Available online at www.sciencedirect.com
SCIENCE DIRECT®
PERGAMON
Phytochemistry 63 (2003) 315–334
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Preparation and biological assessment of hydroxycinnamic acid amides of polyamines

Solomon Fixon-Owoo[a], Frédéric Levasseur[a], Keith Williams[b], Thomas N. Sabado[b], Mike Lowe[c], Markus Klose[c], A. Joffre Mercier[c], Paul Fields[d], Jeffrey Atkinson[a,*]

[a]*Department of Chemistry, Brock University, St.Catharines, Ontario, Canada L2S3A1*
[b]*Department of Physiology and Pharmacology, SUNY Downstate Medical Center, Brooklyn, NY 11203-2098, USA*
[c]*Department of Biological Sciences, Brock University, St. Catharines, Ontario, Canada L2S 3A1*
[d]*Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, Manitoba, Canada R3T 2M9*

Received 17 December 2002; received in revised form 27 January 2003

Abstract

Many plants contain hydroxycinnamic acid conjugates of polyamines that are remarkably similar in general structure to the acylated polyamines found in spider and wasp toxins. In an effort to determine whether these compounds might play a role in the chemical defense of plants against arthropod pests we synthesized a variety of analogues of the coumaric (4-hydroxycinnamic) acid conjugates of di-, tri-, and tetraamines using common protection and acylation strategies. N^1- and N^8-coumaroyl spermidine were tested in feeding trials with insect larvae including the European corn borer (*Ostrinia nubilalis*), the tobacco budworm (*Heliothis verescens*) and the oblique banded leaf roller (*Choristoneura rosaceana*). Antifeedant assays with the rice weevil *Sitophilus oryzae* were also performed. Neither the naturally occurring coumaric acid conjugates of polyamines nor their analogues showed notable toxicity towards insects, despite precautions to maintain these easily oxidized materials in the wet diet. However, more direct bioassays of these compounds on glutamate dependent neuroreceptors including the deep abdominal extensor muscles of crayfish, or mammalian NMDA, δ2, and AMPA receptors, clearly showed that these compounds were inhibitory. N^1-Coumaoryl spermine, a dodecyl and a cyclohexyl analogue were especially active at NMDA NR1/NR2B receptors. The latter had an IC_{50} of 300 μM in the crayfish. N^1-Coumaroyl spermine had an IC_{50} in the crayfish preparation of 70–300 μM and against the mammalian NR1/NR2B receptor of 38 nM. Structure–activity variations show similar trends of length and hydrophobicity as has been seen previously with analogues of spider toxins. We conclude from this work that while the coumaric acid polyamine conjugates are active when directly applied to neuroreceptors, they show no overt toxicity when ingested by insect larvae.

Keywords: Polyamines; Hydroxycinnamic acids; Neurotoxins; Glutamate receptor antagonist

1. Introduction

It has been known for more than 25 years that plants can conjugate polyamines with various phenolic acids creating a mixture of mono-, di- and triamides. Some of the most common conjugates are those that contain the hydroxycinnamic acids coumaric, ferulic and caffeic acids (Smith et al., 1983). Although the roles of these conjugates in normal plant physiology are far from clear, it is interesting to note their similarity to other polyamine conjugates found in the venoms of predaceous spiders and wasps (Blagbrough et al., 1994; McCormick and Meinwald, 1993; Quicke and Usherwood, 1990; Schafer et al., 1994). The invertebrate polyamine toxins are usually composed of an aromatic acid, often derived from tryptophan or tyrosine, and a long polyamine tail of varied structure. (Fig. 1) These compounds comprise a portion of the low molecular weight components in invertebrate venoms that inhibit neurotransmission and presumably have evolved to immobilize prey for capture. Some of these toxins are active at glutamate regulated ion-channels and the molecules have attracted a great deal of interest from

* Corresponding author. Tel.: +1-905-688-555x3967; fax: +1-905-682-9020.
E-mail address: jatkin@brocku.ca (J. Atkinson).
URL: http://chemiris.labs.brocku.ca/~chemweb/faculty/atkinson/

0031-9422/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0031-9422(03)00133-X

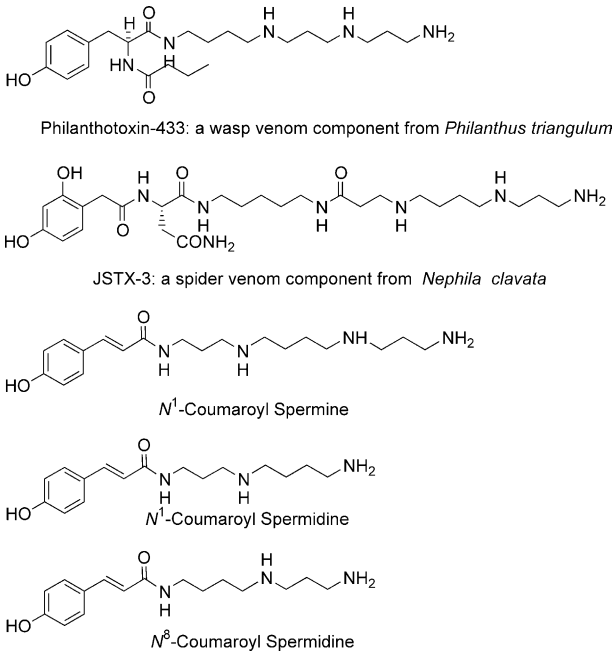


Fig. 1. Comparison of acylated polyamine toxins from invertebrates (PhTX-433 and JSTX-3) with common plant derived coumaric acid conjugates of spermidine and spermine.

neurophysiologists (Blagbrough et al., 1994; Moya and Blagbrough, 1995; Quicke and Usherwood, 1990; Schafer et al., 1994; Usherwood and Blagbrough, 1991).

The similarity of the plant derived hydroxycinnamic acid (i.e coumaric) amides and the invertebrate polyamine toxins has been noted before by Blagbrough (Blagbrough and Usherwood, 1990, 1992) who prepared and tested N^1-coumaroyl spermine and analogues against a locust nerve-muscle preparation. We became interested in this family of compounds after recognizing that plant protection against insect herbivory can at least in part be described by the quantity and type of phenolic compounds present in tissue (Duffey and Stout, 1996). Many chemical analyses of the total phenolic content of plants, however, liberate the free phenolics from conjugated forms by hydrolysis with strong acids and bases (Sharma and Rajam, 1995; Torrigiani et al., 1987). A great deal of information concerning the chemical identity of the phenolic compounds is lost during such a procedure. We have devised a method for extraction and analysis of hydroxycinnamic acid amides from plant tissue (Panagabko et al., 2000) and have shown that N^8- and N^1-coumaroyl spermidine, shorter polyamine conjugates than N^1-coumaroyl spermine, are active at the glutamate dependent neuromuscular junction of crustaceans (Klose et al., 2002; Mercier et al., 1998). We have prepared a number of aryl substituted cinnamic acid amides by methods essentially identical to those of Hu and Hesse (1996) and in the present study we expand on this family by preparing coumaric acid monoamides with a variety of di-, tri- and tetraamines.

2. Results

2.1. Synthesis

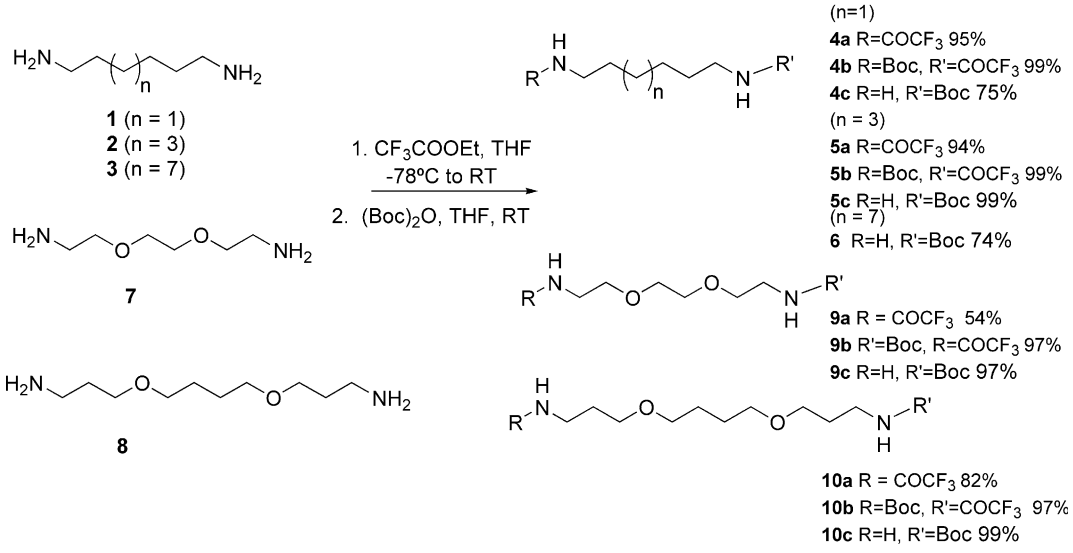
The results of structure–activity studies using analogues of the polyamine toxins from spiders have determined that the aromatic chromophore is an absolute requirement for activity at the quisqualate class of glutamate receptors (Blagbrough and Usherwood, 1990; Bruce et al., 1990). With this in mind we are interested in the mono-coumaroyl (4-hydroxycinnamoyl) conjugates of polyamines. Plants are known to produce a complex mixture of mono-, di-, and tri-conjugates of the more common polyamines with differing acyl groups and differing numbers of hydroxyl and methoxyl groups (Bokern et al., 1995; Meurer-Grimes, 1995; Werner et al., 1995). For simplicity, we prepared only the mono-coumaroyl conjugates in keeping with the common occurrence of a phenolic group in the invertebrate toxins (McCormick and Meinwald, 1993). With this portion of the molecule remaining constant, we designed a select number of analogues to determine whether the chain length, degree of heteroatom substitution, or extra hydrophobic substituents in the sidechain would have any effect on the biological activity assessed at crustacean and mammalian glutamate receptors.

Many of the synthetic procedures that involve polyamines incorporate a chemoselective step at some point and this has prompted the use of a large variety of protecting group strategies and reagents (Karigiannis and Papaioannou, 2000). Our requirement of selectively acylating di- and polyamines was fairly easily met by the mono-trifluoroacetylation of diamines and the selective mono-trifluoracetylation of primary over secondary amines (Blagbrough and Geall, 1998; Osullivan and Dalrymple, 1995). Many diamines can be mono-Boc protected using $(Boc)_2O$ in dioxane (Krapcho and Kuell, 1990) and we sometimes use this method as in the preparation of **6** (Scheme 1). The success of this method relies on the spontaneous precipitation of the di-Boc amine product and the easy removal of remaining excess diamine, usually by distillation. When the diamines are not available in such large excess, or have high boiling points, then retrieval of the mono-Boc protected amine is much less efficient and it is better to first mono-trifluoroacetylate, carbamoylate the remaining amine group, then remove the trifluoroacetyl protecting group. For example, in the case of **6**, the diamine was readily available and it could be easily removed by washing the crude reaction with water and extracting the mono-protected material (Scheme 1).

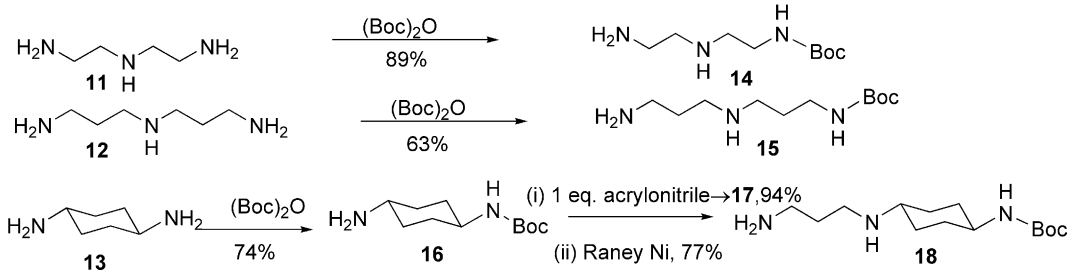
Protection of the triamines **11** and **12** could be achieved directly with $(Boc)_2O$, whereas treatment of these amines with ethyl trifluoroacetate consistently provided diacylated materials as the major product (Scheme 2). Acylation of **14** with **28** (Scheme 3) provided

the desired **34** in low yields (16%), and also gave a significant amount of the 1,3-dicoumaroyl conjugate **34c** (30%). Very little of such diacylation (8%) was observed to occur with **15**.

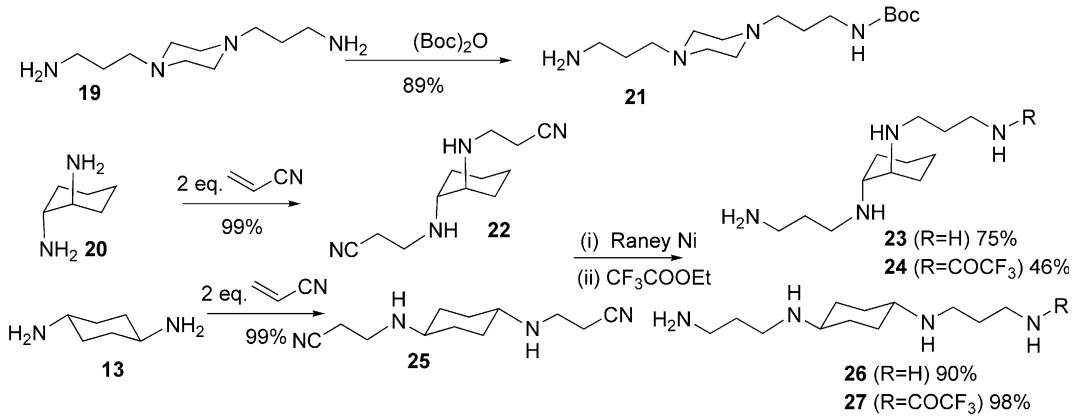
The tetraamine **19** is commercially available (Scheme 3) and **23** and **26** could be prepared by dicyanoethylation of the diaminocyclohexanes **20** and **13**. During one attempt of a reduction of **25** on a fairly high scale (19 g) the reduction was quite slow, presumably due to an insufficient amount of Raney nickel catalyst and a signifcant by product was observed and isolated. This material was identified to be the half-hydrolyzed primary amide **26a**.



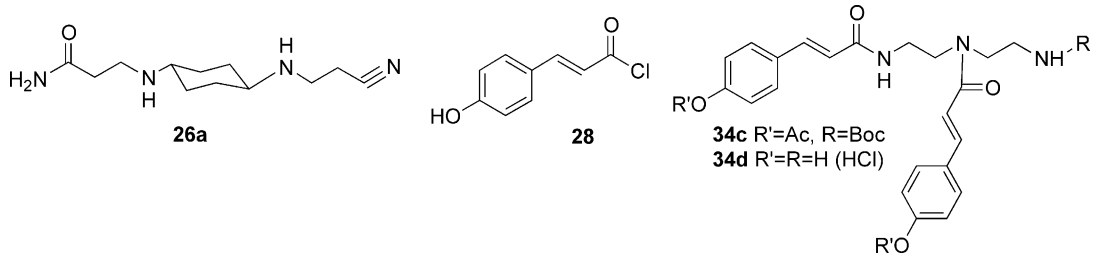
Scheme 1. Monoprotection of selected diamines.



Scheme 2. Monoprotection of selected triamines.

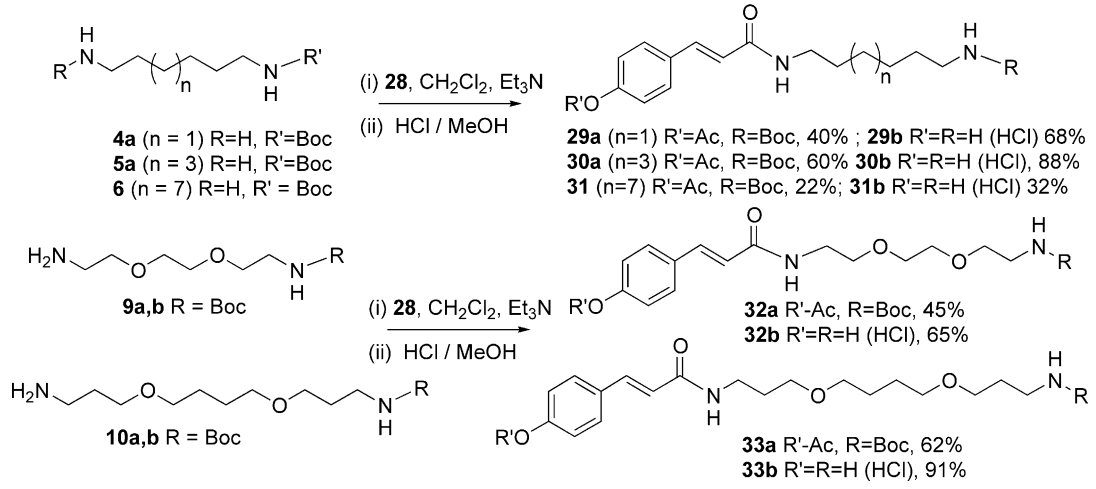


Scheme 3. Preparation and derivatization of tetraamines.

