnow, R. A.; Kallimopoulos, T.; Konno, K.; Nakanishi, K.; Niwa, M.; Usherwood, P. N. R. *Toxicon* **1990**, *28*, 1333.

[89] Anis, N.; Sherby, S.; Goodnow Jr.,R.; Niwa, M.; Konno, K.; Kallimopoulos, T.; Bokownik, R.; Naknishi, K.; Usherwood, P. N. R.; Eldefrawi, A.; Eldefrawi, M. E. *J. Pharmacol. Exp. Ther.* **1990**, *254*, 764.

[90] Goodnow, R. A.; Konno, K.; Kallimopoulos, T.; Bukownik, R.; Lenares, D.; Nakanishi, K. *Tetrahedron* **1990**, *46*, 3267.

[91] Goodnow, R. A.; Bukownik, R.; Nakanishi, K.; Usherwood, P. N. R.; Eldefrawi, A. T.; Anis, N. A.; Eldefrawi, M. E. *J. Med. Chem.* **1991**, *34*, 2389.

[92] Choi, S.-K.; Goodnow, R. A.; Kalivretenos, A. G.; Chiles, G. W.; Fushiya, S.; Nakanishi, K. *Tetrahedron* **1992**, *48*, 4793.

[93] Nakanishi, K.; Goodnow, R. A.; Kalivretenos, A. G.; Choi, S.-K.; Fushiya, S.; Chiles, G. W.; Eldefrawi, A. T.; Eldefrawi, M. E.; Usherwood, P. N. R. In *New leads and targets in drug research*; Krogsgaard-Larsen, P., Christensen, S. B., Kofod, H., Eds.; Munksgaard: Copenhagen, **1992**; pp 138-148.

[94] Kalivretenos, A. G.; Nakanishi, K. *J. Org. Chem.* **1993**, *58*, 6596.

[95] Choi, S. K.; Nakanishi, K.; Usherwood, P. N. R. *Tetrahedron*, **1993**, *49*, 5777.

[96] Nakanishi, K.; Choi, S.-K.; Hwang, D.; Lerro, K.; Orlando, M.; Kalivretenos, A. G.; Eldefrawi, A. T.; Eldefrawi, M. E.; Usherwood, P. N. R. *Pure Appl. Chem.* **1994**, *66*, 671.

[97] Sudan, H. L.; Kerry, C. J.; Mellor, I. R.; Choi, S.-K.; Huang, D.; Nakanishi, K.; Usherwood, P. N. R. *Inver. Neurosci.* **1995**, *1*, 159.

[98] Schäfer, A.; Benz, H.; Fiedler, W.; Guggisberg, A.; Bienz, S.; Hesse, M. In *The Alkaloids. Chemistry and Pharmacology*; Cordell, G. A. and Brossi, A., Eds.; Academic Press: San Diego, **1994**; *Vol. 45*; pp 1-125.

[99] Usherwood, P. N. R. *Il Farmaco*, **2000**, *55*, 202.

[100] Kuksa, V.; Buchan, R.; Lin, P. K. T. *Synthesis*, **2000**, 1189.

[101] Karigiannis, G.; Papaioannou, D. *Eur. J. Org. Chem.* **2000**, 1841.

[102] Itagaki, Y.; Nakajima, T. *J. Toxicol. Toxin Rev.*, **2000**, *19*, 23.

[103] Huang, D.; Jiang, H.; Nakanishi, K.; Usherwood, P. N. R. *Tetrahedron* **1997**, *53*, 12391.

[104] Huang, D.; Matile, S.; Berova, N.; Nakanishi, K. *Heterocycles* **1996**, *42*, 723.

[105] Nakanishi, K.; Huang, X.; Jiang, H.; Liu, Y.; Fang, K.; Huang, D.; Choi, S.-K.; Katz, E.; Eldefrawi, M. E. *Bioorg. Med. Chem.* **1997**, *5*, 1969.

[106] Hashimoto, M.; Liu, Y.; Fang, K.; Li, H.; Campiani, G.; Nakanishi, K. *Bioorg. Med. Chem.* **1999**, *7*, 1181.

[107] Rosini, M.; Budriesi, R.; Bixel, M.G.; Bolognesi, M.L.; Chiarini, A.; Hucho, F.; Krogsgaard-Larsen, P.; Mellor, I.R.; Minarini, A.; Tumiatti, V.; Usherwood, P. N. R.; Melchiorre, C. *J. Med. Chem.*, **1999**, *42*, 5212.

