

Small Molecules from Spiders Used as Chemical Probes

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allomones · pheromones · polyamines · spiders · toxins

Spiders are important species in ecological systems and as major predators of insects they are endowed with a plethora of low-molecular-weight natural products having intriguing biological activities. The isolation and biological characterization of these entities are well established, however, only very recently have these compounds been used as templates for the design, synthesis, and biological evaluation of synthetic analogues. In contrast, the investigation of compounds responsible for chemical communication between spiders is far less developed, but recently new light has been shed onto the area of pheromones and allomones from spiders. Herein, we recapitulate these recent results, put them into perspective with previous findings, and provide an outlook for future studies of these chemotypes.

1. Introduction

Spiders (Araneae) are air-breathing arthropods with eight legs and chelicerae with fangs that inject venom (Figure 1). They are found worldwide and approximately 40 000 different species and more than 100 families have been characterized. One of the most well-known characteristics of spiders is their ability to extrude silk and form webs, which are frequently used to capture prey. Almost all spiders are predators, preying mostly upon insects and other spiders by using a variety of different strategies, including injection of toxic venom into the prey.

Spiders have become common symbols in the arts and mythology because of their wide range of behaviors that symbolize combinations of patience, cruelty, and creative powers. This dates back to the ancient Egyptian and Greek mythologies and continues into present day culture, with characters such as Shelob from *The Lord of the Rings* and the comic book superhero Spider Man. Spiders are also related to one of the most common specific phobias, arachnophobia, which is an abnormal fear of spiders or anything reminiscent

of spiders. However, the venom of only a few spider species is dangerous to humans.

Both spider venom and silk have been the subjects of intense investigation in recent years. Spider silk is characterized by a combination of lightness, resiliency, and unusual strength, all of which have potential use, for example, in medical sutures and artificial cartilage. These and other applications of (artificial) spider silk are currently being pursued by a number of biotech companies. Spider silk is mainly composed of peptides/proteins, and although the specific composition varies within different species, the spider silk is generally composed of peptide regions that self-assemble into a β -sheet conformation. The β sheets stack to

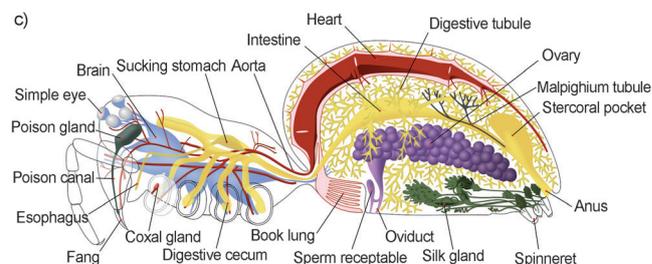
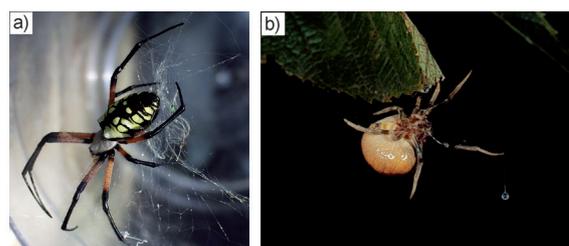


Figure 1. a) Argiope spider. b) Bolas spider. c) Cartoon representation of the spider anatomy.

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form crystals, whereas other segments of the proteins form amorphous domains, and the interplay between these regions gives spider silk its extraordinary properties. The generation, composition, and application of spider silk have recently been extensively reviewed,^[1] and will not be covered in further detail here.

The venom of spiders contain mixtures of proteins and peptides as well as low-molecular-weight (< 1000 Da) compounds such as biogenic amines, amino acids, neurotransmitters, and notably, acylpolyamine toxins. Generally, spider venom has been studied for more than 50 years^[2] with early attempts at separating venom constituents using paper chromatography reported in the late 1950s,^[3] followed by partial characterization and subsequent isolation of venom components.^[4] In the years that followed, a substantial number of peptide and protein toxins were isolated from spider venom. Biological investigations revealed that several of these toxins had intriguing pharmacological properties, particularly as ligands for various classes of ion channels. Thus, peptide and protein spider toxins have found unparalleled use in studies of ion channels and have aroused interest in understanding their composition and structure, and in particular, their mechanism of action when targeting ion channels.^[5] Since this fertile and exciting area of spider peptide and protein toxins has also been reviewed recently,^[5f-h,6] we will only highlight a few representative examples herein (see Section 4). We will thus focus on two classes of low-molecular-weight components from spiders, namely acylpolyamine toxins isolated from the venom, and small-molecule cues involved in inter- as well as intra-species communication.

Spiders, like many other arthropods, insects, and animals, use chemical cues for highly sophisticated chemical communication. In the case of spiders, they either exploit small-molecule cues that are distributed throughout the silk used in their webs or more volatile, airborne compounds for communication. In particular two types of cues have received substantial interest, allomones and pheromones;^[7] allomones are small molecules involved in inter-species communication for the benefit of the emitting species, whereas pheromones are used in communication within species.