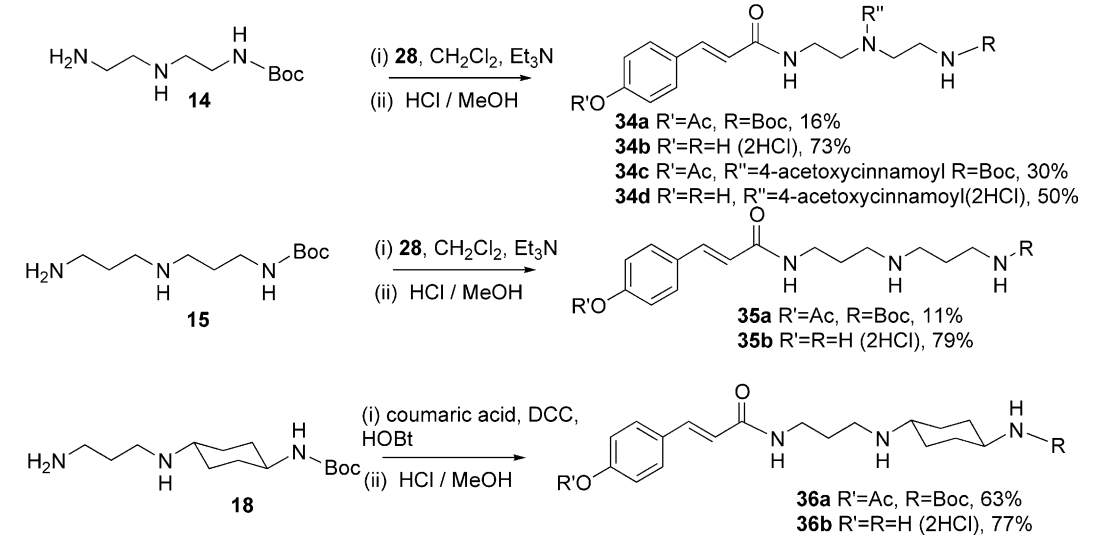


Acylation of the protected amines with (*E*)-3-(4-acetoxyphenyl)prop-2-enoyl chloride **28** (Scheme 3) was straightforward although recovered yields are variable depending on the amine. While the mono-trifluoroacetates themselves can be acylated with **28**, removal of the trifluoroacetyl group requires treatment with strong base and we have found this to reduce yields and quality of final phenolic products. It was thus straightforward to prepare mono-protected diamines that could be acylated directly with **28**, and deprotected under acidic conditions (Schemes 4–6).

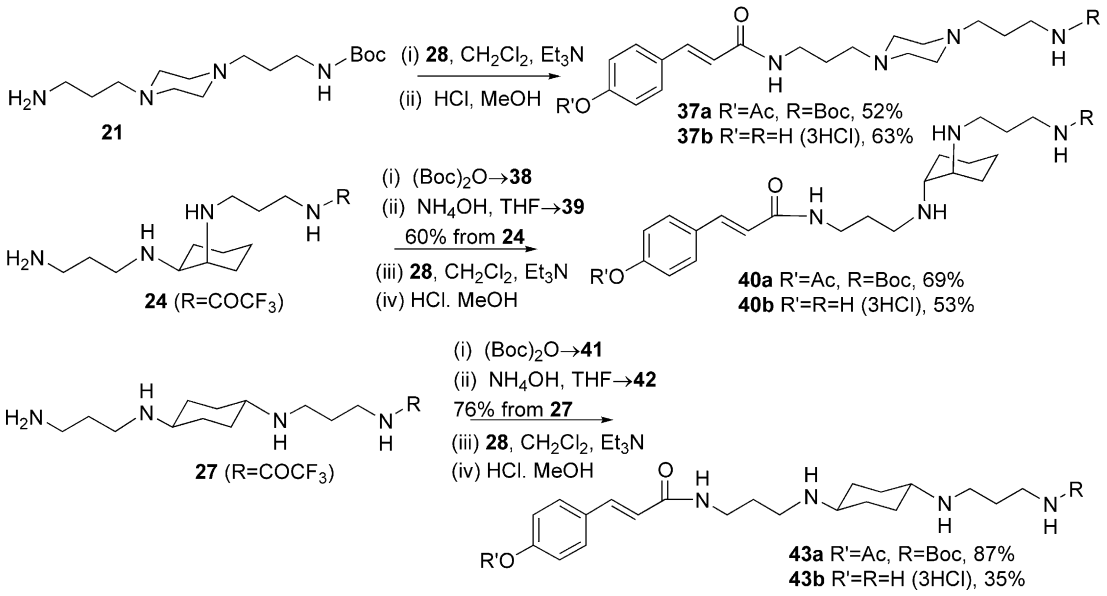
The final deprotected coumaric acid conjugates were all hygroscopic solids that showed a tendency to isomerize at the coumaroyl double bond if exposed to room light for even short periods of time. Careful handling of the hydrochloride salts is required to obtain accurate weights for feeding trials and stock solutions.



Scheme 4. Acylation of protection diamines.



Scheme 5. Acylation of selected triamines.



Scheme 6. Acylation of selected tetraamines.

2.2. Biological activity assessment

Glutamate is the neurotransmitter at most neuromuscular synapses in crayfish (Atwood, 1982) and glutamate receptor antagonists block excitatory post-synaptic potentials (EPSPs) in the crayfish muscles used in this investigation (Mercier et al., 1998). We have previously reported the inhibitory activity of N^8-coumaroyl spermidine (Mercier et al., 1998) and N^1-coumaroyl spermidine (Klose et al., 2002) at the neuromuscular junction of the deep abdominal extensor muscles of the crayfish *Procambarus clarkii*. The naturally occurring conjugates N^1- and N^8-coumaroyl spermidine had IC_{50} values of 80 and 50–150 μM respectively. N^1-coumaroyl spermine was less potent having an IC_{50} of 70–300 μM. A synthetic analogue **43b** (Scheme 6) that was designed as an analogue of N^1-coumaroyl spermine with a more bulky and hydrophobic polyamine chain, was tested on this same crayfish preparation. EPSP amplitudes were recorded for 5 min before toxin application and for 10 min after the toxin was first introduced. Introduction of the toxin to the bathing solution reduced EPSP amplitude within approximately 2–3 min (depending on the concentration), and the effect reversed rapidly when the toxin was washed out with saline. Control trials were performed by recording EPSPs for the same time but without applying the toxin. The response to the toxin was estimated in each trial by comparing the amplitude of the EPSP at 10 min of toxin exposure to the average EPSP amplitude of seven control trials at the corresponding time. The response in each trial was calculated as a percentage of the control value, and data from individual trials were used to construct a dose–response curve (Fig. 2). The IC_{50} value for **43b** was estimated at approximately 300 μM.

2.3. Mammalian glutamate receptors

Naturally occurring and synthetic conjugates were also tested against recombinant mammalian glutamate receptors expressed in *Xenopus* oocytes. We studied three classes of mammalian receptors—NMDA, AMPA, and δ receptors. Native NMDA and AMPA receptors are gated by glutamate, and a family of subunits that can assemble to form these receptors has been

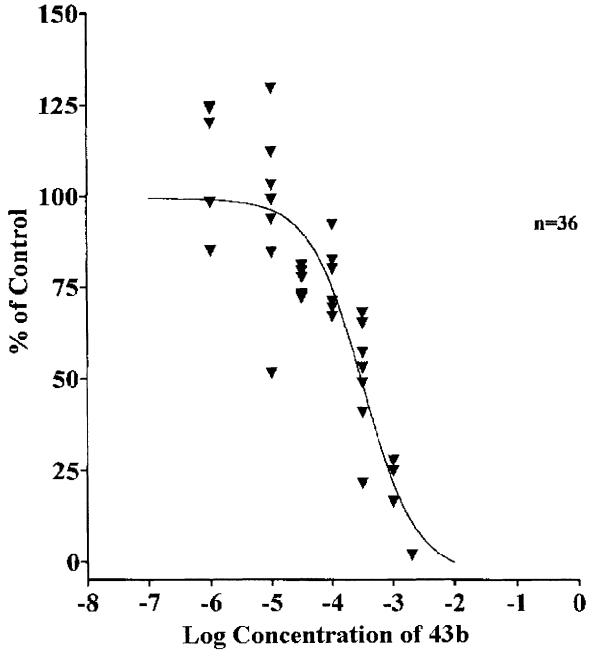
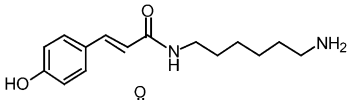
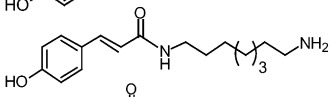
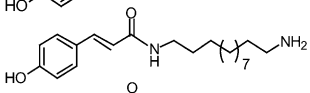
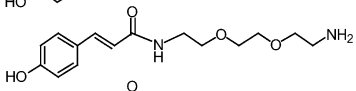
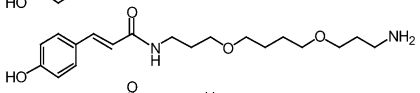
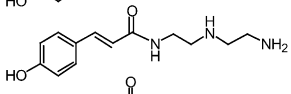
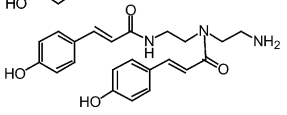
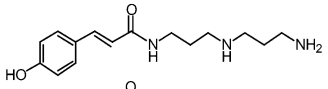
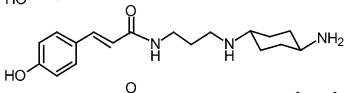
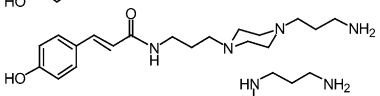
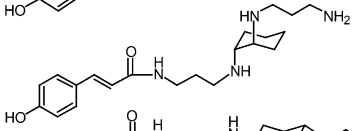
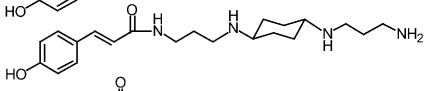
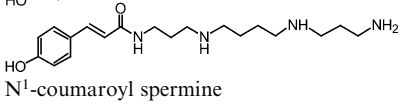


Fig. 2. Dose–response curve for the reduction of excitatory post-synaptic potentials at a crayfish neuromuscular preparation by **43b**. Non-linear regression analysis was performed to obtain a curve of best fit for the data and produced a sigmoidal curve.

cloned (Dingledine et al., 1999; Hollmann and Heinemann, 1994). Two "orphan" subunits, termed δ1 and δ2, which have homology to NMDA and AMPA receptor subunits have been identified, although their function is still unknown as they are not directly gated by glutamate. A mutation in the δ2 subunit creates a constitutively open cation channel, and this mutation is responsible for the neurological phenotype of the *Lurcher* mouse (Zuo et al., 1997). Recent studies have suggested that the pore of δ2 channels may be similar to that of AMPA channels (in particular those containing Q at the Q/R editing site), but there is as yet no detailed pharmacology of the δ2 channels (Wollmuth et al., 2000). Studies with a number of traditional glutamate receptor blockers suggest that the pore has properties in common with both AMPA and NMDA channels (Williams et al., 2003). For the current studies, we used heteromeric NMDA receptors expressed from the NR1a and NR2B subunits (NR1/NR2B receptors), homomeric AMPA receptors expressed from the GluR2(Q) subunit, which contains Q at the Q/R editing site in the M2 loop region (Hume et al., 1991), heteromeric GluR1/GluR2(Q) receptors, and δ2 receptors expressed from the constitutively active δ2(A654T) mutant (Zuo et al., 1997).

We screened the activities of thirteen compounds at a concentration of 10 μM at NMDA, AMPA and δ2

Table 1
Effects of coumaroyl–amine conjugates on NMDA, AMPA, and δ2 channels

Compound	Number	NMDA NR1/NR2B % Inhibition	δ2 δ2(A654T) % Inhibition	AMPA GluR2(Q) % Inhibition	AMPA GluR1/GluR2(Q) % Inhibition
	**29b**	8	1	0	1
	**30b**	11	4	0	1
	**31b**	98	22	13	11
	**32b**	8	1	2	2
	**33b**	3	0	2	1
	**34b**	12	1	6	6
	**34d**	59	−19	10	10
	**35b**	37	−17	11	11
	**36b**	26	4	18	16
	**37b**	12	4	8	9
	**40b**	28	0	15	13
	**43b**	95	43	52	50
	–	100	14	84	78
N^1-coumaroyl spermine					

The percentage inhibition by 10 μM of each compound is shown. Values are mean from 2–4 oocytes. In some cases, the compound produced a small potentiation of the current (indicated by a negative value).

receptors. The results are shown in Table 1. Many compounds had little or no effect on glutamate channels, but **34d** inhibited NMDA receptors by about 60% and compounds **31b**, **43b** and N^1-coumaroyl spermine inhibited NMDA receptors by 95–100%. Compounds **31b** and **43b** also inhibited AMPA and δ2 channels, but most showed some selectivity for NMDA receptors, producing a larger inhibition at NMDA than at AMPA or δ2 channels. N^1-Coumaroyl spermine was a potent blocker of both NMDA and AMPA channels but not of δ2 channels.

The concentration–inhibition relationship for N^1-coumaroyl spermine at NR1/NR2B NMDA receptors was also studied. N^1-Coumaroyl spermine inhibited NMDA receptors with an IC_{50} of 38 nM (Fig. 3). The blocking effects of polyamines are voltage-dependent, but the potency of N^1-coumaroyl spermine at NR1/NR2B receptors at −70 mV is similar to that of the spider toxins argiotoxin$_{636}$ and Agel-505 at NMDA receptors (Williams, 1993a) and about 10-fold higher than the polyamine derivatives N^1,N^4,N^8-tribenzylspermidine and N^1-dansyl-spermine (Chao et al., 1997; Igarashi et al., 1997).

2.4. Insect feeding trials

N^1- and N^8-Coumaroyl spermidine have also been tested against insects in feeding assays using semi-synthetic diets doped with varying amounts of the conjugates. European corn borer (ECB, *Ostrinia nubilalis Hübner*) were raised on a commercial corn meal base diet. Larvae were selected at second instar based on weight and reared on diet that contained 10–500 μg conjugate per gram of wet diet. Initial control experiments showed that the hydroxycinnamic acid polyamine conjugates did not remain in the diet for more than a day as followed by extracting the diet and analyzing for conjugates by ion-pair HPLC (Panagabko et al., 2000). We suspected that peroxidase activity in the grain-base diets was responsible for a large portion of this loss (Converso and Fernández, 1995) and indeed, peroxidase activity could be easily assayed (Makinen and Tenovuo, 1982) (data not shown). This loss could be greatly attenuated by heating the dry diet material at 120 °C for 1 h prior to making up the wet diet. With this treatment about half of the conjugate concentration could be maintained after one day. For full growth studies this necessitated replacing the diet each day. Even after these precautions, there was no discernable difference between the growth rate, fifth instar larval mass, nor mortality when compared to control insects raised on diet similarly heat treated but not containing any spermidine conjugate. Similar experiments were also performed on tobacco budworm (*Heliothis verescens*) and the oblique banded leaf roller (*Choristoneura rosaceana*) with the same results. Further trials with adults of the rice weevil (*Sitophilus oryzae*) using wheat flour wafers (Xie et al., 1996) impregnated with **36b**, **37b**, **40b**, **43b**, N^1-feruloyl spermidine, N^1- and N^8-coumaroyl spermidine at 1.6 and 3.2 mg/200 mg wheat flour also showed no mortality after 7, 14 or 21 days. However, **43b** at the highest dose tested did cause 50% mortality after 21 days. Furthermore, 3-day tests showed that the conjugates, even **43b**, had no antifeedant (Xie et al., 1996) properties.

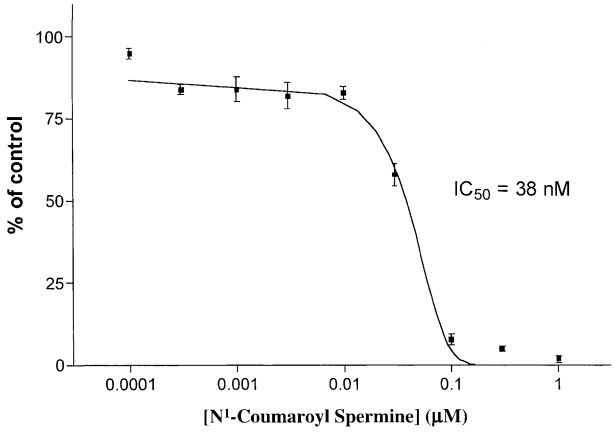


Fig. 3. Effects of N^1-coumaroyl spermine on NMDA receptors. The effects of N^1-coumaroyl spermine were studied on NMDA receptors expressed in *Xenopus* oocytes from NR1/NR2B subunits. Receptors were activated by 10 μM glutamate + 10 μM glycine, and oocytes were voltage-clamped at −70 mV.