[108] Bixel, M. G.; Krauss, M.; Liu, Y., Bolognesi, M. L.; Rosini, M.; Mellor, I. S.; Usherwood, P. N. R.; Melchiorre, C.; Nakanishi, K.; Hucho, F. *Eur. J. Biochem*. **2000**, *267*, 110.

[109] Moe, S. T.; Smith, D. L.; Chien, Y.; Raszkiewicz, J. L.; Artman, L. D.; Mueller, A. L. *Pharm. Res.* **1998**, *15*, 31.

[110] Strømgaard, K.; Brierly, M. J.; Andersen, K.; Sløk, F. A.; Mellor, I. R.; Usherwood, P. N. R.; Krogsgaard-Larsen, P.; Jaroszewski, J. W. *J. Med. Chem*. **1999**, *42* 5224.

[111] Strømgaard, K.; Brier, T. J.; Andersen, K.; Mellor, I. R.; Saghyan, A.; Tikhonov, D.; Usherwood, P. N. R.; Krogsgaard-Larsen, P.; Jaroszewski, J. W. *J. Med. Chem*. **2000**, *43*, 4526.

[112] Goodnow, R. A.; Nakanishi, K.; Sudan, H. L.; Usherwood, P. N. R. *Neurosci. Lett.*, **1991**, *125*, 62.

[113] Choi, S.-K.; Kalivretenos, A. G.; Usherwood, P. N. R.; Nakanishi, K. *Chem. Biol.* **1995**, *2*, 23.

[114] Bixel, M. G.; Wiese, C.; Bolognesi, M. L.; Rosini, M.; Brierly, M. J.; Mellor, I. R.; Usherwood, P. N. R.; Melchiorre, C.; Hucho, F. *J. Biol. Chem.* **2001**, *276*, 6151.

[115] Fang, K.; Hashimoto, M.; Jockosch, S.; Turro, N. J.; Nakanishi, K. *J. Am. Chem. Soc.* **1998**, *120*, 8543.

[116] Li, H.; Liu, Y.; Fang, K.; Nakanishi, K. *Chem. Commun.* **1999**, 365.

[117] Huang, X.; Borhan, B.; Matile, S.; Nakanishi, K. *Bioorg. Med. Chem.* **1999**, *7*, 811.

[118] Jaroszewski, J. W.; Matzen, L.; Frølund, B.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1996**, *39*, 515.

[119] Jayaraman, V.; Usherwood, P. N. R.; Hess, G. P. *Biochemistry*, **1999**, *38*, 11406.

[120] Bähring, R.; Bowie, D.; Benveniste, M.; Mayer, M. L. *J. Physiol. (Lond.)* **1997**, *502*, 575.

[121] Cui, C.; Bähring, R.; Mayer, M. L. *Neuropharmacology*. **1998**, *37*, 1381.

[122] Bähring, R.; Mayer, M. L. *J. Physiol. (Lond.)* **1998**, *509*, 635.

[123] Bowie, D.; Bähring, R.; Mayer, M. L. In *Ionotropic Glutamate Receptors in the CNS*, Jonas, Monyer, Ed; Springer-Verlag Berlin Heidelberg, **1999**, Vol. *141*, pp. 251-273.

[124] Scott, R. H.; Gibson, M. T. *Biochem. Soc. Trans.* **1998**, *26*, 614.

[125] Scott, R. H.; Thatcher, N. M.; Ayar, A.; Mitchell, S.J.; Pollock, J., Gibson, M. T.; Duce, I. R.; Moya, E.; Blagbrough, I. S. *Neuropharmacology*, **1998**, *37*, 1563.

[126] Kerry, C. J.; Sudan, H.-L.; Nakanishi, K.; Usherwood, P. N. R. *Inver. Neurosci*. **1997**, *2*, 223.

[127] Brundell, P.; Goodnow, R. A.; Kerry, C. J.; Nakanishi, K.; Sudan, H. L.; Usherwood, P. N. R. *Neurosci. Lett*, **1991**, *131*, 196.

[128] Bycroft, B. W.; Chan, W. C.; Hone, N. D.; Millington, S.; Nash, I. A *J. Am. Chem. Soc.* **1994**, *116*, 7415.

[129] Bycroft, B. W.; Chan, W. C.; Hone, N. D.; Millington, S.; Nash, I. A *J. Chem. Soc. Chem. Commun.* **1993**, 778.

[130] Nash, I. A.; Bycroft, B. W.; Chan, W. C. *Tetrahedron Lett.* **1996**, *37*, 2625.