We first discuss the recent progress in the isolation and characterization of allomones and pheromones, which so far have yielded about two dozen small molecules for which the structure and biological significance have been elucidated. Recent progress in modern isolation techniques, however, will most likely change this dramatically in the years to come and provide important advances towards understanding the molecular details of communication of spiders.

Next, we discuss the acylpolyamine toxins, with a particular emphasis on the application of these compounds and their analogues as chemical tools to probe biological systems. The first member of the class of acylpolyamine toxins was isolated and structurally characterized in the late 1980s.^[8] Subsequently, these toxins have been extensively studied.^[4c,9] Recent synthetic and biological advances, however, have enabled novel insights into their mode of action and their potential use in future drug discovery efforts, particularly those related to diseases in the brain.



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The subjects covered as well as the structure of this Minireview article are reminiscent of the excellent full Review by Schulz published in this journal in 1997,^[10] and may therefore be regarded as an update of said review.

2. Chemical Communication

Chemical signaling plays an important role in the behavior of most, if not all, organisms. Arthropods and insects communicate through odors in the form of small molecules, and in fact they rely more heavily on chemical signals than on any other form of communication such as vision, sound, or vibrations. These small molecules, termed semiochemicals, serve as a chemical language that mediates interactions between organisms. Semiochemicals are generally divided into two groups, pheromones and allelochemicals. The pheromones are chemical signals that carry information from one individual to another member of the same species. Allelochemicals on the other hand are used in communication

between different species and are subdivided into three groups, allomones, kairomones, and synomones.

Chemical communication between spiders has been known for almost a century and extensive studies of the behavior of several spiders have been performed using live animals, silk, and silk extracts as the source of the chemical cues.^[7,11] A partial isolation of the chemicals involved in these communicative processes were reported in 1986,^[12] but the chemical identity of these compounds remained elusive until 1993 when the first spider pheromone was isolated from *Linyphia triangulis* and structurally characterized.^[13] In contrast, allomones used by the unusual orb-weaver spiders, the Bolas spiders, for hunting their prey were isolated and characterized in the 1980s.^[14] Therefore, we describe how the molecular details of chemical communication in spiders have emerged in recent years, focusing specifically on allelochemicals and pheromones. The recent progress has provided vital insights into the chemical structures underlying this communication.

2.1. Allelochemicals

The Bolas spiders have been instrumental in studies of spiders and their allomones. Bolas spiders are species of three related genera: American (*Mastophora*), African (*Cladomelea*), and Australian (*Ordgarius*) Bolas spiders. They are characterized by an intriguing hunting technique, where they swing a string of silk having a sticky ball/droplet (bolas) which sticks to passing prey and thereafter the spider paralyzes and encapsulates or feeds on the prey.^[15]

It was discovered that adult females of *Mastophora* sp. attract prey with volatile substances emitted from their bodies rather than the sticky silk droplets.^[24] It was suggested that these volatile substances mimic female sex pheromones of a subfamily of moths, the noctuid moths (*Noctuidae*), as all identified prey were male noctuid moths.^[24] A decade later, it was demonstrated that the hunting female Bolas spider *Mastophora cornigera* indeed emits volatile compounds that are identical to the sex pheromones found in female noctuid moths.^[14] This provided, to the best of our knowledge, the first molecular evidence for the chemical mimicry theory described above,^[24] and was also the first study describing the characterization of any volatile semiochemical compound from spiders. Volatiles from eight *Mastophora cornigera* Bolas spiders, kept in a green house under approximately natural hunting conditions, were collected by transferring each individual spider to a collection chamber once they entered hunting mode. Spectroscopic analysis of pools of the volatiles dissolved in hexanes, and comparison with synthetic standards confirmed the presence of at least three noctuid moth sex pheromones (**1–3**; Table 1), as well as indicated the presence of a fourth pheromone (**4**). Furthermore, both intra- and inter-individual variations in the composition of the emitted mixtures were demonstrated by examining several different pools. Notably, none of the identified compounds were found in webs or glue droplets, thus underpinning these allomones as being most likely emitted from the bodies of the spiders.^[14]

Mastophora hutchinsoni Bolas spiders have subsequently been shown to emit volatile mixtures containing (*Z*)-tetradec-9-enyl acetate (**2**), as well as its bis-unsaturated analogue **5**.^[17a] Both compounds are present as a female sex pheromone in their primary source of prey, the bristly cutworm *Lacinipolia renigera*.^[19] Interestingly, the spider not only produces the same chemicals as the prey, but also mimics the ratio of pheromones used by the female moths.^[17a] Biosynthetic studies confirmed that all these structures, compounds **1–5**, are found in the noctuid moth *Trichoplusia ni* (Noctuidae).^[18] Similarly, (*Z*)-octadec-11-enyl acetate (**6**), which is structurally closely related to **2**, **4**, and **5** was identified as a sex pheromone in flies,^[21] and was subsequently isolated from the webs of Linyphiid spiders.^[10] Compounds **7** and **8** are also known female moth sex pheromones from *Tetanolita mynesalis*,^[22] and although these have not yet been isolated from spiders, compelling evidence suggests that the Bolas spider *Mastophora hutchinsoni* may produce the compounds. These studies involved trapping of moths having either a mixture of **2** and **5** (*Lacinipoli arenigera*) or a mixture of **7** and **8** (*Tetanolita mynesalis*) as their sex pheromone, by using both Bolas spiders and synthetic pheromone blends in the trapping event.^[17b]