3. Discussion

The conjugates of hydroxycinnamic acids with polyamines have been found in a wide variety of plants and have been proposed to have a number of biological activities including anti-viral to antifungal effects. The similarity of these plant derived compounds with the polyamine toxins from invertebrates has been noted previously (Blagbrough et al., 1992; Blagbrough and Usherwood, 1990), but little evidence has been given as to their relevance in chemical ecology. We were interested first in whether hydroxycinnamic acid–polyamine conjugates such as N^1-coumaroyl spermidine were toxic to larvae of certain Lepidopteran crop pests. Since we had significant quantities of some analogues on hand from synthesis, feeding trials were attempted using larvae raised on semi-synthetic diets. Despite much effort in maintaining these readily oxidized compounds in the diets, the results of these trials, and those done with the rice weevil *Sytophilus oryzae,* showed a near lack of toxicity except for **43b** at higher doses and long exposure. Either these compounds are not toxic to the organisms tested, or they do not manifest such toxicity with insects reared on synthetic diets in the laboratory. This latter caveat is mentioned since there are reliable indications that such feeding trials miss the complexity of whole plant tissues and the milieu of enzymes present

as an insect feeds (Duffey and Stout, 1996). However, these effects do not usually mask the presence of potent toxins in the diet and we conclude that these conjugates, when ingested, are not toxic to the pests.

The inability of these coumaric acid–polyamine conjugates to arrest larval feeding may be due in part to the fact that they are relatively poor blockers of the glutamate receptors found at arthropod neuromuscular junctions. IC_{50} values for N^8-coumaroyl spermidine (Mercier et al., 1998), N^1-coumaroyl spermidine (Klose et al., 2002) and **43b** (present results) range from 70 to 300 μM. The IC_{50} values for effect of *Joro* spider toxin on crustacean neuromuscular synapses is approximately 0.02–0.1 μM (Abe et al., 1983; Aonuma et al., 1998; Shudo et al., 1987). Aside from such differences in potency, it is not known how effectively the toxins are absorbed through the digestive system, if at all, and this could be an important reason for the lack of larval growth inhibition. Spider and wasp toxins, by contrast, are injected directly into the hemolymph and, thus, elicit paralysis very rapidly.

Although the hydroxycinnamic acid conjugates that we studied are relatively poor blockers of arthropod glutamate receptors, they are effective in blocking mammalian glutamate receptors. The IC_{50} for N^1-coumaroyl spermine was 38 nM at NMDA receptors. The same compound blocks arthropod glutamate receptors with an IC_{50} value of about 80 μM, indicating a difference in potency of more than three orders of magnitude. Some of this difference is probably due to the fact that arthropod glutamate receptors are more closely related to the AMPA-type, being activated by quisqualate (Abe et al., 1983; Shinozaki and Shibuya, 1974; Usherwood and Blagbrough, 1991).

3.1. Structure–activity

The nature of the assay with crayfish meant that only a few compounds could easily be assessed. This, coupled with the relatively small number of compounds made in this study, cannot support a complete analysis of the variation of biological activity with chemical structure, but a few salient points can be made. The IC_{50}s of N^1- and N^8-coumaroyl spermidine for reduction of crustacean glutamate receptor excitatory postsynaptic potentials (EPSPs) are 70 and 200 μM respectively (Klose et al., 2002). These short chain amine conjugates are much less active than the longer chain *Joro* spider toxin JSTX-3 which has an IC_{50} in the crustacean preparations of 0.02–0.1 μM. Higher potencies with longer chain acylpolyamine toxins has been noted previously for philanthotoxins (Anis et al., 1990; Benson et al., 1993; Bruce et al., 1990). Treating nerve preparations with 50 μM of N^1-coumaroyl spermidine or N^1-coumaroyl spermine gave EPSP reductions of near equal magnitude. This was unusual, as the longer chain spermine conjugates would have been expected to be more potent given their greater similarity to the spider toxins. A similar assay with **43b** gave an IC_{50} of 300 μM, approximately 4 times that of N^1-coumaroyl spermine. Both N^1-coumaroyl spermine and **43b** are much less effective on the crustacean receptor than the mammalian glutamate receptors (see below).

The percent inhibition of mammalian glutamate receptors by 10 μM of the coumaroyl polyamines is shown in Table 1. All compounds show selectivity for the NMDA NR1/NR2B receptor but range from 3 to 100% in the degree of inhibition. Compounds with the largest separation between the coumaroyl amide nitrogen and the terminal (primary) amine tended to have higher activity as has been noted for the biological activity against mammalian nicotinic acetylcholine receptors (Li et al., 2001). Thus compounds **31b**, **43b**, and N^1-coumaroyl spermine all inhibited NR1/NR2B receptors >95% at 10 μM, and were also active at the AMPA and δ receptors. The activity of the dodecyldiamine analogue **31b** is remarkable given that it has no internal nitrogens. Counterpoint this with the near complete inactivity of the oxa-analogues **32b** and **33b**. Philanthotoxin analogues that contain oxa-amine sidechains retain their activity against acetylcholine receptors (nAChR), but are inactive against non-NMDA receptors expressed in oocytes, although the structure–activity descriptions in this case are complicated by a dependency on the acyl group attached to the α-nitrogen of the tyrosine moiety of the toxin (Strømgaard et al., 2000). The high inhibitory activity of **31b** suggests that the terminal amine is more important than the internal ones and may also reflect the role that hydrophobic forces have to play in polyamine binding (Cu et al., 1998) although a similar analogue of philanthotoxin showed no inhibition of the glutamatergic neuromuscular synapse in larvae of the house fly, *Musca domestica* (Benson et al., 1992).

A model of mammalian glutamate receptor channels based on the structure of the potassium channel KscA from *Streptomyces lividans* has been proposed. The model is supported by the results of studies on mutations within the glutamate channel that affect block by polyamines (Panchenko et al., 2001). The model includes a glutamate residue in a solvent accessible loop on the cytoplasmic side of the channel that is required for block by intracellular polyamines. Some of the long-chain polyamines such as **31b** may bind in such as way that their polyamine tail passes through the channel to interact at the intracellular site, but the head group remains lodged in the channel unable to pass through the narrow selectivity filter about halfway through the channel. Consistent with this are previous observations that some unsubstituted polyamines or analogs with small head groups can permeate the channel when

applied extracellularly, whereas analogs with larger headgroups are unable to easily permeate the channel (Chao et al., 1997; Igarashi et al., 1995, 1997).

If hydrophobic forces play a key role in polyamines block, the dodecylamine analogue **31b** may take advantage of hydrophobic forces during entry into the channel pore, whereas the oxa-analogues may be too polar for favorable binding. Polyamines that have inappropriate distances between nitrogen atoms in the chain, i.e. having too few or too many methylene groups between cationic sites to find matching cluster of tryptophans in the channel, would also bind poorly. This may explain the lack of activity of **34b** and **37b**. Similar results have been seen with non-acylated polyamines that bind to NMDA receptors (Subramaniam et al., 1994) where inhibitory potency was correlated with carbon chain length and length of methylene spacer between positive charges. Compounds **35b** and **36b** have some residual activity, but are clearly too short.

The requirement for mammalian receptor binding and inhibition would thus appear to be a 12-atom separation between the amide nitrogen and the terminal amine. This is equivalent to a distance of about 16–17 Å (amide N-atom to terminal N-**H**) in a fully extended conformation for N^1-couamroyl spermine and **43b**. This does not necessarily mean that this is the conformation on binding, although NMR studies at varying pH support a near linear conformation of argiotoxin$_{636}$ (Raditsch et al., 1996) and Monte-Carlo minimization methods show that the vast majority of low energy structures populate linear polyamine conformations (Tikhonov et al., 2000). The lower activity of **40b** with respect to **43b** against the NMDA receptor may arise because of the lack of conformational freedom in the side chain of **40b** due to the 1,2-disposition of the cyclohexyldiamine moiety in the side chain.

Curiously, the diacylated compound **34d** retains some activity (although it is stimulatory at δ2-receptors). If this compound binds in a fashion analogous to the other compounds then there must be enough space in the channel for the second bulky group to bind. This may be reasonable to conclude given the ability of NMDA receptors to bind tribenzylspermidine (Igarashi et al., 1997; Kashiwagi et al., 2002).

4. Conclusions

The original hypothesis for this work was that the plant hydroxycinnamoyl conjugates of polyamines such as N^1- and N^8-coumaroyl spermidine and N^1-coumaroyl spermine may mimic the arthropod toxins and be in part responsible for the chemical protection of plants. We have shown that indeed these compounds are active inhibitors of glutamatergic receptors in crustaceans as evidenced by the reduction in EPSPs in crayfish extensor muscle preparations. However, since these compounds would necessarily be ingested by herbivorous insects, we also tested for toxicity towards a variety of lepidopteran larvae using semi-synthetic diets laced with N^1- and N^8-coumaroyl spermidine. There was no evidence of mortality, growth reduction or, in the case of the rice weevil *Sitophilus oryzae,* any activity as an antifeedant. Since the amount of acylpolyamine compound required for these insect feeding trials is large, due to oxidative decomposition and the necessity of replacing diet every day over a 10 day feeding trial, not all of the compounds could be tested. Only the shorter chain spermidine conjugates were tested in this manner and their lower inhibitory potency may have meant that little observable biological activity was to be witnessed. Alternatively, there is a great deal of difference between in vitro cell based assays, where toxins can be readily applied close to the site of action, and whole animal trials where multiple barriers exist between ingestion and a binding event at a remote tissue. In effect, the highly charged nature of these compounds may make them completely inactive as ingested toxins, requiring them to be injected into tissues, something a spider already practices.

The expectation that the acylpolyamines prepared in this work are similar enough to spider and wasp toxins to be active in receptor based assays was clearly met as evidenced by the strong activities at mammalian glutamate receptors, especially the NR1/NR2B NMDA receptors. The structure–activity description largely parallels that seen with philanthotoxin, argiotoxin, and non-acylated polyamine analogues at various receptor preparations.

5. Experimental procedures

5.1. Physiological studies

Freshwater crayfish (*Procambarus clarkii*) were obtained from Atchafalaya Biological Supply Co., Inc. (Raceland, LA). Crayfish size were 1–3 inches in length. The crayfish were fed a diet of "Tender Vittle" catfood and kept in circulating, filtered freshwater tanks. Physiological effects were examined using deep abdominal extensor muscles of crayfish, as described by Mercier and Atwood (1989). Briefly, the dorsal abdominal shell was dissected away with the extensor muscles intact and was pinned out in a dish containing a physiological saline based on that of van Harreveld (1936), but with reduced Ca_{2+} and elevated Mg_{2+} levels to prevent muscle twitching. This saline solution contained 205 mM NaCl, 5.3 mM KCl, 6.25 mM $CaCl_2.2H_2O$, 12.5 mM $MgCl_2$, and 5 mM HEPES, and the pH was adjusted to 7.4 with NaOH. Excitatory postsynaptic potentials (EPSPs) in the muscle were elicited by applying electrical stimuli to the nerve using

a suction electrode. EPSPs were recorded from muscle L1 (Parnas and Atwood, 1966) using intracellular microelectrodes filled with 3 M KCl. The nerve was stimulated at a frequency of 0.2 Hz, and responses were acquired and averaged using a 386-compatible microcomputer and software designed specifically for this purpose (Technical Services Division, Brock University, St. Catharines, ON). The recording dish had a volume of approximately 300 ml and was perfused continuously at a rate of 1 ml/min throughout each experiment. The toxin was applied by changing the perfusate from the standard physiological saline to one containing the toxin. All experiments were performed at room temperature (21 °C).

5.2. Recombinant mammalian glutamate receptors

Mammalian glutamate receptors were studied using voltage-clamp recording of recombinant receptors expressed in *Xenopus* oocytes. Plasmids with clones encoding glutamate receptor subunits were linearized at a site 3′ to the insert using an appropriate restriction endonuclease. cRNAs were synthesized from cDNAs using the mMessage mMachine kit (Ambion, Austin, TX), and oocytes were injected with the appropriate cRNA or mixture of cRNAs (0.2–10 ng per oocyte). The preparation and maintenance of oocytes was carried out as described previously (Williams, 1993b; Williams et al., 1993). Macroscopic currents were recorded with a two-electrode voltage-clamp using glass microelectrodes filled with 3 M KCl and having resistances of 0.5–3 MΩ (Williams, 1993b). Oocytes were continuously superfused with a saline solution (96 mM NaCl, 2 mM KCl, 1.8 mM $BaCl_2$, 10 mM HEPES, pH 7.5) that contained $BaCl_2$ rather than $CaCl_2$ to minimize Ca^{2+}-activated Cl^- currents, and in experiments with NMDA receptors, oocytes were injected with K^+-BAPTA (100 nl of 40 mM, pH 7.0–7.4) on the day of recording. Oocytes were voltage-clamped at −70 mV. NMDA receptors were activated by 10 μM glutamate plus 10 μM glycine. AMPA receptors were activated by 100 μM kainate. To study currents through δ2(A654T) channels, we determined the difference in the holding current measured in an extracellular solution containing Na^+ (composition as above) and one in which the Na^+ was replaced by an equimolar concentration of the large organic cation *N*-methyl-D-glucamine (NMDG). The constitutively open δ2(A654T) channels are permeable to Na^+ but not to NMDG (Wollmuth et al., 2000; Zuo et al., 1997). The δ2(A654T) channels produced macroscopic currents (defined as the difference in holding current between Na^+ and NMDG) of several hundred nA. In control experiments, uninjected oocytes, or oocytes injected with the wild-type (inactive) δ2 clone had a difference of 5–15 nA in the Na^+ versus NMDG holding current.

5.3. Syntheses

N^1-Coumaroyl spermine was prepared following the published method (Blagbrough and Geall, 1998) as were N^1- and N^8-coumaroyl spermidines (Hu and Hesse, 1996).

5.4. Reagents and solvents

Reagent grade solvents were used for all extraction and work-up procedures without further purification. THF was dried over sodium metal in the presence of benzophenone. Dichloromethane was distilled from calcium hydride. Methanol was distilled from magnesium turnings and a catalytic amount of iodine.

5.5. General methods

Unless otherwise stated, all starting materials were obtained from commercial suppliers and were used without further purification. Chromatography was carried out on Aldrich silica gel (230–400 mesh) with the indicated solvent systems. Analytical TLC was performed on 0.25 mm, precoated silica gel plates (EM Science, Silica Gel 60 F-254). Visualization was achieved using a UV lamp at 254 nm or exposure to iodine vapor, or by immersion in a solution of ninhydrin in ethanol (0.2 g in 100 ml) followed by heating. Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Low resolution mass spectra were obtained by electron impact (EI) at 70 eV or fast atom bombardment (FAB) using *m*-nitrobenzyl alcohol (NBA) as the matrix. ^1H NMR were recorded at 300 MHz and ^{13}C NMR at 75.6 MHz with deuterated-chloroform as the solvent unless otherwise noted. The chemical shifts, δ, for the ^1H were recorded in ppm relative to $CDCl_3$ ($\delta = 7.26$ ppm) and that for ^{13}C was relative to $CDCl_3$ ($\delta = 77.0$ ppm) or DMSO ($\delta = 39.5$ ppm).

5.6. General procedure for trifluoracetylation of di- and polyamines

Di- and polyamines were mono-trifluoracetylated following the published method (Osullivan and Dalrymple, 1995). Briefly, the amine is dissolved in MeOH or THF, cooled to −78 °C and to this is added 1.0 equivalent of ethyltrifluoroacetate. After 1 h the solution is allowed to warm to room temperature and the product is recovered by simple evaporation of the solvent. Further purification is dependent on the compound and is described in the relevant sections below.

5.7. General procedure for mono-t-butoxy carbonylation of di- and polyamines

Mono-protection of di- and polyamines with $(Boc)_2O$ was performed using the method of Krapcho and Kuell (1990).