[131] Strømgaard, K.; Bjørnsdottir, I.; Andersen, K.; Brierly, M. J.; Rizoli, S.; Eldursi, N.; Mellor, I. R.; Usherwood, P. N. R.; Hansen, S. H.; Krogsgaard-Larsen, P.; Jaroszewski, J. W. *Chirality*, **2000**, *12*, 93.

[132] Carpino, L. A.; El-Faham, A. *J. Org. Chem.* **1994**, *59*, 695.

[133] Carpino, L. A.; Ionescu, D.; El-Faham, A. *J. Org. Chem.* **1996**, *61*, 2460.

[134] Wang, F.; Manku, S.; Hall, D. G. *Organic Lett.* **2000**, *2*, 1581.

[135] Hall, D. G.; Laplante, C.; Manku, S.; Nagendran, J. *J. Org. Chem.* **1999**, *64*, 698.

[136] Manku, S.; Wang, F.; Hall, D. G. Pacifichem 2000 meeting, **2000**, ORGN 763.

[137] Hidai, Y.; Kan, T.; Fukuyama, T. *Tetrahedron Lett.* **1999**, *40*, 4711.

[138] Hidai, Y.; Kan, T.; Fukuyama, T. *Chem. Pharm. Bull*. **2000**, *48*, 1570.

[139] Fukuyama, T.; Jow, C.-K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373.

[140] Fukuyama, T.; Cheung, M.; Jow, C.-K.; Hidai, Y.; Kan, T. *Tetrahedron Lett.* **1997**, *38*, 5831.

[141] Hone, N. D.; Payne, L. J. *Tetrahedron Lett*., **2000**, *41*, 6149.

[142] Chhabra, S. R.; Khan, A. N.; Bycroft, B. W. *Tetrahedron Lett.* **1998**, *39*, 3585.

[143] Chhabra, S. R.; Khan, A. N.; Bycroft, B. W. *Tetrahedron Lett.* **2000**, *41*, 1095.

[144] Chhabra, S. R.; Khan, A. N.; Bycroft, B. W. *Tetrahedron Lett.* **2000**, *41*, 1099.

[145] Strømgaard, K.; Andersen, K.; Ruhland, T.; Krogsgaard-Larsen, P.; Jaroszewski, J. W. *Synthesis*, **2001**, 877.

[146] Tsunoda, T.; Yamamiya, Y.; Itô, S. *Tetrahedron Lett*. **1993**, *34*, 1639.

[147] Shao, Z.; Mellor, I. R.; Brierly, M. J.; Harris, J.; Usherwood, P. N. R. *J. Pharmacol. Exp. Ther.* **1998**, *286*, 1269.

[148] Zeng, L.; Burton, L.; Yung, K.; Shushan, B.; Kassel, D. B. *J. Chromatogr. A*, **1998**, *794*, 3.

[149] Hsu, B. H.; Orton, E.; Tang, S.-Y.; Carlton, R. A. *J. Chromatogr. B*, **1999**, *725*, 103.

[150] Marsh, I. R.; Bradley, M. *Tetrahedr*on, **1997**, *53*, 17317.

[151] Byk, G.; Frederic, M.; Sherman, D. *Tetrahedron Lett.* **1997**, *38*, 3219.

[152] Page, P.; Burrage, S.; Baldock, L.; Bradley, M. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1751.

[153] Ostresh, J. M.; Schoner, C. C.; Hamashin, V. T.; Nefzi, A.; Meyer, J.-P.; Houghten, R. A. *J. Org. Chem.* **1998**, *63*, 8622.

[154] Tomasi, S.; Roch, M. L.; Renault, J.; Corbel, J-C.; Uriac, P.; Carboni, B.; Moncoq, D.; Martin, B.; Delcross, J-G. *Bioorg. Med. Chem. Lett*. **1998**, *8*, 635.

[155] Karigiannis, G.; Mamos, P.; Balayiannis, G.; Katsoulis, I.; Papaioannou, D. *Tetrahedron Lett.* **1998**, *39*, 5117.

[156] Kellam, B.; Bycroft, B. W.; Chan, W. C.; Chhabra, S. R. *Tetrahedron* **1998**, *54*, 6817.

[157] Carrington, S.; Renault, J.; Tomasi, S.; Sinbandhit, S.; Blagbrough, I. S.; Uriac, P. *Pharm. Pharmacol. Commun.* **1999**, *5*, 151.

[158] Nefzi, A.; Ostresh, J. M.; Houghten, R. A. *Tetrahedron*, **1999**, *55*, 335.

[159] Chitkul, B.; Bradley, M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2367.

[160] Manku, S.; Laplante, C.; Kopac, D.; Chan, T., Hall, D. G., *J. Org. Chem.* **2001**, *66*, 874.