Some spiders also have the ability to take advantage of chemical cues emitted from their prey. An example of this phenomenon has revealed that the spider *Habronestes bradleyi* use an alarm pheromone, 6-methyl-hept-5-en-2-one (**9**), emitted from *Iridomyrmex purpureus* ants to locate this prey. Thus, **9** is classified as a kairomone,^[7a] that is, a compound that is emitted by a different species (in this case the ant) but also benefits the spider.^[23]

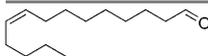
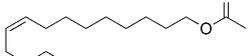
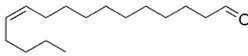
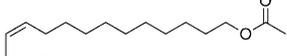
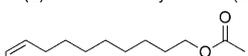
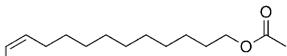
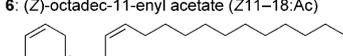
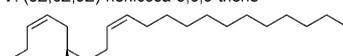
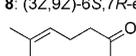
Lipid matrices on spider webs have revealed a wide variety of fatty acids and long chain hydrocarbons also found in spider cuticles,^[25] and these chemicals seem to be involved in the pheromone-based communication as either a solvent or vehicle for administration of the pheromone chemotypes to the silk.^[13,25a] However, the exact functions of these chemotypes still remain to be investigated in further detail, as they have also been hypothesized to play a role in recognition.

The 20-hydroxyecdysone steroidal hormone **10** (Scheme 1), which has been shown to affect ovarian development,^[26] cuticular contact signal levels,^[27] and cannibalistic behavior^[27] was recently shown to change the levels of several fatty acids and esters that elicited sexual behavior in male *Tegenaria atrica* spiders.^[28] This steroid has also previously been found in other species, such as *Phalagiu mopilio*, *Leiobonum limbatum*, *Opilio parietinus*, and *Opilio ravennae*.^[29]

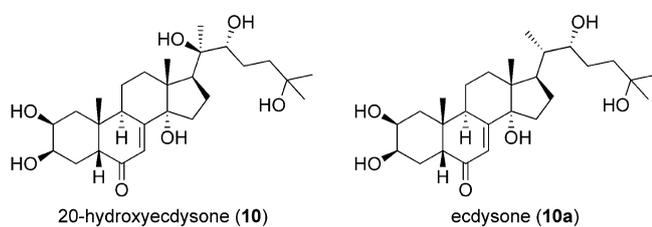
2.2. Pheromones

The first isolation and characterization of a small-molecule pheromone was a monumental achievement by Bute-nandt and co-workers who discovered bombykol, (10*E*,12*Z*)-hexadeca-10,12-dien-1-ol, from the silkworm moth, *Bombyx mori*.^[30] Interestingly, this compound is structurally very similar to the majority of the allomones described above, one of the most notable differences being that bombykol

Table 1: Allelochemicals from spiders.^[a]

| Compound | Type | Spider taxa ^[b] | Origin ^[c] |
|---|-----------|---|--|
|  1: (Z)-tetradec-9-enal (Z9–14:Al) | allomone | <i>Mastophora cornigera</i> ^[14] | Noctuid moths ^[16] |
|  2: (Z)-tetradec-9-enyl acetate (Z9–14:Ac) | allomone | <i>Mastophora cornigera</i> , ^[14] <i>Mastophora hutchinsoni</i> ^[17] | Noctuid moths, ^[16] <i>Trichoplusia ni</i> (Noctuidae), ^[18] <i>Lacinipolia renigera</i> ^[19] |
|  3: (Z)-hexadec-11-enal (Z11–16:Al) | allomone | <i>Mastophora cornigera</i> ^[14] | Noctuid moths, ^[16] <i>Nephelodes minians</i> ^[20] |
|  4: (Z)-hexadec-11-enyl acetate (Z11–16:Ac) | allomone | inconclusive data ^[14] | Noctuid moths, ^[16] <i>Trichoplusia ni</i> (Noctuidae), ^[18] <i>Nephelodes minians</i> ^[20] |
|  5: (9Z,12E)-tetradeca-9,12-dienyl acetate (Z,E-9,12–14:Ac) | allomone | <i>Mastophora hutchinsoni</i> ^[17] | <i>Lacinipolia renigera</i> ^[19] |
|  6: (Z)-octadec-11-enyl acetate (Z11–18:Ac) | allomone | various <i>linyphiids</i> ^[10] | <i>Drosophila simulans</i> ^[21] |
|  7: (3Z,6Z,9Z)-hencicosa-3,6,9-triene | allomone | <i>Mastophora hutchinsoni</i> ^[17b] | <i>Tetanolita mynesalis</i> ^[22] |
|  8: (3Z,9Z)-6S,7R-epoxy-henicosa-3,9-diene | allomone | <i>Mastophora hutchinsoni</i> ^[17b] | <i>Tetanolita mynesalis</i> ^[22] |
|  9: 6-methyl-hept-5-en-2-one | kairomone | <i>Habronestes bradleyi</i> ^[23] | <i>Iridomyrmex purpureus</i> ^[23] |

[a] The table contains known small molecules from spiders that are not spider sex pheromones. [b] Species from which the compounds have been isolated or have shown an effect in behavioral studies. [c] For the allomones shown here are the species from which the chemicals mimicked by the spiders arise.