5.8. General procedure for acylation with (*E*)-3-(4-acetoxyphenyl) prop-2-enoyl chloride

Under an argon atmosphere (*E*)-3-(4-acetoxyphenyl) prop-2-enoic acid (1.0 eq., 2.15 g, 10.4 mmol) was suspended in 20 ml of CH_2Cl_2 and a solution of oxalyl chloride (1.2 eq., 1.76 g, 1.21 ml) in CH_2Cl_2 (20 ml) was added drop wise. Approximately 70 μL of DMF was added as a catalyst. The reaction mixture was refluxed for 1 h, cooled and partially evaporated to remove HCl. The (*E*)-3-(4-acetoxyphenyl) prop-2-enoyl chloride so produced was added drop wise to a solution of the appropriate amine (1.0 eq.) and 3 eq. of Et_3N at RT over 10 min. After the reaction was judged complete the solvent was removed, and the residue dissolved in distilled water. The aqueous phase was then extracted with CH_2Cl_2 and the combined organic fractions were washed with 0.25 M Na_2CO_3 to remove the any coumaric acid in solution. The organic phase was dried with anhydrous Na_2SO_4 and evaporated to dryness to afford the various amides described below.

5.9. Estimation of purities

The impurities of chief concern are residual amounts of free amines, which could confound the glutamate receptor assays, and *E*/*Z* isomerism of the coumaric acid moiety. TLC easily shows whether any free amines remain since for these samples the origin stains purple with ninhydrin. No sample destined for bioassay contained any free amines as determined by this method. The extent to which the coumaroyl group had undergone *E*/*Z* isomerization was best determined by NMR, and judging by the larger *J*-values recorded all samples contained >95% of the *E*-isomer.

5.10. 2(*E*)-*N*-(6-aminohexyl)-3-(4-hydroxyphenyl)prop-2-enamide (**29b**)

5.10.1. Synthesis of *N*-(6-aminohexyl)-2,2,2-trifluoroacetamide (**4a**)

Following the general method, 9.75 g (83.9 mmol) of 1,6-hexanediamine (**1**), was dissolved in MeOH (50 ml) and cooled to −78 °C. A solution of ethyl trifluoroacetate (CF_3COEt), (1 eq., 11.9 g, 9.98 ml) was added drop wise to the diamine with a gas tight syringe and the reaction temperature maintained for 1.5 hr. After warming to 0 °C over 1 h, the MeOH solvent was evaporated to afford 16.9 g (95%) of crude **4a** as a clear, viscous pale yellow oil containing only trace of hexanediamine. TLC R_f = 0.47 (CH_2Cl_2/MeOH/NH_4OH 30% = 10:4:1); ^1H NMR spectral data ($CDCl_3$) δ 8.27 (*br s*, 1H), 3.28 (*t*, 2H), 2.65–2.61 (*t*, 2H), 1.53 (2H), 1.40–1.38 (*m*, 4H), 1.30–1.28 (*m*, 4H); ^{13}C NMR spectral data ($CDCl_3$) 157.79 (*q*, 36 Hz), 116.43 (*q*, 286 Hz), 42.36, 40.04, 33.92, 33.62, 29.15, 29.04; FAB-MS ($CDCl_3$) *m*/*z* 213 ($[M+1]^+$, 100), 117 (10).

5.10.2. Synthesis of tert-butyl 6-[(trifluoroacetyl)amino]-hexylcarbamate (**4b**)

A solution of $(Boc)_2O$ (1 eq., 16.5 g, 75.5 mmol) in THF was added dropwise to 16.0 g (75.5 mmol) of **4a** in 35 ml of THF stirred overnight at room temp. The solvent was removed under reduced pressure to give **4b** as a clear yellow viscous oil (23.3 g, 99%) which solidified on standing. TLC R_f = 0.85 (CH_2Cl_2/MeOH/NH_4OH 30% = 10:4:1); ^1H NMR spectral data ($CDCl_3$) δ 6.5 (*br s*, 1H), 4.52 (*br s*, 1H), 3.39 (*t*, 2H), 3.11 (*br s*, 2H), 1.45 (*s*, 9H, *t*-Bu), 1.39–1.28 (*m*, 8H); ^{13}C NMR spectral data ($CDCl_3$) 160.0, 39.87, 30.43, 29.17, 29.07, 28.78, 26.74, 26.16, 25.90; FAB-MS *m*/*z* 313 ($[M+1]^+$, 8), 256 (46), 239 (29), 161 (65).

5.10.3. Synthesis of tert-butyl 6-aminohexylcarbamate (**4c**)

4b (23.0 g) was suspended in MeOH (50 ml) and concentrated NH_4OH (1:1). After stirring for 48 h the reaction mixture was evaporated to dryness, water (60 ml) was added and the aqueous phase extracted with CH_2Cl_2. Compound **4c** remained in the aqueous phase and was concentrated under reduced pressure to give a colorless oil (12.0 g, 75%). R_f = 0.57 (CH_2Cl_2/MeOH/NH_4OH 30% = 10:4:1); ^1H NMR spectral data ($CDCl_3$) δ 4.63 (*br s*, 1H), 3.09 (*t*, 2H), 2.70 (*m*, 2H), 2.25 (2H, -–NH_2), 1.42 (*s*, 9H, *t*-Bu), 1.40 (*m*, 4H), 1.29–1.20 (*m*, 4H); ^{13}C NMR spectral data ($CDCl_3$) 156.4, 79.4, 42.2, 40.8, 33.4, 30.4, 28.8, 26.8; FAB-MS *m*/*z* 433 ($[2M+1]^+$, 7), 217 ($[M+1]^+$, 100), 161 (56), 117 (19).

Acylation of 2.50 g (11.6 mmol) of **4c** with **29** afforded 1.74 g of a yellow spongy voluminous product. The crude product was purified on silica gel with CH_2Cl_2/MeOH = 20:1, followed by CH_2Cl_2/MeOH = 10:1 as eluant to give 1.63 g of **29a**. The yield was 40%. TLC R_f = 0.50 (CH_2Cl_2/MeOH = 10:1); ^1H NMR spectral data ($CDCl_3$) δ 7.64 (*d*, *J* = 16 Hz), 7.54 (*d*, 2H, *J* = 8 Hz), 7.12 (*d*, 2H, *J* = 8 Hz), 6.4 (*d*, 1H, *J* = 16 Hz) 5.90 (*br s*, 1H), 4.55 (*br s*, 1H) 3.42 (*m*, 2H), 3.1 (*m*, 2H), 2.34 (*s*, 3H, OCH_3), 1.61 (*m*, 2H), 1.46 (*s*, 9H, *t*-Bu), 1.39 (*m*, 4H); ^{13}C NMR spectral data ($CDCl_3$) 169.4, 162.7, 153.3, 147.9, 131.8, 130.2, 129.2, 122.4, 121.5, 117.2, 42.2, 40.8, 33.4, 30.4, 28.8, 21.5; FAB-MS *m*/*z* 809 ($[2M+1]^+$, 6), 405 ($[M+1]^+$, 22) 349 (24), 305 (63), 189 (49), 147 (100).

Deprotection of 1.63 g (4.50 mmol) of **29a** in 40 ml dry MeOH/HCl (2.15 g) at 0 °C provided **29b** as yellow crystals after solvent evaporation and recrystallization from absolute EtOH. The yield was 800 mg (68%).

TLC R_f = 0.30 (CH_2Cl_2/MeOH = 10:1); ^1H NMR ($CDCl_3$) δ 8.02 (*br s*, 1H), 7.37 (*d*, 2H, *J* = 8 Hz), 7.31 (*d*, 1H, *J* = 16 Hz), 3.94 (*br s*, 1H), 3.14–3.13 (*m*, 2H), 2.74

(*m*, 2H), 1.53–1.08 (*m*, 8H); ^{13}C NMR ($CDCl_3$) 165.15, 159.65, 139.27, 129.93, 126.81, 119.74, 116.59, 31.51, 29.88, 27.76, 26.78, 26.35; FAB-MS *m/z* 525 ($[2M+1]^+$, 40), 263 ($[M+1]^+$, 100), 147 (78) HRMS (FAB, NBA). Calculated mass: 263.1760 for $C_{15}H_{23}N_2O_2$, found: 263.1718.

5.11. 2(*E*)-(8-aminooctyl)-3-(4-hydroxyphenyl)prop-2-enamide (**30b**)

5.11.1. Synthesis of N-(8-aminooctyl)-2,2,2-trifluoroacetamide (**5a**)

Following the general procedure1,8-octanediamine **2** (7.60 g, 52.7 mmol) was mono-trifluoracetylated to afford **5a**. Purification of the crude product on silica gel ($MeOH/CH_2Cl_2/AcOH = 60:40:2$) gave 11.9 g (94%) of a pale yellow oil. TLC $R_f = 0.48$ ($CH_2Cl_2/MeOH/NH_4OH$ 30% = 10:4:1); ^{1}H NMR spectral data ($CDCl_3$) δ 7.68 (*br s*, 1H) 3.29 (*t*, 2H), 2.65 (*t*, 2H), 1.73 (2H), 1.55 (2H), 1.42 (2H), 1.28 (*m*, 8H); ^{13}C NMR spectral data ($CDCl_3$) 157.79 (*q*, $J = 36$ Hz), 116.38 (*q*, $J = 286$ Hz), 50.38, 42.45, 40.2, 33.96, 29.78, 29.43, 27.17, 27.06; FAB-MS *m/z* 241 ($[M+1]^+$, 100), 185 (7), 145 (34), 126 (10), 69 (22), 56 (21), 30 (52).

5.11.2. Synthesis of tert-butyl 8-[(trifluoroacetyl)-amino]octylcarbamate (**5b**)

A solution of 1 eq. $(Boc)_2O$ (10.85 g) in THF (30 ml) was added to a solution of **5a** (11.94 g, 49.75 mmol) in THF (30 ml) via syringe at room temp. After stirring overnight, the solvent was evaporated and the product purified on silica gel (CH_2Cl_2/MeOH/ammonia water 30% = 10:4:1) to afford **5b** (16.71 g, 99%) as a yellow viscous oil. TLC $R_f = 0.75$ ($CH_2Cl_2/MeOH/NH_4OH = 10:4:1$); ^{1}H NMR spectral data ($CDCl_3$) δ 6.48 (*br s*, 1 H), 4.53 (*br s*, 1 H), 3.38–3.33 (*m*, 2H), 3.13–3.07 (*m*, 2H), 1.66–1.57 (*m*, 2 H), 1.45 (*s*, 9H, *t*-Bu), 1.43–1.32 (10H); ^{13}C NMR spectral data ($CDCl_3$) 175.4, 157.64 (*q*, $J = 36$ Hz), 116.32 (*q*, $J = 287$ Hz), 40.9, 40.3, 30.4, 29.6, 29.3, 29.2, 28.8, 27.0, 26.9, 26.8; FAB-MS *m/z* 363 ($[M+Na]^+$, 82), 341 ($[M+1]^+$, 17), 285 (74), 241 (100), 57 (96).

5.11.3. Synthesis of tert-butyl 6-aminooctylcarbamate (**5c**)

16.6 g (2.15 mmol) of **5b** was suspended in 50 ml of MeOH and concentrated NH_4OH (1:1). After stirring at reflux for 48 h a white precipitate was filtered off, the solvent was evaporated, water (60 ml) was added and the aqueous phase extracted with CH_2Cl_2. Compound **5c** remained in the aqueous phase and was concentrated under reduced pressure to give 11.78 g (99%) of a colorless oil which solidified at room temp. TLC $R_f = 0.50$ ($CH_2Cl_2/MeOH/NH_4OH$ 30% = 10:4:1); ^{1}H NMR spectral data ($CDCl_3$) δ 7.79 (*br s*, 2H), 4.6 (*br s*, 1H) 3.08 (*t*, 2H), 2.93 (*t*, 2H), 1.67 (*t*, 2H), 1.45 (*s*, 9H, *t*-Bu), 1.40–1.30 (*m*, 10H); ^{13}C NMR spectral data ($CDCl_3$) 175.4, 50.6, 40.9, 40.3, 30.2, 29.0, 28.8, 27.6, 26.7, 26.4. FAB-MS *m/z* 489 ($[2M+1]^+$, 7), 245 ($[M+1]^+$, 100), 189 (93), 145 (18), 57 (83).

5.11.4. Synthesis of 4-[(1*E*)-3-({8-[tert-butoxy-carbonyl)amino]octyl}amino)-3-oxoprop-1-enyl]phenyl acetate

Acylation of 4.00 g of **5c** (16.4 mmol) with **28** following the general method afforded **30a** (4.28 g, 60%) as a yellow spongy solid. TLC $R_f = 0.37$ ($CH_2Cl_2/MeOH = 10:1$); ^{1}H NMR spectral data ($CDCl_3$) δ 7.63 (*d*, 1H, $J = 16$ Hz), 7.53 (*d*, 2H, $J = 8$ Hz), 7.11 (*d*, 2H, $J = 8$ Hz), 6.39 (*d*, 1H, $J = 16$ Hz), 5.78 (*s*, br, 1H), 4.54 (*br s*, 1H) 3.41–3.32 (*m*, 2H), 3.14 (*m*, 2H), 2.34 (*s*, 3H, OCH_3), 1.58 (*m*, 2H), 1.45 (*s*, 9H), 1.32 (10H); ^{13}C NMR spectral data ($CDCl_3$) 166.3 159.8, 139.7, 130.0, 129.9, 126.9, 119.5, 116.6, 70.1, 69.9, 30.1, 29.0, 28.8, 27.6, 26.9; FAB-MS *m/z* 865 ($[2M+1]^+$, 5), 433 ($[M+1]^+$, 17), 377 (14), 359 (5) 333 (31), 189 (20), 147 (40).

Deprotection of 1.10 g of **30a** (2.54 mmol) was accomplished using dry HCl (g) in MeOH (30 ml) at 0 °C. Fine yellow crystals of **30b** (650 mg, 88%) were isolated by filtration. TLC $R_f = 0.36$ ($CH_2Cl_2/MeOH = 10:1$); ^{1}H NMR (DMSO) δ 7.37 (*d*, 2H, $J = 8$ Hz), 7.31 (*d*, 1H, $J = 16$ Hz), 6.81 (*d*, 2H, $J = 8$ Hz), 6.45 (*d*, 1H, $J = 16$ Hz), 3.16 (2H) 2.88 (2H), 1.54–1.43 (4H), 1.26 (8H); ^{13}C NMR (DMSO) 166.1, 159.7, 139.2, 129.9, 119.7, 116.6, 30.0, 29.3, 29.1, 27.7, 27.2, 26.5; FAB-MS *m/z* 581 ($[2M+1]^+$, 5), 291 ($[M+1]^+$, 100), 176 (26), 147 (89) 145 (41). HRMS (FAB, NBA). Calculated mass: 291.2073 for $C_{17}H_{27}N_2O_2$, found: 291.1998.

5.12. 2(*E*)-*N*-(12-aminododecyl)-3-(4-hydroxyphenyl)-prop-2-enamide (**31b**)

5.12.1. Synthesis of tert-butyl 12-aminododecylcarbamate (**6**)

Mono-tert-butoxycarbonylation of 10.0 g of 1,12-dodecanediamine 3 (50.0 mmol) with $(Boc)_2O$ (1.36 g, 6.25 mmol) following the general procedure afforded **6** (1.36 g, 74%) as yellow oil. TLC $R_f = 0.40$ ($CH_2Cl_2/MeOH/NH_4OH = 10:4:1$); ^{1}H NMR spectral data ($CDCl_3$) δ 4.52 (*br s*, 1H), 2.71 (*t*, 2H), 1.45 (*s*, 9H, *t*-Bu), 1.28 (22H); ^{13}C NMR spectral data ($CDCl_3$) 176.5, 79.1, 42.9, 40.4, 34.3, 30.1, 29.9, 29.8, 29.7, 29.3, 29.0, 26.9, 26.5, 26.1; FAB-MS *m/z* 601 ($[2M+1]+$, 2), 301 ($[M+1]^+$, 100), 245 (79), 201 (36).

5.12.2. Synthesis of 4-[(1*E*)-3-({12-[(tert-butoxy-carbonyl)amino]dodecyl} amino)-3-oxoprop-1-enyl]-phenyl acetate

Acylation of **6** with **28** (1.38 g, 4.60 mmol) following the general procedure gave 540 mg (22%) of **31a** as a yellow spongy solid. TLC $R_f = 0.46$ ($CH_2Cl_2/MeOH = 10:1$); ^{1}H NMR spectral data ($CDCl_3$) δ 7.65 (*d*, 1H, $J = 16$ Hz), 7.54 (*d*, 2H, $J = 9$ Hz), 7.13 (*d*, 2H,

$J=9$ Hz), 6.37 (*d*, 1H, $J=16$ Hz), 5.62 (*br s*, 1H), 4.51 (*br s*, 1H), 3.41 (*t*, 2H), 3.11 (*m*, 2H), 2.33 (*s*, 3H, OCH_3), 1.58 (*m*, 2H), 1.46 (*s*, 9 H, *t*-Bu). ^{13}C NMR spectral data ($CDCl_3$) 169.8, 166.5, 156.7, 152.1, 139.7, 133.3, 129.7, 129.1, 122.6, 121.9, 79.3, 71.4, 42.9, 40.6, 34.5, 30.2, 29.8, 29.7, 29.6, 29.4, 29.1, 28.9, 27.0, 26.8; FAB-MS *m/z* 489 ($[M+1]^+$, 7), 433 (5), 389 (31), 245 (48), 189 (20), 147 (60), 57 (100). **31a** (520 mg, 0.107 mmol) was treated with dry HCl (2.15 g) in MeOH and afforded **31b** as a yellow solid (120 mg, 32%) after recrystallization from absolute EtOH; TLC $R_f=0.66$ (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); ^1H NMR (d_6-DMSO) δ 7.60 (*d*, 1H, $J=15$ Hz), 7.41 (*d*, 2H, $J=9$ Hz), 6.87 (*d*, 2H, $J=9$ Hz), 6.29 (*d*, 1H, $J=16$ Hz), 5.69 (*br s*, 1H), 3.43 (*m*, 2H), 3.11 (*m*, 2H), 1.60–1.57 (*m*, 4H), 1.27 (16H). ^{13}C NMR (d_6-DMSO) 172.2, 165.5, 144.2, 134.6, 131.0, 124.2, 121.4, 42.7, 40.4, 34.3, 30.0, 29.9, 29.8, 29.7, 29.5, 29.4, 29.3, 27.3, 27.0; FAB-MS *m/z* 347 ($[M+1]^+$, 100), 330 ($[M+1\text{-}NH_3$, 2), 371 (2), 201 (6), 147 (83), 86 (4), 72 (3). HRMS (FAB, NBA). Calculated mass: 347.2699 for $C_{21}H_{35}N_2O_2$, found: 347.2679.

*5.13. 2(E)-N-{2-[2-(2-aminoethoxy)ethoxy]ethyl}-3-(4-hydroxyphenyl)prop-2-enamide (**32b**)*

*5.13.1. Synthesis of N-{2-[2[(2-aminoethoxy)ethoxy]-ethyl}-2,2,2-trifluorocaetamide (**9a**)*

Monotrifluoroacetylation of 30.0 g (29.5 ml, 202 mmol) of 2,2′-(ethylenedioxy) bis (ethylamine) **7** following the general procedure gave 26.5 g (54%) of **9a**, following evaporation of the THF, dissolution in water (100 ml) and extraction with CH_2Cl_2. The organic phase was concentrated and purified on silica gel (CH_2Cl_2/MeOH = 20:1 followed by 10:1) TLC $R_f=0.58$ (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); ^1H NMR spectral data ($CDCl_3$) δ 8.75 (*br s*, 1H), 3.72 (*t*, 2H), 3.64 (*m*, 6H), 3.54 (*t*, 2H), 3.12 (*t*, 2H); ^{13}C NMR spectral data ($CDCl_3$) 158.16 (*q*, $J=36$ Hz), 116.37 (*q*, $J=286$ Hz), 70.5, 69.5, 67.4, 40.1, 39.7, 22.3; FAB-MS *m/z* 245 ($[M+1]^+$, 100), 149 (22), 140 (49), 44 (50).

*5.13.2. Synthesis of N-tert butyl 2-(2-{2-[(trifluoro-acetyl)amino] ethoxy}ethoxy) ethylcarbamate) (**9b**)*

A solution of $(Boc)_2O$ (1 eq., 21.97 g) in THF (60 ml) was added to **9a** (24.56 g, 100.7 mmol) in THF (100 ml) at room temp. and stirred for 3 h. The reaction mixture was evaporated to dryness to afford **9b** as a pale yellow oil (33.25 g, 97%) which was used for the next step of the reaction without further purification. TLC $R_f=0.73$ (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); ^1H NMR spectral data ($CDCl_3$) δ 3.69 (*m*, 6H), 3.54 (*m*, 4H), 3.31 (*t*, 2H), 1.43 (*s*, 9H, *t*-Bu); ^{13}C NMR spectral data ($CDCl_3$) 174.98, 157.29 (*q*, 36 Hz), 116.37 (*q*, $J=286$ Hz), 70.5, 70.4, 69.2, 67.2, 40.0, 39.6, 22.3; FAB-MS *m/z* 367 ($[M+Na]^+$, 100), 345 ($[M+1]^+$, 5), 289 (13), 245 (42), 57 (33).

*5.13.3. Synthesis of tert-butyl 2-[2-(2-aminoethoxy)-ethoxy]ethylcarbamate (**9c**)*

9b (33.0 g) was suspended in MeOH (100 ml) and 40 ml conc. NH_4OH was added to the solution. The reaction mixture was stirred and heated to reflux for 24 h. After evaporation to dryness water (100 ml) was added and the aqueous phase extracted with CH_2Cl_2. Evaporation of the aqueous phase gave **9c** as yellow oil (23.1 g, 97%). TLC $R_f=0.53$ (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); ^1H NMR spectral data ($CDCl_3$) δ 5.38 (*br s*, 1H), 3.72 (*t*, 2H), 3.69 (*m*, 4H), 3.54 (*t*, 2H), 3.27 (*t*, 2H), 3.14 (2H), 1.42 (*s*, 9H, *t*-Bu); ^{13}C NMR spectral data ($CDCl_3$) 158.5, 114.1, 65.7, 65.6, 65.4, 62.4, 62.1, 61.9, 35.9, 35.1, 23.9, 23.6; FAB-MS *m/z* 497 $[2M+1]^+$, 5), 249 ($[M+1]^+$, 84), 193 (30), 149 (100), 106 (30), 57 (40), 44 (96).

*5.13.4. Synthesis of 4-[(1E)-16,16-dimethyl-3,14-dioxo-7,10,15-trioxa-4,13-diazaheptadec-1-en-1-yl]phenyl acetate(**32a**)*

Acylation of **9c** with **28** (10.0 g, 40.3 mmol) following the general procedure gave 12.9 g of a crude product that was purified by on silica gel (CH_2Cl_2/MeOH = 20:1, 10:1) to give **32a** as a yellow viscous oil (7.90 g, 45%). TLC $R_f=0.52$ (CH_2Cl_2/MeOH = 10:1); ^1H NMR spectral data ($CDCl_3$) δ 7.65 (*d*, 1H, $J=15$ Hz), 7.54 (*d*, 2H, $J=8$ Hz), 7.12 (*d*, 2H, $J=8$ Hz), 6.46 (*d*, 1 H, $J=15$ Hz), 3.64 (*m*, 6H), 3.59 (*m*, 4H), 3.34 (2H), 2.34 (*s*, 3H, OCH_3), 1.46 (*s*, 9H, *t*-Bu); ^{13}C NMR spectral data ($CDCl_3$) 166.32, 159.70, 139.57, 130.02, 126.71, 119.42, 116.57, 70.33, 70.03, 29.07; FAB-MS *m/z* 459 ($[M+Na]^+$, 19), 437 $[M+1]^+$, 13), 339 (6), 337 (62), 245 (22), 189(46), 147 (100).

Deprotection of **32a** (2.00 g, 4.60 mmol) in 40 ml MeOH using dry HCl (2.15 g) gave, after evaporation of the MeOH, a crude product, that was washed with Et_2O to afford 880 mg (65%) of **32b** as a yellow viscous oil. TLC $R_f=0.20$ (CH_2Cl_2/MeOH/NH_4OH = 10:4:0.5); ^1H NMR ($CDCl_3$) δ 7.37 (*d*, 2 arom H, $J=8.36$), 7.31 (*d*, 1 olef H, $J=16$), 6.81 (*d*, 2 arom H, $J=8.40$), 6.52 (*d*, 1 olef H, $J=16$), 3.66 (*t*, 2H), 3.53 (*m*, 4 H), 3.45 (*t*, 2H), 3.30 (*t*, 2H), 2.93 (*t*, 2H); ^{13}C NMR ($CDCl_3$) 171.2, 164.5, 144.3, 134.7, 131.4, 124.2, 121.4, 75.3, 75.1, 74.8, 73.4, 72.2; FAB-MS *m/z* 589 ($[2M+1]^+$, 5), 295 $[M+1]^+$, 96), 178 (14), 149 (48), 147 (7), 89 (16), 44 (53). HRMS (FAB, NBA). Calculated mass: 295.1658 for $C_{15}H_{23}N_2O_4$, found: 295.1612.

*5.14. 2(E)-N{3-[4-(3-aminopropoxy)butoxy]propyl}-3-(4-hydroxyphenyl)prop-2-enamide (**33b**)*

*5.14.1. Synthesis of N-{3-[4-(3-aminopropoxy)-butoxy]propyl})-2,2,2-trifluoroacetamide (**10a**)*

Following the general procedure12.0 g (58.8 mmol, 12.5 ml) of 4,9-dioxa-1,12-dodecanediamine, **8**, was mono-trifluoroacetylated to give a crude product that was purified on silica gel (MeOH/CH_2Cl_2/

AcOH = 10:20:1, followed by 20:20:1) to afford **10a** as a yellow viscous oil (14.6 g, 82%). TLC $R_f = 0.60$ (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); ^1H NMR spectral data ($CDCl_3$) δ 8.20 (*br s*, $NHCOCF_3$), 3.61–3.51 (*m*, 4H), 3.40 (*t*, 4H), 3.06 (*m*, 2H), 1.94 (*m*, 6H), 1.85 (*m*, 2H), 1.61 (4H); ^{13}C NMR spectral data ($CDCl_3$) 157.5 (*q*, $J = 36$ Hz), 116.4 (*q*, $J = 286$ Hz), 71.5, 70.2, 69.1, 39.3, 38.6, 28.5, 27.7, 26.7, 23.8; FAB-MS *m/z* 301 ($[M+1]^+$, 100), 205 (18), 154 (68), 126 (13), 58 (26), 30 (18).

5.14.2. Synthesis of tert-butyl 3-(4-{3-[(trifluoroacetyl)amino]propoxy} butoxy)propylcarbamate (10b)

A solution of $(Boc)_2O$ (1 eq., 9.13 g) in THF (35 ml) was added to **10a** (12.6 g, 41.8 mmol) in THF (45 ml) at RT and the reaction mixture stirred overnight. Evaporation of the solvent afforded **10b** as a pale yellow oil (16.3 g, 97%) which was used for the next step of the synthesis without purification. TLC $R_f = 0.80$ (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); ^1H NMR spectral data ($CDCl_3$) δ 7.54 (*br s*, 1H), 4.48 (*br s*, 1H), 3.61–3.57 (*m*, 2H), 3.49–3.45 (*m*, 10H), 3.2 (*t*, 2H), 1.88–1.81 (*m*, 4H), 1.72 (2H), 1.43 (*s*, 9H, *t*-Bu); ^{13}C NMR spectral data ($CDCl_3$) 176.0, 157.3 (*q*, 36 Hz), 116.4 (*q*, $J = 286$ Hz), 71.6, 70.7, 69.8, 68.7, 39.8, 31.5, 30.1, 28.8, 28.3, 26.8, 25.9, 21.1; FAB-MS *m/z* 423 ($[M+Na]^+$, 17), 401 ($[M+1]^+$, 10), 345 (12), 301 (100), 263 (6), 249 (21), 126 (11), 102 (64), 74 (18), 57 (83).

5.14.3. Synthesis of tert-butyl 3-[4-(3-aminopropoxy)-butoxy]propyl carbamate (10c)

To a solution of 16.2 g (40.5 mmol) of **10b** in MeOH (100 ml) was added 40 ml of concentrated NH_4OH. After heating to reflux for 20 h and evaporation to dryness, the residue was purified on silica gel (CH_2Cl_2/MeOH = 20:1, then 10:1) to give **10c** as a yellow oil (12.2 g, 99%). TLC $R_f = 0.70$ (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); ^1H NMR spectral data ($CDCl_3$) δ 5.01 (*br s*, 1H), 3.59 (*t*, 2H), 3.49–3.42 (*m*, 6H), 3.20 (*t*, 2H), 3.12 (*t*, 2H), 1.96 (*m*, 2H), 1.92 (*m*, 2H), 1.76 (*t*, 2H), 1.62 (*m*, 4H), 1.43 (*s*, 9H, *t*-Bu); ^{13}C NMR spectral data ($CDCl_3$) 176.6, 71.5, 71.1, 70.9, 69.7, 69.3, 39.4, 38.9, 30.1 28.8, 27.4, 26.8, 26.7; FAB-MS *m/z* 327 ($[M+Na]^+$, 14), 305 ($[M+1]^+$, 100), 249 (23), 231 (6), 205 (16), 146 (10), 130 (6), 102 (30), 74 (20), 58 (84), 57 (67).

5.14.4. Synthesis of 4-[(1E)-20,20-dimethyl-3,18-dioxo-8,13,19-trioxa-4,17-diazahenicos-1-en-yl]phenyl acetate (33a)

Acylation of **10c** (10,0 g, 32.9 mmol) with 28 following the general procedure gave **33a** as a yellow oil (10.0 g, 62%). TLC $R_f = 0.52$ (CH_2Cl_2/MeOH = 10:1); ^1H NMR spectral data ($CDCl_3$) δ 7.56 (*d*, 1H, $J = 16$ Hz), 7.46 (*d*, 2H, $J = 8$ Hz), 7.05 (*d*, 2H, $J = 16$ Hz), 6.38 (*d*, 1H, $J = 8$ Hz), 5.03 *br*, 1H), 3.51–3.47 (*t*, 2H,), 3.45–3.38 (*m*, 8H), 3.17 (*m*, 2H), 2.26 (*s*, 3H, OCH_3), 1.81 (*m*, 2H), 1.78 (*m*, 2H), 1.64 (*m*, 4H), 1.40 (*s*, 9H, *t*-Bu); ^{13}C NMR spectral data ($CDCl_3$) 169.6, 166.3, 156.5, 151.8, 139.7,133.1, 129.6, 129.1, 122.5, 121.7, 79.3, 71.1, 71.0, 69.9, 69.5, 39.1, 38.6, 30.0, 29.5, 28.8, 26.9, 21.5; FAB-MS *m/z* 493 ($[M+1]^+$, 14), 393 (64), 305 (15), 249 (35), 189 (20), 147 (55), 102 (68), 74 (25), 57 (100).

5.14.5. Synthesis of (2E)-N-{3-[4-(3-aminopropoxy)-butoxy]propyl}-3-(4-hydroxyphenyl)prop-2-enamide (33b)

Deprotection of **33a** (8.30 g, 16.9 mmol) using dry HCl (2.15 g) in MeOH at 0 °C gave a crude product after solvent removal that was dissolved in 1N HCl (80 ml) and extracted with CH_2Cl_2. Evaporation of the aqueous phase gave **33b** as a bright yellow oil (5.38 g, 91%). TLC $R_f = 0.57$ (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); HPLC Rt = 10.8 min; ^1H NMR spectral data (d_6-DMSO) δ 7.38 (*d*, 2H, $J = 8$ Hz), 7.31 (*d*, 1H, $J = 16$ Hz), 6.81 (*d*, 2H, $J = 8$ Hz), 6.46 (*d*, 1H, $J = 16$ Hz), 3.42 (*m*, 10H), 3.18 (*t*, 2H), 2.83–2.78 (*m*, 2H), 1.83 (*m*, 2H), 1.70 (*q*, 2H), 1.51 (*m*, 4H); ^{13}C NMR spectral data (d_6-DMSO) 166.3, 159.7, 139.4, 129.9, 126.7, 119.6, 70.7, 68.5, 67.7, 37.4, 36.8, 30.3, 28.1, 26.8, 23.4; FAB-MS *m/z* 351 ($[M+1]^+$, 100), 247 (41), 205 (51), 147 (64), 130 (7), 74 (12), 58 (79), 30 (60). HRMS (FAB, NBA). Calculated mass: 351.2284 for $C_{19}H_{31}N_2O_4$, found: 351.2278.

5.15. Synthesis of (2E)-N-{2-[(2-aminoethyl)amino]-ethyl}-3-(4-hydroxyphenyl)prop-2-enamide (34b)

5.15.1. Synthesis of tert-butyl 2-[(2-aminoethyl)-amino]ethylcarbamate (14)

Mono-protection of 10.0 g (97.1 mmol, 10.5 ml) of diethylenetriamine **11** with $(Boc)O_2$ (0.13 eq., 2.83 g, 12.9 mmol) following the general procedure gave **14** as a colorless viscous oil (2.30 g, 89%). TLC $R_f = 0.83$ (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); ^1H NMR spectral data ($CDCl_3$) δ 4.48 (2H), 3.35 (2H), 3.28 (2H), 2.18 (2H), 1.43 (9H); ^{13}C-NMR spectral data ($CDCl_3$) 156.6, 79.5, 70.4, 53.2, 49.8, 39.7, 28.8; FAB-MS *m/z* 204 ($[M+1]^+$, 76), 148 (54), 131 (16), 104 (40), 87 (34), 73 (35), 57 (100).