Scheme 1. Structures of the major molting hormone 20-hydroxyecdysone (**10**) and its prohormone precursor ecdysone (**10a**).

contains a free hydroxy group, whereas the related spider allomones are generally acetylated or oxidized to the aldehyde oxidation state. Since those early reports from Butenandt and co-workers, the development of spectroscopic methods and analytical tools have been revolutionized, and numerous pheromones from various species have been structurally elucidated.^[31]

Schulz and Toft isolated and characterized the first female sex pheromones from a spider in 1993.^[13] The isolation of the pheromones from webs of female *Linyphia triangulis* spiders was guided by the observation of male spiders and their web reduction behavior. This behavior was originally observed by Watson, who noticed that the webs of unmated female spiders

attracted male spiders, who would start cutting lines and packing part of the web, presumably to minimize evaporation of volatile sex pheromones, thereby minimizing attraction of other males.^[32] The sex pheromones isolated by Schultz and Toft were (*R*)-3-hydroxybutyric acid (**11**) and its dimeric adduct, compound **12** (Table 2). These compounds were shown to elicit web reduction by spraying them onto webs of mated females that had not previously given rise to this response. Indications of the presence of trace amounts of the trimeric compound **13** were also observed in the extracts, together with minute amounts of crotonic acid (**14**). Compound **13** was not investigated further and **14** did not elicit web reduction behavior. However, since large amounts of **12** were found on the webs, it seems likely that **12** is applied to the web by the spider, and that **11** and **14** are volatile degradation products from a retro-Michael reaction taking place on the web.^[13] The two aldehydes **15** and **16** (Table 2) have not been confirmed as spider pheromones either, but they did enable trapping of male *Xysticus* sp. spiders as observed in a field study.^[33]

In 2000, the discovery of another spider pheromone, cupilure (**17**), from the silk dragline of the tropical wandering spider *Cupiennius salei* was reported.^[34] To verify the correct stereochemistry of **17**, chemical synthesis of both stereois-

mers combined with spectroscopic investigations verified that the biologically active form is the *S* enantiomer **17**.^[34] In addition, it was shown that courtship behavior of male spiders

was induced when they encountered male silk threads treated with the synthetic pheromone in behavioral bioassays, thus confirming biological activity of the synthetic sample.^[35] To

Table 2: Spider pheromones.^[a]

| Compounds | Spider taxa | Male behavior | Mode of conveying |
|--|---|---|-------------------|
| 11: (<i>R</i>)-3-hydroxybutyric acid (HBA) | <i>Linyphia triangulis</i> ^[13] | attraction and web reduction | contact with silk |
| 12: (<i>R,R</i>)-3-(3-hydroxybutyryloxy)-butyric acid (HBBA) | <i>Linyphia triangulis</i> ^[13] | attraction and web reduction | contact with silk |
| 13: (<i>R,R,R</i>)-3-[3-(3-hydroxybutyryloxy)-hydroxybutyryloxy]-butyric acid (HBBBA) | <i>Linyphia triangulis</i> , inconclusive data ^[13] | not tested | not known |
| 14: crotonic acid (CA) | <i>Linyphia triangulis</i> ^[13] | none observed | contact with silk |
| 15: (<i>E</i>)-dec-2-enal and 16: (<i>E</i>)-oct-2-enal | <i>Xysticus ferox</i> , <i>X. discursans</i> , <i>X. triguttatus</i> , <i>X. auctificus</i> | trapping of males with synthetic compound in a field study ^[33] | airborne |
| 17: (<i>S</i>)-1,1'-dimethylcitrate ("cupilure") | <i>Cupiennius salei</i> ^[34] | attraction and courtship ^[34] electrophysiology on male sensilla ^[35] | contact with silk |
| 18: 8-methyl-2-nonanone | <i>Agelenopsis aperta</i> ^[36] | attraction and courtship | airborne |
| 19: hexadecyl acetate and 20: (<i>E,E</i>)-farnesyl acetate | <i>Pholcus beijingensis</i> ^[37] | attraction | contact with silk |
| 21: trimethyl (<i>2R,3S</i>)-methylcitrate | <i>Argiope bruennichi</i> ^[38] | trapping of males with a confirmed pheromone in a field study | airborne |
| 22: <i>N</i> -3-methylbutyryl- <i>O</i> -2-(<i>S</i>)-methylbutyryl- <i>L</i> -serine methylester | <i>Latrodectus hasselti</i> ^[39] | attraction | contact with silk |
| 23: <i>N</i> -3-methylbutyryl- <i>O</i> -2-methylpropyl- <i>L</i> -serine methylester | <i>Latrodectus hasselti</i> ^[39] | identified in the active silk from this species, but its resynthesis and effect on male behavior was not reported ^[39] | contact with silk |