5.15.2. Synthesis of 4-[(1E)-13,13-dimethyl-3,11-dioxo-12-oxa-4,7,10-triazatetradec-1-en-1-yl]-3-(4-hydroxyphenyl acetate (34a)

Acylation of **14** (1.00 g, 4.93 mmol) following the general procedure gave 1.31 g of a fluffy yellow crude product which was suspended in water (50 ml) and extracted with CH_2Cl_2. The organic phase was then dried with Na_2SO_4. Purification on silica gel (CH_2Cl_2/MeOH = 20:1) gave **34a** (0.32 g, 0.82 mmol, 16%) and a diacylated product 34c (0.86 g, 1.48 mmol, 30%).

Compound **34a** TLC $R_f = 0.45$ (CH_2Cl_2/MeOH = 10:1); ^1H NMR spectral data ($CDCl_3$) δ 7.30 (*d*, 2H, $J = 8$ Hz), 7.10 (*d*, 1H, $J = 16$ Hz), 6.80 (*d*, 2H, $J = 8$ Hz), 6.48 (*d*, 1 olef H, $J = 15.6$), 3.16 (2H), 2.97 (2H), 2.73 (4H), 2.31 (s, 3H, OCH_3), 1.43 (*s*, 9H, *t*-Bu); ^{13}C NMR spectral data ($CDCl_3$) 169.5, 166.8, 158.3, 151.7, 139.8, 133.4, 129.7, 129.0, 127.1, 121.9, 79.3, 70.4,

56.8, 49.9, 39.5, 36.8; FAB-MS *m/z* 392 ($[M+1]^+$, 45), 335 (20), 318 (83), 189 (15), 147 (45), 57 (100).

Compound **34c** TLC $R_f = 0.66$ (CH_2Cl_2/MeOH = 10:1); FAB-MS *m/z* 580 ($[M+1]^+$, 2), 480 (10), 438 (6), 189 (33), 147 (100), 57 (57).

Deprotection of **34a** (300 mg, 0.77 mmol) in 30 ml MeOH was accomplished by adding 20 drops of concentrated HCl and stirring at 35 °C for 1.5 h. After evaporation of solvent followed by chromatography (CH_2Cl_2/MeOH/NH_4OH = 10:4:0.5), **34b** was isolated as a yellow solid (140 mg, 73%).

Similarly, 0.59 g of **34c** (1.02 mmol) suspended in MeOH (20 ml) was reacted with 20 drops of concentrated HCl. The reaction mixture was stirred at 35 °C for 1.5 h. After evaporation of solvent followed by column chromatography (CH_2Cl_2/MeOH/NH_4OH = 10:4:0.5), **34d** was isolated as a bright yellow solid (200 mg, 50%).

Compound **34b** TLC $R_f = 0.44$ (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); ^1H NMR (d_6-DMSO) δ 7.30 (*d*, 2H, *J* = 8 Hz), 7.10 (*d*, 1H, *J* = 16 Hz), 6.80 (*d*, 2H, *J* = 8 Hz), 6.48 (*d*, 1H, *J* = 16 Hz), 3.81 (*br s*, 1H), 3.60 (1H), 3.41–3.39 (*m*, 4H), 3.07 (2H); ^{13}C NMR (d_6-DMSO) 166.7, 159.9, 139.8, 130.0, 126.5, 119.5, 116.4, 55.8, 47.6, 45.3, 36.9; FAB-MS *m/z* 499 ($[2M+1]^+$, 5%), 250 ($[M+1]^+$, 100), 233 (13), 219 (5), 190 (22), 176 (10), 147 (94). HRMS (FAB, NBA). Calculated mass: 250.1556 for $C_{13}H_{20}N_3O_4$, found: 250.1517.

Compound **34d** TLC $R_f = 0.76$ (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); FAB-MS *m/z* 396 ($[M+1]^+$, 58), 379 (6), 250 (40), 190 (21), 147 (100).

*5.16. 2(E)-N-{3-[(3-aminopropyl)amino]propyl}-3-(4-hydroxyphenyl)prop-2-enamide (**35b**)*

*5.16.1. Synthesis of tert-butyl 3-[(3-aminopropyl)-amino]propyl carbamate (**15**)*

Mono-protection of 10.0 g (76.3 mmol, 10.7 ml) of 3,3′-iminobispropylamine **12** with $(Boc)_2O$ (0.13 eq., 2.22 g, 10.2 mmol) following the general procedure gave a crude product which, after removal of the dioxane, was dissolved in water (60 ml) and extracted with CH_2Cl_2. The organic phase was dried with Na_2SO_4 and concentrated. The crude product was purified on silica gel (CH_2Cl_2/MeOH/NH_4OH = 10:4:1). Compound **15** was obtained as a clear yellow oil (1.48 g, 63%). TLC $R_f = 0.26$ (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); ^1H NMR spectral data ($CDCl_3$) δ 5.20 (*br s*, 1H), 3.21 (*m*, 2H), 2.78 (*t*, 2H), 2.67–2.62 (*m*, 4H), 1.69–1.59 (*m*, 4H), 1.43 (*s*, 9H, *t*-Bu); ^{13}C NMR spectral data ($CDCl_3$) 156.54, 79.86, 53.81, 48.10, 40.72, 39.52, 33.99, 30.26, 28.81; FAB-MS *m/z* 232 ($[M+1]^+$, 100), 186 (5), 132 (36), 102 (11), 87 (8), 74 (9), 57(80), 44 (48).

*5.16.2. Synthesis of 4-[(1E)-15,15-dimethyl-3,13-dioxo-14-oxa-4,8,12-triazahexadec-1-en-1yl]phenyl acetate (**35a**)*

Acylation of **15** with (*E*)-3-(4-acetoxyphenyl) chloride **28** (1.10 g, 4.72 mmol) following the general procedure gave 480 mg of a fluffy yellow crude product which was suspended in water (50 ml) and extracted with CH_2Cl_2. The organic phase was then dried with Na_2SO_4 and evaporated.

Purification on silica gel gave the desired monoacylated product **35a** (0.22 g, 0.52 mmol, 11%) and a diacylated product **35c** (0.23 g, 0.38 mmol, 8%).

Compound **35a** TLC $R_f = 0.60$ (CH_2Cl_2/MeOH = 10:1); ^1H NMR spectral data ($CDCl_3$) δ 7.37 (*d*, 2H, *J* = 8 Hz), 7.30 (*d*, 1H, *J* = 16 Hz), 6.98 (*d*, 2H, *J* = 8 Hz), 6.38 (*d*, 1H, *J* = 116 Hz), 3.16 (*m*, 2H), 2.99–2.95 (*m*, 4H), 2.59 (*m*, 2H), 1.89 (*s*, 3H, OCH_3), 1.56 (2H), 1.50 (2H), 1.43 (*s*, 9H, *t*-Bu); ^{13}C NMR spectral data ($CDCl_3$) 169.5, 164.8, 153.3, 147.9, 130.9, 130.2, 129.2, 122.4, 121.5, 117.2, 79.1, 53,9, 48.6, 41.7, 36.7, 34.0, 30.9, 28.6; FAB-MS *m/z* 420 ($[M+1]^+$, 8), 320 (15), 216 (24), 147 (100), 129 (18), 100 (33), 57 (96).

Deprotection of **35a** (200 mg, 0.48 mmol) was accomplished with dry HCl (2.15 g) in MeOH at 0 °C and gave **35b** (105 mg, 79%) after recrystallization from absolute EtOH. TLC $R_f = 0.39$ (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); ^1H NMR (DMSO) δ 7.37 (*d*, 2H, *J* = 8 hz), 7.30 (*d*, 1H, *J* = 16 Hz), 6.98 (*d*, 2H, *J* = 8 Hz), 6.38 (*d*, 1H, *J* = 16 Hz), 3.10 (*m*, 2H), 2.99–2.85 (*m*, 4H), 2.52 (*m*, 2H), 1.56 (2H), 1.49 (2H); ^{13}C NMR (d_6-DMSO) 165.2, 159.7, 139.3, 129.9, 126.8, 119.7, 116.6, 15.6, 47.2, 40.8, 56.0, 33.8, 24.9; FAB-MS *m/z* 278 $[M+1]^+$, 65), 260 (15), 190 (21), 162 (45), 176 (25), 147 (80), 57 (100). HRMS (FAB, NBA). Calculated mass: 278.1869 for $C_{15}H_{24}N_3O_2$, found: 278.1829.

*5.17. 2(E)-N-{3-[4-aminocyclohexyl)amino]propyl}-3-(4-hydroxyphenyl)prop-2-enamide (**36b**)*

*5.17.1. Synthesis of tert-butyl 4-aminocyclohexyl-carbamate (**16**)*

Monoprotection of 30.0 g (263 mmol) of 1,4-diaminocyclohexane **13** with 0.125 eq. of $(Boc)_2O$ (32.8 mmol, 7.17 g) following the general procedure gave, after removal of the reaction solvent, a residue that was partitioned between water and dichloromethane. The mono-Boc and di-Boc derivatives were extracted into the organic phase leaving the unreacted starting material in the aqueous phase. Separation of the mixture on silica gel with CH_2Cl_2/MeOH/NH_4OH = 10:4:1 gave 5.18 g (74%) of 16. TLC $R_f = 0.58$ (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); ^1H NMR spectral data ($CDCl_3$) δ 4.39 (*br s*, 1 H), 3.44–3.36 (*m*, 1H), 2.62–2.59 (*m*, 1H), 2.17 (2H), 1.99 (2H), 1.47 (2H), 1.43 (9H), 1.25–1.06 (4H); ^{13}C NMR spectral data ($CDCl_3$) 155.6 (C=O), 79.5 (C–O), 50.3, 49.6, 35.7, 32.5, 28.8; FAB-MS *m/z* 429 ([2M + 1]+, 11), 215($[M+1]^+$, 61), 159 (45), 142 (100), 96 (26), 81 (36), 57 (65).

*5.17.2. Synthesis of tert-butyl 4-[(2-cyanoethyl)-amino]cyclohexylcarbamate (**17**)*

16 5.00 g, 23.4 mmol) was dissolved in MeOH (60 ml) and 1 eq. of acrylonitrile (1.24 g, 1.54 ml) in MeOH

(40 ml) was added slowly over 1 h and stirred for 6 h. Evaporation of the solvent afforded 5.84 g (94%) of crude **17**. TLC $R_f = 0.63$ (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); ^1H NMR spectral data ($CDCl_3$) δ 4.38 (*br s*, 1H), 3.48 (1H), 3.42 (1H), 2.96 (*t*, 2H), 2.50 (*t*, 2H), 2.04 (2H), 1.95–1.92 (2H), 1.45 (*s*, 9H, *t*-Bu), 1.2–1.07 (*m*, 5H); ^{13}C NMR spectral data ($CDCl_3$) 155.6 (C=O), 119.1 (CN), 79.6 (C–O), 55.8, 49.8, 42.7, 32.4, 32.3, 28.8, 19.6; FAB-MS *m*/*z* 535 ($[2M+1]^+$, 5), 268 ($[M+1]^+$, 48), 212 (35), 171 (17), 142 (82), 96 (22), 81 (44), 57 (100).

5.17.3. Synthesis of tert-butyl 4-[(3-aminopropyl) amino]cyclohexy l carbamate (18)

17 (5.00 g, 18.7 mmol) was dissolved in 150 ml of 95% EtOH/1N NaOH. One gram of Raney-Ni catalyst was added and the mixture subjected to hydrogenation at 60 psi for 24 h. After filtering through Celite® 521, the reaction mixture was concentrated to a volume of about 40 ml. After a few hours an oil separated on top which was removed. Purification on silica gel (5:2 = CH_2Cl_2/MeOH) gave **18** (3.90 g, 77%) as a white fluffy solid. TLC $R_f = 0.39$ (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); ^1H NMR spectral data ($CDCl_3$) δ 4.44 (*br s*, 1H), 3.45 (1H), 2.78 (*t*, 2H), 2.68 (*t*, 2H), 2.30 (1H), 2.15 (2H), 2.10–1.96 (5H), 1.65–1.60 (*m*, 2H), 1.43 (*s*, 9H, *t*-Bu), 1.19–1.13 (*m*, 4H); ^{13}C NMR spectral data ($CDCl_3$) 155.7 (C=O), 79.5 (C–O), 56.5, 49.9, 45.4, 40.8, 34.1, 32.4, 32.3, 28.8; FAB-MS *m*/*z* 272 ($[M+1]^+$, 100), 216 (30), 171(7), 142 (25), 96 (14), 81 (30), 57 (58).

5.17.4. Synthesis of 4-((1E)-3-{[3-([4-[(tert-butoxy-carbonyl)amino] cyclohexyl}amino)propyl]amino}-3-oxoprop-1-enyl)phenyl acetate (36a)

(*E*)-3-4-(4-Acetoxyphenyl) prop-2-enoic acid (1.14 g, 5.54 mmol) of was suspended in CH_2Cl_2 (50 ml) to which was added 1.10 eq. (1.26 g) of DCC in CH_2Cl_2 and 0.25 eq. of 1-hydroxybenzotriazole. The solution was then added to a mixture of 1.5 g (5.53 mmol) of **18** in CH_2Cl_2 (80 ml) and the reaction mixture refluxed for 36 h. Evaporation of the solvent gave a yellow fluffy crude product which was purified on silica gel (CH_2Cl_2/MeOH = 10:1) to give 1.60 g (63%) of **36a**. TLC $R_f = 0.1$ (CH_2Cl_2/MeOH = 10:1); ^1H NMR spectral data (MeOH) δ 7.51 (*d*, 1H, $J = 16$ Hz), 7.43 (*d*, 2H, $J = 8$ Hz), 6.82 (*d*, 2H, $J = 8$ Hz), 6.45 (*d*, 1H, $J = 16$ Hz), 3.72 (*t*, 1H), 2.92–2.85 (*m*, 4H), 2.07 (2H), 2.03 (*s*, 3H, OCH_3), 1.96 (1H), 1.92–1.87 (*m*, 4H), 1.43 (*s*, 9H, *t*-Bu), 1.35–1.24 (*m*, 6H); ^{13}C NMR spectral data (*d*-MeOH) 168.9, 159.9, 156.8, 141.3, 129.7, 126.4, 124.2, 117.7, 116.8, 115.9, 111.5, 79.0, 67.9, 56.3, 51.1, 43.1, 36.5, 31.0, 29.3, 27.9, 25.5; FAB-MS *m*/*z* 460 ($[M+1]^+$, 100), 404 (18), 356 (12), 147 (49), 96 (17), 57 (65).

Deprotection of **36a** (1.50 g, 3.27 mmol) was accomplished in MeOH (50 ml) containing 20 drops of conc. HCl. After stirring at room temp. for 5 h, evaporation of the solvent, followed by recrystallization from absolute EtOH gave 0.75 g (77%) of **36b**. TLC $R_f = 0.10$ (CH_2Cl_2/MeOH = 10:1); ^1H NMR (d_6-DMSO) δ 7.51 (*d*, 1H, $J = 16$ Hz), 7.42 (*d*, 2H, $J = 9$ Hz), 6.78 (*d*, 2H, $J = 9$ Hz), 6.45 (*d*, 1H, $J = 16$ Hz), 3.62 (*t*, 2H), 2.96 (*m*, 4H), 2.70–2.64 (2H), 1.60 (*m*, 4H), 1.30–1.26 (*m*, 4H); ^{13}C NMR (d_6-DMSO) 166.7, 159.8, 139.7, 130.0, 126.5, 119.8, 116.6, 49.1, 49.0, 36.7, 28.8, 27.1 FAB-MS *m*/*z* 318 ($[M+1]^+$, 48), 214 (59), 176 (10), 160 (22), 147 (17), 142 (9), 136 (100), 113 (6), 81 (22). HRMS (FAB, NBA). Calculated mass: 318.2182 for $C_{18}H_{28}N_3O_2$, found: 318.2188.

5.18. 2(E)-N-{3-[4-(3-aminopropyl)piperazin-1-yl}-propyl}-3-(4-hydroxyphenyl)prop-2-enamide (37b)

5.18.1. Synthesis of tert-butyl 3-[4-(3-aminopropyl) piperazin-1-yl]propyl carbamate (22)

Mono-protection of 12.0 g of 1,4-bis (3-aminopropyl) piperazine **19** (60.0 mmol, 12.3 ml) with $(Boc)_2O$ (1.75 g, 8 mmol) following the general conditions provided a residue after evaporation that was suspended in water (80 ml) and extracted with CH_2Cl_2. Removal of the solvent provided **21** as a colorless oil (2.13 g, 89%) which formed needle-like crystals on standing. TLC $R_f = 0.33$ (CH_2Cl_2/MeOH/NH_4OH 30% = 10:4:1); ^1H NMR spectral data ($CDCl_3$) δ 5.52 (*br s*, 1H, NHBoc), 3.17 (*q*, 2H), 2.76 (*q*, 2H), 2.44–2.35 (*m*, 10H), 1.67–1.57 (*m*, 6H), 1.42 (*s*, 9H, *t*-Bu); ^{13}C NMR spectral data ($CDCl_3$) 156.5, 67.5, 57.2, 56.9, 53.7, 53.6, 41.3, 40.4, 30.8, 28.8, 26.7; FAB-MS *m*/*z* 301 ($[M+1]^+$, 92), 270 (10), 256 (14), 242 (11), 201 (68), 127 (20), 97 (39), 70 (42), 57 (100).

5.18.2. Synthesis of 4-(1E)-3-{[3-(4-{3-[tert-butoxy-carbonyl)amino]propyl} piperazin-1-yl)propyl]amino}-3-oxoprop-1-enyl)phenyl acetate (37a)

Acylation of **21** (1.80 g, 6 mmol) with **28** following the general procedure gave a crude product that was purified by chromatography on silica gel (CH_2Cl_2/MeOH/NH_4OH = 10:4:0.5) and yielded **37a** as a yellow spongy solid (1.36 g, 52%). TLC $R_f = 0.25$ (CH_2Cl_2/MeOH = 10:1); ^1H NMR spectral data ($CDCl_3$) δ 7.57 (*d*, 1H, $J = 16$ Hz), 7.49 (*d*, 2H, $J = 8$ Hz), 7.32 (*d*, 2H, $J = 8$ Hz), 6.35 (*d*, 1H, $J = 16$ Hz), 5.43 (*br s*, 1H), 3.48 (*m*, 2H), 3.16 (*m*, 2H), 2.51–2.47 (*m*, 8H), 2.43–2.38 (*m*, 4H), 2.29 (*s*, 3H, OCH_3), 1.74 (*t*, 2H), 1.68 (*m*, 2H), 1.43 (*s*, 9H, *t*-Bu); ^{13}C NMR spectral data ($CDCl_3$) 169.7, 166.0, 156.5, 151.5, 139.5, 133.2, 129.7, 129.1, 122.4, 121.9, 116.5, 79.2, 57.9, 57.2, 53.8, 46.3, 40.2, 28.8, 26.7, 25.4, 21.5; FAB-MS *m*/*z* 489($[M+1]^+$, 28), 389(10), 256 (11), 201 (17), 147 (42), 102 (18), 74 (14), 57 (100).

Deprotection of **37a** (1.36 g, 2.79 mmol) was accomplished in MeOH (30 ml) by treating with 20 drops of concentrated HCl. After 1.5 h the reaction was evaporated to dryness, the residue was dissolved in absolute ethanol and filtered. The ethanolic solution was evaporated

giving, **37b** (687 mg, 63%) as a pale yellow solid. TLC R_f = 0.20 (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); ^1H NMR (d_6-DMSO) δ 7.40 (*d*, 2H, *J* = 8 Hz), 7.36 (*d*, 1H, *J* = 16 Hz), 6.81 (*d*, 2H, *J* = 8 Hz), 6.44 (*d*, 1H, *J* = 16 Hz), 3.73 (*m*, 8H), 3.45 (*m*, 2H), 3.23–3.09 (4H), 2.91 (2H), 2.02 (*m*, 4H); ^{13}C NMR (d_6-DMSO) 166.1, 159.3, 139.2, 129.5, 126.1, 118.7, 116.1, 55.0, 41.4, 36.5, 26.5, 24.1 FAB-MS *m*/*z* 369 ($[M+Na]^+$, 5), 347 ($[M+1]^+$, 100), 204 (17), 176 (8), 147 (49), 144 (6), 127 (14), 113 (16), 86 (13). HRMS (FAB, NBA). Calculated mass: 347.2447 for $C_{19}H_{231}N_4O_2$, found: 347.2409.

5.19. 2(*E*)-*N*-[3-({2-[(3-aminopropyl)amino]-cyclohexyl}amino)propyl]-3-(4-hydroxyphenyl)prop-2-enamide (**40b**)

5.19.1. Synthesis of *N*,*N*′-bis(2-cyanoethyl)cyclohexane-1,2-diamine (**22**)

trans-1,2-Diaminocyclohexane **20** (10.0 g (87.6 mmol, 6.72 ml) was dissolved in MeOH (65 ml) and 2.50 eq. of acrylonitrile (14.4 ml, 11.6 g) in 50 ml of MeOH was added drop wise over 30 min. After stirring overnight at room temp. and evaporation of the solvent **22** was recovered as a yellow oil (19.1 g, 99%). TLC R_f = 0.88 (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); ^1H NMR spectral data ($CDCl_3$) δ 3.04–2.98 (*m*, 2H), 2.79–2.75 (*m*, 2H), 2.53–2.48 (*t*, 4H), 2.17–2.15 (*t*, 2H), 2.14 (2H), 1.96 (*br s*, 2H), 1.75–1.72 (*m*, 2H); ^{13}C NMR spectral data ($CDCl_3$) 119.3, 61.4, 42.8, 32.0, 25.2, 19.7; FAB-MS *m*/*z* 221 ($[M+1]^+$, 100), 194 (4), 180 (12), 151 (33), 109 (14), 81 (13)

5.19.2. Synthesis of *N*,*N*′-bis(3-aminopropyl)cyclohexane-1,2-diamine (**23**)

Compound **23** was obtained by reducing 19.0 g (86.4 mmol) of **22** with hydrogen at 50 psi using 95% EtOH/1N NaOH as solvent and Raney-Ni (3 g) as catalyst. The reduction was complete after 24 h. The mixture was filtered using Celite® 521 and concentrated by rotary evaporation to ~45 ml. A pale yellow viscous oil separated on top and this was removed. The oil was then purified by cc on silica gel (CH_2Cl_2/MeOH/NH_4OH = 10:4:0.5) to afford 14.7 g (75%) of **23**. TLC R_f = 0.25 (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); ^1H NMR spectral data ($CDCl_3$) δ 4.76 (*br s*, 1H), 2.99 (*br s*, 1H), 2.75–2.71 (*m*, 6H), 2.48–2.44 (*m*, 2H), 2.09–2.05 (*m*, 4H), 1.70 (2H), 1.67–1.59 (4H), 1.57 (1H), 1.20 (3H), 1.17 (2H); ^{13}C NMR spectral data ($CDCl_3$) 62.0, 44.9, 40.5, 34.5,31.9, 25.5; FAB-MS *m*/*z* 229 ($[M+1]^+$, 100), 155 (25), 110 (10), 81 (13), 58 (11).

5.19.3. Synthesis of *N*-[3-({2-[(3-aminopropyl)amino]-cyclohexyl}amino) propyl]-2,2,2- trifluoroacetamide (**24**)

Mono-trifluoracetulation of **23** (5.00 g, 21.9 mmol) following the general procedure gave a crude product that was purified on silica gel (CH_2Cl_2/MeOH/AcOH = 10:20:1). The yield of **24** was 3.25 g (46%). TLC R_f = 0.48 (CH_2Cl_2/MeOH/AcOH = 60:40:2); ^1H NMR spectral data ($CDCl_3$) δ 3.56–3.52 (*m*, 1H), 3.29–3.27 (*m*, 1H), 2.82–2.68 (*m*, 5H), 2.47–2.44 (*q*, 1H), 1.71–1.62 (*t*, 2H), 1.19 (2H), 0.93 (2H); ^{13}C NMR spectral data ($CDCl_3$) 157.4 (*q*, *J* = 36 Hz), 116.5 (*q*, *J* = 285 Hz), 62.3, 61.7, 45.8, 44.9, 40.7, 40.3, 34.1, 31.8, 31.7, 28.3, 25.5, 25.1; FAB-MS *m*/*z* 325 ($[M+1]^+$, 100), 251 (27), 155 (37), 81 (33), 56 (35).

5.19.4. Synthesis of triBoc-*N*-[3-({2-[(3-aminopropyl)-amino]cyclohexyl}amino)propyl]-2,2,2-trifluoroacetamide (**38**) and triBoc-*N*,*N*′-bis(3-aminopropyl)cyclohexane-1,2-diamine (**39**)

24 (3.10 g, 9.57 mmol) was suspended in THF (60 ml) and 3.0 eq. of $(Boc)_2O$ (28.7 mmol, 6.26 g) in THF (40 ml) was added at room temp.. When the formation of **38** was complete as judged by TLC, 15 ml of concentrated. NH_4OH was added followed by refluxing at 60 °C for 24 h to remove the TFA protecting group. After evaporation of the solvent the crude material was purified by cc to give 3.28 g (60%) of the triBoc amine **39**. TLC R_f = 0.70 (CH_2Cl_2/MeOH = 10:1); ^1H NMR spectral data ($CDCl_3$) δ 5.87 (*br s*, 1H), 4.90 (4H), 4.59 (2H), 4.03 (4H), 3.82 (1H), 3.03 (6H), 1.75–1.66 (7H), 1.45 (*s*, 27H); ESI-MS *m*/*z* 1057.8 ($[2M+1]^+$, 8), 529.4 ($[M+1]^+$, 100), 429.4 (80), 329.4 (35), 229.2 (15).

5.19.5. Synthesis of compound **40a**

Acylation of **39** (3.00 g, 5.68 mmol) following the general procedure afforded, after evaporation of the reaction solvent, a residue which was dissolved in distilled water and extracted with CH_2Cl_2. Evaporation of the organic phases gave **40a** as a yellow spongy voluminous solid (2.54 g, 69%). TLC R_f = 0.53 (CH_2Cl_2/MeOH = 10:1); ^1H NMR spectral data ($CDCl_3$) δ 7.70 (*d*, 1H, *J* = 16 Hz), 7.63 (*d*, 2H, *J* = 8 Hz), 7.13 (*d*, 2H, *J* = 8 Hz), 6.43 (*d*, 1H, *J* = 16 Hz), 3.33–3.17 (*m*, 6H), 2.31 (*s*, 3H, OCH_3), 1.77 (*m*, 6H), 1.45–1.43 (27H); FAB-MS *m*/*z* 717 ($[M+1]^+$, 2), 617 (5), 529 (65), 429 (18), 254 (13), 147 (13), 81 (11), 57 (100).

Deprotection of **40a** (3.0 g, 4.2 mmol) was accomplished with HCl (2.15 g) in 50 ml of dry MeOH at 0 °C. Evaporation of the solvent, followed by washing with diethyl ether and recrystallization from absolute EtOH gave 830 mg (53%) of **40b**. TLC R_f = 0.10 (CH_2Cl_2/MeOH = 10:1); ^1H NMR (d_6-DMSO) δ 7.40 (*d*, 2H, *J* = 8 Hz), 7.37 (*d*, 1H, *J* = 16 Hz), 6.82 (*d*, 2H, *J* = 8 Hz), 6.48 (*d*, 1H, *J* = 16 Hz), 3.26 (3H), 2.92 (4H), 2.11 (6H), 1.82–1.72 (4H), 1.21 (2H); ^{13}C NMR (d_6-DMSO) 165.9, 159.0, 139.0, 129.2, 125.8, 118.4, 115.8, 56.2, 48.6, 41.8, 36.2, 25.4, 23.7, 22.1 FAB-MS *m*/*z* 375 ($[M+1]^+$,40), 325 (92), 316 (42), 229 (100), 155 (68), 111 (25), 98 (42), 81 (60), 57 (62). HRMS (FAB, NBA). Calculated mass: 375.2760 for $C_{21}H_{35}N_4O_2$, found: 375.2780.

*5.20. 2(E)-N-[3-({4-[(3-aminopropyl)amino]cyclohexyl}amino) propyl]-3-(4-hydroxyphenyl)prop-2-enamide (**43b**)*

*5.20.1. Synthesis of N,N′-bis(2-cyanoethyl)cyclohexane-1,4-diamine (**25**)*

trans-1,4-Diaminocyclohexane **13** (10.0 g, 87.6 mmol, 10.6 ml) was dissolved in MeOH (65 ml) and 2.5 eq. of acrylonitrile (14.4 ml, 11.6 g) in MeOH (50 ml) was added drop wise over 30 min with continuous stirring. After stirring overnight at room temperature, evaporation of the solvent followed by purification by cc (silica, 2:1 CH_2Cl_2: MeOH) gave 9.1 g (99%) of **25** as off-white crystals. TLC $R_f = 0.87$ (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); ^1H NMR spectral data ($CDCl_3$) δ 2.98–2.93 (*m*, 4H), 2.53–2.49 (*m*, 6H), 1.97–1.95 (4H), 1.20–1.07 (*m*, 6H); ^{13}C NMR spectral data ($CDCl_3$) 119.1, 56.3, 42.8, 32.3, 19.6; FAB-MS *m*/*z* 441 ($[2M+1]^+$, 13), 221 ($[M+1]^+$, 84), 180 (12), 151 (100), 109 (21), 81 (34).

*5.20.2. Synthesis of N,N′-bis(3-aminopropyl)cyclohexane-1,4-diamine (**26**)*

Compound **26** was obtained by reducing 6.00 g (27.3 mmol) of **26** with hydrogen at 50 psi for 24 h using 95% EtOH/1 N NaOH as solvent and 2 g of Raney-Ni as catalyst. When complete the mixture was filtered through Celite® 521 and concentrated to a volume of about 35 ml. A pale, yellow viscous oil separated on top of the mixture and this was removed. The oil was then purified by cc on silica gel to afford 5.60 g (90%) of **27**. TLC $R_f = 0.20$ (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); ^1H NMR spectral data ($CDCl_3$) δ 3.16 (*br s*, 1H), 2.64–2.50 (*m*, 8H), 2.30 (*m*, 2H), 1.84–1.82 (*m*, 4H), 1.54–1.46 (*q*, 4H), 1.09–0.96 (*m*, 6H); ^{13}C NMR spectral data ($CDCl_3$) 56.2, 43.7, 39.1, 32.3,30.8, 17.2; FAB-MS *m*/*z* 229 ($[M+1]^+$, 100), 176 (19), 155 (22), 57 (28).

*5.20.3. Synthesis of **26a** primary amide nitrile of **26***

During reduction of **25** at larger scale (i.e. 19.0 g, 86.4 mmol) using 3.6 g Raney-Ni, TLC (CH_2Cl_2/MeOH/NH_4OH = 10:4:1) showed a spot that migrated with an $R_f = 0.36$, between the dinitrile starting material **25** ($R_f = 0.60$) and fully reduced amine product **26** ($R_f = 0.2$). Recovery of this material by silica chromatography eluting by with the same solvent as for TLC provided 1.50 g of a viscous oil that solidified on standing. ^1H NMR spectral data ($CDCl_3$) δ 7.02 (*bs*, 2H), 5.47 (*bs*, 2H) 2.95 (*m*, 2H), 2.53 (*m*, 4H), 2.25 (broad multiplet, 6H), 2.13 (*m*, 6H), 1.96 (*m*, 3H), 1.71 (*m*, 1H), 1.55 (*bs*, 4H), 0.75 (*m*, 4H). ^{13}C NMR spectral data (d_6-DMSO) 174.2, 120.5, 56.4, 55.9, 43.1, 42.4, 36.1, 31.8, 18.6, 18.2 FAB-MS *m*/*z* 239 ([M+1, 98], 225 (100), 180 (10), 169 (20), 167 (14), 151 (72), 149 (45), 110 (42).

*5.20.4. Synthesis of N-[3-({4-[(3-aminopropyl)amino]-cyclohexyl}amino)propyl]-2,2,2-trifluoroacetamide (**27**)*

Mono-trifluoracetylation of **26** (3.70 g, 16.2 mmol) following the general procedure gave a crude product which was purified on silica gel (CH_2Cl_2/MeOH/AcOH = 10:20:1) and provided 5.16 g, (98%) of 27. TLC $R_f = 0.48$ (CH_2Cl_2/MeOH/AcOH = 60:40:2); ^1H NMR spectral data ($CDCl_3$) δ 3.1–6–3.12 (*m*, 12H), 2.52 (*m*, 2H), 2.23 (2H), 1.79–(4H), 1.58 (1H), 1.45–1.39 (2H), 0.92 (4H); ^{13}C NMR spectral data ($CDCl_3$) 57.4, 57.2, 49.4, 45.4, 44.9, 34.7, 32.5, 29.4; FAB-MS *m*/*z* 325 ($[M+1]^+$, 8), 251 (22), 191 (13), 155 (21), 107 (29), 96 (21), 81 (50), 65 (100), 56 (42).