[a] The table includes spider pheromones as well as chemotypes that were isolated in pheromone studies, but were not fully characterized or failed to show an effect. Compounds **11**, **12**, **17**, **18**, **21**, and **22** were confirmed to act as individual pheromones; compounds **19** and **20** were shown to act as a two-component pheromone; compounds **15** and **16** were able to trap male spiders in a field study, but have not yet been isolated from a spider; compounds **13**, **14**, and **23** were identified on active spider silk samples.

provide additional evidence for this phenomenon, electrophysiological experiments were performed with electrodes placed in the sensilla of male spiders. In these recordings, similar traces were obtained for the treatment with either female silk or the synthetic pheromone **17**.^[35]

The spider sex pheromones discussed so far are transferred through contact with the web; however, indications of airborne pheromones have been demonstrated for several spider species.^[7,40] The first airborne spider pheromone, 8-methylnonan-2-one (**18**) was isolated from sexually mature virgin female desert spiders, *Agelenopsis aperta*.^[36] Comparison of headspace extracts collected from juvenile virgin females and sexually mature virgin females by gas chromatography/mass spectrometry (GC/MS) analysis showed that there were two peaks in the chromatogram of the extracts from the mature females that were not present in the extracts from the juveniles. Spectroscopic characterization, and a combination of resynthesis and chromatography confirmed that the two components were 6-methylheptan-2-one and 8-methylnonan-2-one (**18**), respectively. In subsequent tests for biological activity, however, only **18** could attract males and induce courtship behavior.^[36]

Compounds **19** and **20** were isolated from the webs of sexually receptive female *Pholcus beijingensis* spiders, and the structures were confirmed by comparison with authentic samples. However, conspecific male spiders were not attracted to the samples of either of the isolated compounds, but instead showed a preference for a 2:1 mixture of **19** and **20**, thus suggesting that an appropriate combination of **19** and **20** serve as a two-component pheromone.^[37]

Recently, Schulz and co-workers reported the isolation and synthesis of the first sex pheromone (**21**) from an orb-weaver spider *Argiope bruennichi*.^[38] This compound was first discovered by comparison of headspace extracts from virgin females, adult females, and sub-adult females using GC/MS. Subsequent synthesis furnished an approximately 6:1 diastereomeric mixture of trimethyl-(2*R*,3*S*) methylcitrate (**21**) and trimethyl-(2*S*,3*S*) methylcitrate when using enantiopure (*S*)-malic acid as the starting material. This enabled the confirmation of **21** as the major diastereoisomer in the natural extract by chiral phase GC, and furthermore, proved efficient in attracting conspecific males in the field.^[38] In an accompanying paper, the identification of an unusual serine derivative (**22**) was described as a sex pheromone of the Australian red back spider, *Latrodectus hasselti*.^[39] A behavioral bioassay using all four possible diastereoisomers confirmed that **22** is the active substance, and application of mixtures of **22** with inactive isomers indicated that other stereoisomers may actually inhibit the response.^[39] The structurally related N,O-diacylated serine methylester analogue **23** was also isolated from the active silk extracts, although the biological activity of this compound remains to be verified.

2.3. Chemical Tools

Pheromone chemistry in general has traditionally been closely linked to organic synthesis because of the often minute

amounts of compounds isolated. Synthetic efforts have thus been instrumental in producing material for confirmation of the proposed structures as well as useful amounts for use as chemical tools to probe biological findings.^[31a-c] An excellent example of a pheromone that fuelled fascinating synthetic studies is the potent sex attractant periplanone B from cockroaches, which gave rise to some striking synthetic studies in the late seventies to mid-eighties.^[41]

With regard to spider semiochemicals as tools, the first allomones helped to unequivocally confirm the theory of aggressive chemical mimicry in the Bolas spiders,^[14] and the first sex pheromones provided a chemical explanation for the web reduction behavior in linyphiids.^[13]

The isolation of cupilure (**17**) was instrumental in identifying the contact chemoreceptors involved in detection of the pheromone in *Cupiennius salei*,^[35] these receptors had been elusive to scientists for more than thirty years. It was long anticipated that the so-called tarsal organ was an olfactory chemoreceptor, and scanning electron microscopy studies revealed sensilla that appeared to comprise both mechano- and chemoreceptors in the tarsal organs of the web spider *Araneus diadematus*.^[42] Five years later, in 1978, Dumpert performed electrophysiological studies to provide the first evidence that tarsal organs of male *Cupiennius salei* responded to odors from females of the same species,^[43] whereas no signals were recorded for either males or females in response to odors from males, and no olfactory recognition was recorded for the odor of various prey species.^[43] Those findings, along with behavioral studies indicated that olfactory recognition of prey was unlikely in *C. salei*.^[44] In 1994, however, Ehn and Tichy presented convincing evidence showing that these organs are more likely to be hygro- and thermo-receptors in *C. salei*,^[45] which is in accord with the more recent identification of the actual contact chemoreceptors aided by the use of an isolated pheromone compound.^[34,35] This example goes to show how useful semiochemicals can be as tools in the elucidation of biological phenomena.

Another longstanding challenge in the field has been to gain knowledge about the biosynthetic pathways used to produce these semiochemicals in spiders. The observed trend that half of the characterized pheromones are structurally related to primary metabolites while the other half is structurally related to lipid-based insect pheromones have furnished cautious speculations about their biosynthetic origin; however, the identification of more structures and examination of their biosynthesis will clearly be important for the success of these endeavors.^[39]

With just eight known chemical structures of spider pheromones and about the same number of other semiochemicals with confirmed chemical structures and biological functions, these relatively few compounds have had a major impact in the field already, and hence indicate a promising future for further discoveries. The characterization of new small molecules and investigation of their functions will certainly provide important tools for probing known behavioral phenomena, sites of perception and production, biosynthetic mechanisms, as well as play a major role in the understanding of the underlying biochemical pathways and

molecular targets involved in olfactory recognition in spiders, a subject that recently has seen great progress in insects.^[46]

3. Acylpolyamine Toxins

3.1. Isolation and Structural Diversity

The first studies of acylpolyamine toxins date back more than 50 years to the first discovery of these in the venom of tarantula spiders.^[47] Acylpolyamines are secondary metabolites of spiders and wasps that are only present in the venom glands, and seem to have evolved specifically as tools for paralyzing prey.^[9a] Kawai and colleagues provided the first insight into the physiological action of acylpolyamines many years later by demonstrating that acylpolyamine fractions from the venom of the spider *Nephila clavata* (Joro spider) selectively blocked postsynaptic ionotropic glutamate (iGlu) receptors in invertebrate neuromuscular synapses.^[48] Since glutamate is the primary chemical messenger in insect neuromuscular junctions, it is not surprising that insect iGlu receptors are the primary target of acylpolyamine toxins.^[9e] The active fractions from *Nephila clavata* and *Nephila maculate* spiders were designated Joro spider toxins (JSTX-1, JSTX-2, JSTX-3, and JSTX-4) and nephila spider toxins (NSTX-1, NSTX-2, NSTX-3, and NSTX-4), respectively, with the numerals denoting the elution order in high-performance liquid chromatography (HPLC). Around the same time, the groups of Usmanov and Usherwood independently showed that *Argiope* venom also could block invertebrate as well as vertebrate glutamate receptors.^[49] Shortly after, these findings were followed by a demonstration of similar potent blockades of mammalian iGlu receptors.^[48a,50]

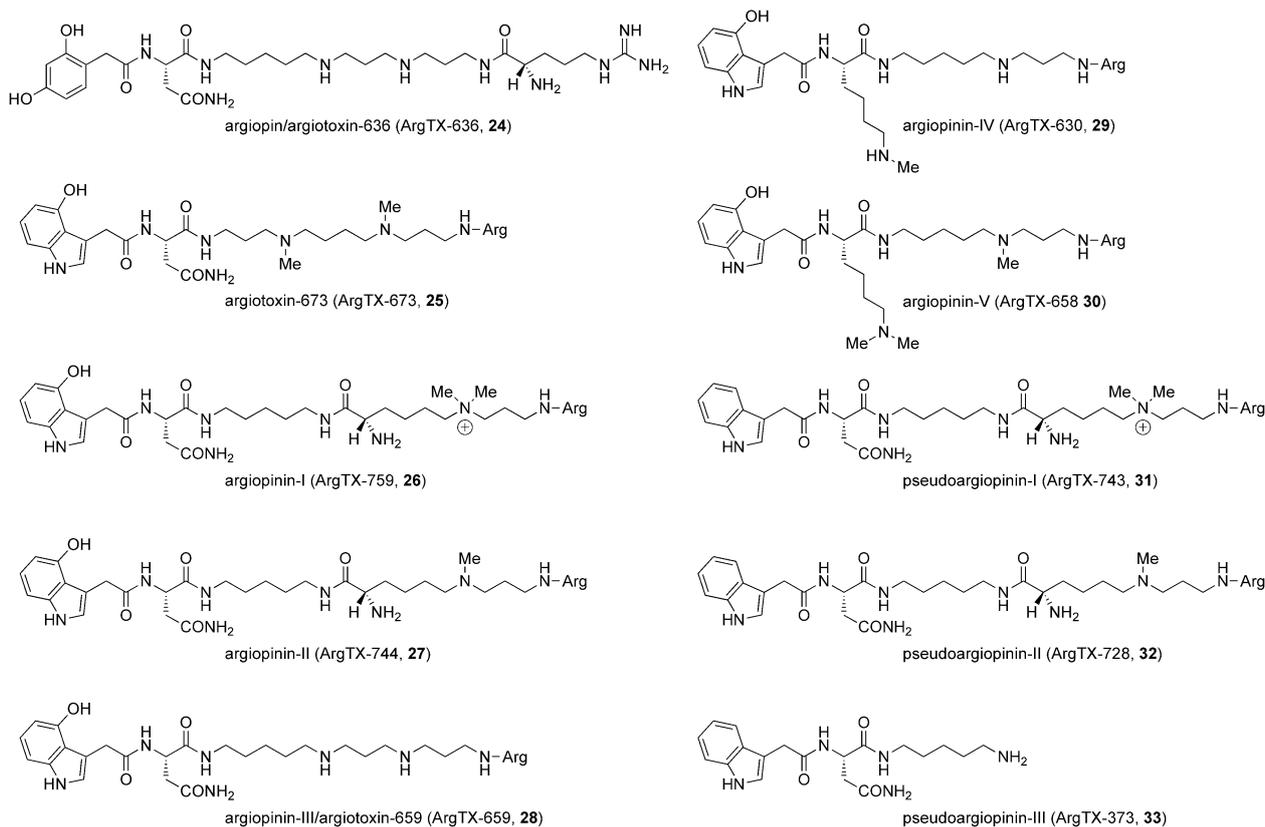
In the mammalian central nervous system (CNS), the iGlu receptors mediate the vast majority of excitatory transmission and are involved in virtually all brain functions as well as numerous neurological diseases including Alzheimer's disease, brain damage following ischemia, and schizophrenia.^[51] The iGlu receptors are divided into three main classes according to the selective action of *N*-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA), and kainate, and are formed by homo- or heteromeric assembly of four subunits. The iGlu receptors contain a centrally located cation channel that conduct Na^+ , K^+ , and Ca^{2+} across the cell membrane, and the channel is controlled by the binding of glutamate to sites on the extracellular domain of iGlu receptors.^[51]