*5.20.5. Syntheses of compounds protected amines **41** and **42***

27 (5.00 g, 15.4 mmol) was dissolved in MeOH (80 ml) and 3 eq. of $(Boc)_2O$ (46.3 mmol, 10.1 g) in THF (40 ml) was added at room temp. The reaction was complete as judged by TLC after 3 h and provided crude **41** that was used without any further purification. In the same flask, 30 ml concentrated NH_4OH was added and the solution refluxed for 24 h to afford 6.20 g (76%) of triBoc protected amine, **42**. TLC $R_f = 0.70$ (CH_2Cl_2/MeOH/NH_4OH = 10:4:1); ^1H NMR spectral data ($CDCl_3$) δ 4.93 (*br s*, 1H), 3.35 (8H), 1.76 (4H), 1.65 (8H), 1.48 (13H), 1.45 (27H); FAB-MS *m*/*z* 529 ($[M+1]^+$, 61), 429 (100), 329 (3), 254 (11), 229 (8), 199 (15), 57 (68).

*5.20.6. Synthesis of **43a***

Acylation of **42** (3.00 g, 5.68 mmol) in CH_2Cl_2 (40 ml) following the general procedure provided, after evaporation of the reaction solvent, a residue which was dissolved in distilled water and extracted with CH_2Cl_2. The organic phase was washed with 0.25 M Na_2CO_3 and then dried with Na_2SO_4. Evaporation of the solvent gave **43a** as a yellow spongy solid (3.20 g, 87%). TLC $R_f = 0.53$ (CH_2Cl_2/MeOH = 10:1); ^1H NMR spectral data ($CDCl_3$) δ 7.60 (*d*, 1H, $J = 16$ Hz), 7.46 (*d*, 2H, $J = 8$ Hz), 7.15 (*d*, 2H, $J = 8$ Hz), 6.45 (*d*, 1H, $J = 16$ Hz), 3.40–3.20 (*m*, 6H), 2.35 (*s*, 3H, OCH_3), 1.75 (*m*, 6H), 1.45–1.43 (27H); FAB-MS *m*/*z* 717 ($[M+1]^+$, 2), 617 (6), 529 (29), 471 (8), 375 (8), 271 (12), 133 (23), 73 (73), 57 (100).

Deprotection of **43a** (3.0 g, 4.2 mmol) in dry MeOH (40 ml) was accomplished by bubbling HCl (2.15 g) through the solution at 0 °C and stirring for 2 h. After evaporation and recrystallization from EtOH, **43b** was obtained as a pale yellow solid (550 mg, 35%). TLC $R_f = 0.15$ (CH_2Cl_2/MeOH = 10:1); ^1H NMR (d_6-DMSO) δ 7.40 (*d*, 2H, $J = 9$ Hz), 7.36 (*d*, 1H, $J = 16$ Hz), 6.82 (*d*, 2H, $J = 9$ Hz), 6.47 (*d*, 1H, $J = 16$ Hz), 4.10 (2H), 2.89 (*m*, 10H), 2.17 (4H), 1.99 (3H), 1.81 (1H), 1.46 (4H); ^{13}C NMR (d_6-DMSO) 165.3, 158.8, 138.4, 129.1, 125.9, 118.8, 115.7, 55.5, 55.1, 52.8, 52.7, 37.1, 26.5, 25.7 FAB-MS *m*/*z* 375

([M + 1]+, 57), 302 (13), 271 (100), 229 (54), 155 (67), 147 (35), 57 (72). HRMS (FAB, NBA). Calculated mass: 375.2760 for $C_{21}H_{35}N_4O_2$, found: 375.2767.

Acknowledgements

This work was funded by an NSERC Strategic Grant (to JKA and AJM), NSERC Operating Grants to JKA and AJM and National Institutes of Health grant NS35047 from NINDS to KW. Tannis Mayert performed the antifeedant trials with *Sitophilus oryzae* at the Cereal Research Centre, Agriculture and Agri-Food Canada, Winnipeg, Manitoba.

References

Abe, T., Kawai, N., Miwa, A., 1983. Effects of a spider toxin on the glutaminergic synapse of lobster muscle. J. Physiol. 339, 243–252.

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

Aonuma, H., Nagayama, T., Takahata, M., 1998. L-Glutamate as an excitatory transmitter of motor giant neurons in the crayfish *Procambarus clarkii*. J. Crustacean Biol. 18, 243–252.

Atwood, H.L., 1982. Synapses and neurotransmitters. In: Atwood, H.L., Sandeman, D.C. (Eds.), The Biology of Crustacea. Academic Press, New York, pp. 105–150.

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

Benson, J.A., Schurmann, F., Kaufmann, L., Gsell, L., Piek, T., 1992. Inhibition of dipteran larval neuromuscular synaptic transmission by analogues of philanthotoxin-4.3.3: a structure–activity study. Comp. Biochem. Physiol. C 102, 267–272.

Blagbrough, I.S., Brackley, P.T.H., Bruce, M., Bycroft, B.W., Mather, A.J., Millington, S., Sudan, H.L., Usherwood, P.N.R., 1992. Arthropod toxins as leads for novel insecticides. Toxicon 30, 303–322.

Blagbrough, I.S., Geall, A.J., 1998. Practical synthesis of unsymmetrical polyamine amides. Tetrahedron Lett. 39, 439–442.

Blagbrough, I.S., Moya, E., Taylor, S., 1994. Polyamines and polyamine amides from wasps and spiders. Biochem. Soc. Trans. 22, 888–893.

Blagbrough, I.S., Usherwood, P.N.R., 1990. Mode of action of polyamine-derived glutamate antagonists. Pest. Sci. 30, 451–453.

Blagbrough, I.S., Usherwood, P.N.R., 1992. Polyamine amide toxins as pharmacological tools and pharmaceutical agents. Proc. R. Soc. Edinburgh 99B, 67–81.

Bokern, M., Witte, L., Wray, V., Nimtz, M., Meurer-Grimes, B., 1995. Trisubstituted hydroxycinnamic acid spermidines from *Quercus dentat* pollen. Phytochemistry 39, 1371–1375.

Bruce, M., Bukownik, R., Eldefrawi, A.T., Eldefrawi, M.E., Goodnow Jr., R., Kallimopoulos, T., Konno, K., Nakanishi, K., Niwa, M., Usherwood, P.N., 1990. Structure–activity relationships of analogues of the wasp toxin philanthotoxin: non-competitive antagonists of quisqualate receptors. Toxicon 28, 1333–3346.

Chao, J., Seiler, N., Renault, J., Kashiwagi, K., Masuko, T., Igarashi, K., Williams, K., 1997. N1-dansyl-spermine and N1-(n-octanesulfonyl)-spermine, novel glutamate receptor antagonists: block and permeation of *N*-methyl-D-aspartate receptors. Mol. Pharmacol. 51, 861–871.

Converso, D.A., Fernández, M.E., 1995. Peroxidase isozymes from wheat germ: purification and properties. Phytochemistry 40, 1341–1345.

Cu, C.H., Bahring, R., Mayer, M.L., 1998. The role of hydrophobic interactions in binding of polyamines to non NMDA receptor ion channels. Neuropharmacology 37, 1381–1391.

Dingledine, R., Borges, K., Bowie, D., Traynelis, S.F., 1999. The glutamate receptor ion channels. Pharmacol. Rev. 51, 7–61.

Duffey, S.S., Stout, M.J., 1996. Antinutritive and toxic components of plant defense against insects. Arch. Insect Biochem. Physiol. 32, 3–37.

Hollmann, M., Heinemann, S., 1994. Cloned glutamate receptors. Annu. Rev. Neurosci. 17, 31–108.

Hu, W.Q., Hesse, M., 1996. The synthesis of p-coumaroylspermidines. Helv. Chim. Acta 79, 548–559.

Hume, R.I., Dingledine, R., Heinemann, S.F., 1991. Identification of a site in glutamate receptor subunits that controls calcium permeability. Science 253, 1028–1031.

Igarashi, K., Koga, K., He, Y., Shimogori, T., Ekimoto, H., Kashiwagi, K., Shirahata, A., 1995. Inhibition of the growth of various human and mouse tumor cells by 1,15-bis(ethylamino)-4,8,12-triazapentadecane. Cancer Research 55, 2615–2619.

Igarashi, K., Shirahata, A., Pahk, A.J., Kashiwagi, K., Williams, K., 1997. Benzyl-polyamines: novel, potent *N*-methyl-D-aspartate receptor antagonists. J. Pharmacol. Exp. Ther. 283, 533–540.

Karigiannis, G., Papaioannou, D., 2000. Structure, biological activity and synthesis of polyamine analogues and conjugates. Eur. J. Org. Chem. 1841–1863.

Kashiwagi, K., Masuko, T., Nguyen, C.D., Kuno, T., Tanaka, I., Igarashi, K., Williams, K., 2002. Channel blockers acting at *N*-methyl-D-aspartate receptors: differential effects of mutations in the vestibule and ion channel pore. Mol. Pharmacol. 61, 533–545.

Klose, M.K., Atkinson, J.K., Mercier, A.J., 2002. Effects of a hydroxy-cinnamoyl conjugate of spermidine on arthropod neuromuscular junctions. J. Comp. Physiol. A. 187, 945–952.

Krapcho, A.P., Kuell, C.S., 1990. Mono-protected diamines. *N*-tert-butoxycarbonyl-α,ω-alkanediamines from α,ω-alkanediamines. Synth. Commun. 20, 2559–2564.

Li, Y., Popaj, K., Lochner, M., Geneste, H., Budriesi, R., Chiarini, A., Melchiorre, C., Hesse, M., 2001. Analogues of polyamine alkaloids and their synthetic advantages. Farmaco 56, 127–131.

Makinen, K.K., Tenovuo, J., 1982. Observations on the use of guaiacol and 2,2′-azino-di(3-ethylbenzthiazoline-6-sulfonic acid) as peroxidase substrates. Anal. Biochem. 126, 100–108.

McCormick, K.D., Meinwald, J., 1993. Neurotoxic acylpolyamines from spider venoms. J Chem. Ecol. 19, 2411–2451.

Mercier, A.J., Atwood, H.L., 1989. Long-term adaptation of a phasic extensor motoneurone in crayfish. J. Exp. Biol. 145, 9–22.

Mercier, A.J., Farragher, S., Schmor, B., Kamau, M., Atkinson, J., 1998. Effect of a plant-derived spider toxin analogue on the crayfish neuromuscular junctions. Can. J. Zool. 76, 2103–2107.

Meurer-Grimes, B., 1995. New evidence for the systematic significance of acylated spermidines and flavonoids in pollen of higher hamamelidae. Brittonia 47, 130–142.

Moya, E., Blagbrough, I.S., 1995. Synthesis and neuropharmacological properties of arthropod polyamine amide toxins. In: Carter, C. (Ed.), Neuropharmacology of Polyamines, pp. 167–183.

Osullivan, M.C., Dalrymple, D.M., 1995. A one-step procedure for the selective trifluoroacetylation of primary amino groups of polyamines. Tetrahedron Lett. 36, 3451–3452.

Panagabko, C., Chenier, D., Fixon-Owoo, S., Atkinson, J.K., 2000. Ion-pair HPLC determination of hydroxycinnamic acid monoconjugates of putrescine, spermidine and spermine. Phytochem. Anal. 11, 11–17.

Panchenko, V.A., Glasser, C.R., Mayer, M.L., 2001. Structural similarities between glutamate receptor channels and K(+) channels

examined by scanning mutagenesis. J. Gen. Physiol. 117, 345–360.

Parnas, I., Atwood, H.L., 1966. Phasic and tonic neuromuscular system in the abdominal extensor muscle of the crayfish and rock lobster. Comp. Biochem. Physiol. 18, 701–723.

Quicke, D.L.J., Usherwood, P.N.R., 1990. Spider toxins as lead structures for novel pesticides. In: Hodgson, E., Kuhr, R.J. (Eds.), Safer Insecticides: Development and Use. Marcel Dekker, New York, pp. 385–452.

Raditsch, M., Geyer, M., Kalbitzer, H.R., Jahn, W., Ruppersberg, J.P., Witzemann, V., 1996. Polyamine spider toxins and mammalian *N*-methyl-D-apsartate receptors: structural basis for channel blocking and binding of argiotoxin(636). Eur. J. Biochem. 240, 416–426.

Schafer, A., Benz, H., Fiedler, W., Guggisberg, A., Bienz, S., Hesse, M., 1994. Polyamine toxins from spiders and wasps. In: Cordell, G.A., Brossi, A. (Eds.), Alkaloids, Vol. 45. Academic Press, San Diego, pp. 1–124.

Sharma, P., Rajam, M.V., 1995. Spatial and temporal changes in endogenous polyamine levels associated with somatic embryogenesis from different hypocotyl segments of eggplant (Solanum melongena). J. Plant Physiol. 146, 658–664.

Shinozaki, H., Shibuya, I., 1974. A new potent excitant, quisqualic acid: effects on crayfish neuromuscular junction. Neuropharmacol. 13, 665–672.

Shudo, K., Endo, Y., Hashimoto, Y., Aramaki, Y., Nakajima, T., Kawai, N., 1987. Newly synthesized analogues of the spider toxin block the crustacean glutamate receptor. Neurosci. Res. 5, 82–85.

Smith, T.A., Negrel, J., Bird, C.R., 1983. The cinnamic acid amides of the di- and polyamines. In: Bachrach, U. (Ed.), Advances in Polyamine Research. Raven Press, New York, pp. 347–370.

Strømgaard, K., Brier, T.J., Andersen, K., Mellor, I.R., Saghyan, A., Tikhonov, D., Usherwood, P.N., Krogsgaard-Larsen, P., Jaroszewski, J.W., 2000. Solid-phase synthesis and biological evaluation of a combinatorial library of philanthotoxin analogues. J. Med. Chem. 43, 4526–4533.

Subramaniam, S., Donevan, S.D., Rogawski, M.A., 1994. Hydrophobic interactions of n-alkyl diamines with the *N*-methyl-D-aspartate receptor: voltage-dependent and-independent blocking sites. Mol. Pharmacol. 45, 117–124.

Tikhonov, D.B., Magazanik, L.G., Mellor, I.R., Usherwood, P.N., 2000. Possible influence of intramolecular hydrogen bonds on the three-dimensional structure of polyamine amides and their interaction with ionotropic glutamate receptors. Receptors Channels 7, 227–236.

Torrigiani, P., Altamura, M.M., Pasqua, G., Monacelli, B., Serafini-Fracassini, D., Bagni, N., 1987. Free and conjugated polyamines during *de novo* floral and vegetative bud formation on thin cell layers of tobacco. Physiol. Plant 70, 453–460.

Usherwood, P.N.R., Blagbrough, I.S., 1991. Spider toxins affecting glutamate receptors: polyamines in therapeutic neurochemistry. Pharmacol. Ther. 52, 245–268.

van Harreveld, A., 1936. A physiological solution for freshwater crustaceans. Proc. Soc. Exp. Biol. Med. 34, 428–432.

Werner, C., Hu, W., Lorenzi-Riatsch, A., Hesse, M., 1995. Dicoumaroylspermidines in anthers of different species of the genus *Aphelandra*. Phytochemistry 40, 461–465.

Williams, K., 1993a. Effects of *Agelenopsis aperta* toxins on the *N*-methyl-D-aspartate receptor- polyamine-like and high-affinity antagonist actions. J. Pharmacol. Exp. Therapeut. 266, 231–236.

Williams, K., 1993b. Ifenprodil discriminates subtypes of the *N*-methyl-D-aspartate receptor: selectivity and mechanisms at recombinant heteromeric receptors. Mol. Pharmacol. 44, 851–859.

Williams, K., Dattilo, M., Sabado, T., Kashiwagi, K., Igarashi, K., 2003. Pharmacology of delta2 glutamate receptors: effects of pentamidine ad protons. J. Pharmacol. Exp. Ther. 305, in press.

Williams, K., Russell, S.L., Shen, Y.M., Molinoff, P.B., 1993. Developmental switch in the expression of NMDA receptors occurs in vivo and in vitro. Neuron 10, 267–278.

Wollmuth, L.P., Kuner, T., Jatzke, C., Seeburg, P.H., Heintz, N., Zuo, J., 2000. The Lurcher mutation identifies delta 2 as an AMPA/kainate receptor-like channel that is potentiated by Ca(2+). J. Neurosci. 20, 5973–5980.

Xie, Y.S., Bodnaryk, R.P., Fields, P.G., 1996. A rapid and simple flour-disk bioassay for testing substances active against stored-product insects. Can. Ent. 128, 865–875.

Zuo, J., De Jager, P.L., Takahashi, K.A., Jiang, W., Linden, D.J., Heintz, N., 1997. Neurodegeneration in Lurcher mice caused by mutation in delta2 glutamate receptor gene. Nature 388, 769–773.