Acylpolyamine toxins are so-called open-channel blockers of iGlu receptors, that is, they bind to the ion-channel region and thereby inhibit the ion flow.^[9d-g] This mechanism of action is highly relevant for the development of drugs targeting iGlu receptors. One of the very few drugs acting at iGlu receptors, memantine (Ebixa) used in the symptomatic treatment of Alzheimer's disease, employs a similar mode of action.^[52] An additional attractive feature of acylpolyamine toxins is their ability to distinguish iGlu receptors that are Ca^{2+} -permeable, from those that are not, as will be described later.

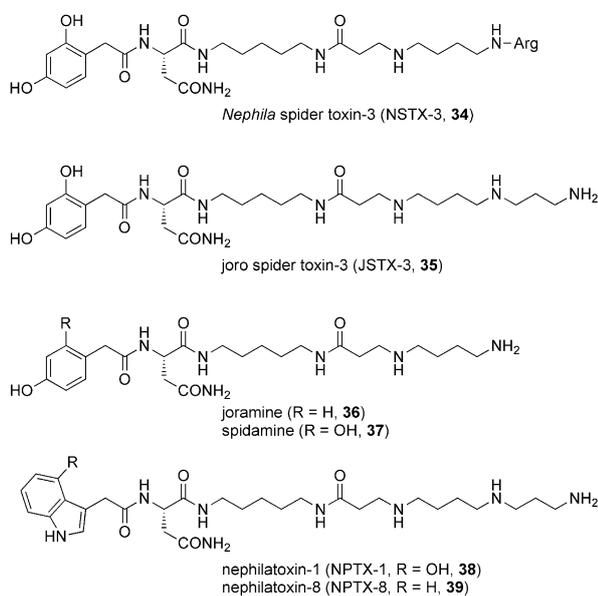
These exciting biological properties were subsequently investigated in more detail using various forms of spider extracts,^[9e,f] and the findings spurred an intense effort into structural characterization of the compounds responsible for these effects. The efforts were driven by the hope that acylpolyamines might provide useful investigational tools for invertebrate and vertebrate nervous systems in addition to serving as lead structures for the synthesis of potential therapeutics targeting brain diseases or for use in agriculture as pesticides.

The first full chemical characterization of an acylpolyamine toxin was achieved for argiotoxin-636 (**24**, Scheme 2) by Grishin and colleagues in 1986.^[8] The compound was originally isolated from the venom of the orb-weaver spider *Argiope lobata*, but was also isolated from the venom mixture of *Argiope aurantia* along with ArgTX-673 (**25**) and ArgTX-659 (**28**) a year later by Adams and co-workers.^[53] The numerals in the compound names for these compounds refer to their molecular weight. In addition, a larger series of structurally related toxins (**26-33**) was identified in the venom of *Argiope lobata* by Grishin and co-workers.^[54] All the argiotoxins contain a head group composed of an arylacetyl moiety connected to the polyamine unit through an asparagine residue (or lysine in the cases of **29** and **30**), and except for the somewhat truncated analogue **33** all the argiotoxins (**24-32**) contain an arginine amino acid tail (Scheme 2).

The first structural assignment for JSTX-3 was published in 1986 by Nakajima and co-workers, but the assignment was slightly revised by the same group a year later when they published the correct structures of both NSTX-3 (**34**)^[55] and JSTX-3 (**35**),^[56] as well as the total syntheses of both, thus unequivocally confirming the structures of the two toxins (Scheme 3).^[57] Two other examples of toxins isolated and characterized from the venom of *Nephila clavata* that were given trivial names are shown in Scheme 3 as well [joramine (**36**) and spidamine (**37**)].^[58] Mass spectrometric (MS) methods developed in the mid-nineties, however, enabled rapid characterization of a plethora of toxin molecules present in very small amounts,^[59] and a large number of toxins were characterized from the venoms of *Nephila clavata*, *Nephila maculate*, *Nephila clavipes*, *Nephilengys borbonica*, and *Nephilengys cruentata* using these novel methods in combination with one- and two-dimensional NMR spectroscopy.^[4c] These toxins, of which the JSTXs and NSTXs comprise subgroups, were named nephilatoxins (NPTX, for examples, see **38** and **39** in Scheme 3).^[4c,60] Following the identification of these large numbers of toxins, a more systematic naming regime was adopted where the numerals denote the molecular weight of the toxin. By the time of the most recent review covering the nephilatoxins by Palma and Nakajima in 2005, approximately 70 of the now 91 known individual acylpolyamines from this spider subfamily had been structurally elucidated.^[4c] With the large number of toxin structures available, it was also found necessary to categorize them into classes, and a classification based on the type of polyamine backbone was chosen (Scheme 4).^[60,61] The toxins were thus divided into four components, of which the "polyamine backbone" and the "aromatic acetyl group" are essential, and the "amino acid linker" and the "amino acid tail" are



Scheme 2. Examples of argiotoxin, argiopin, argiopin, and pseudoargiopin acylpolyamine toxins from *Argiope* spiders.



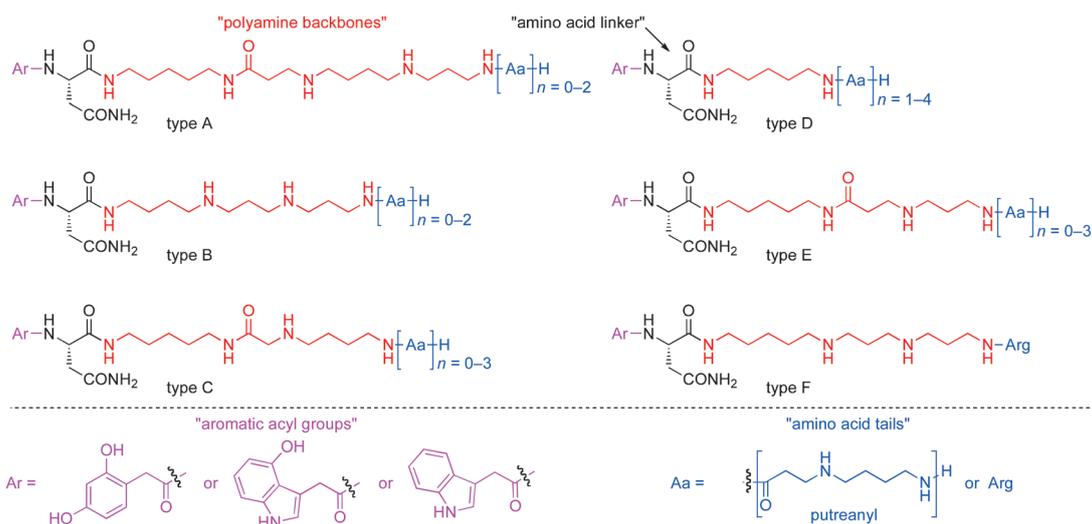
Scheme 3. Examples of nephilatoxins (NPTX) including joro spider toxins (JSTX) and *Nephila maculata* spider toxins (NSTX).

considered optional, albeit most known structures contain the asparagine amino acid linker. In addition to the systematic classes, types A–F shown in Scheme 4, a type G, which has the simple 1,5-diaminopentane backbone as in type D, but may also incorporate ornithine in the tail or in the linker, has been

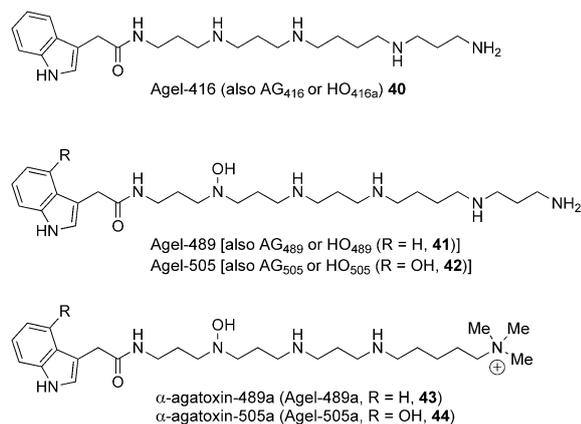
suggested.^[4c] Furthermore, when naming newly discovered toxins, the backbone subtype should be added after the molecular weight in the name. For example NPTX-1, which has a molecular weight of 588 Da, would be NPTX-588A, though for historical reasons it remains NPTX-1.

Interestingly, the spider's ability to produce a wide variety of molecules (at least 91) from this limited number of simple building blocks resembles synthetic efforts in combinatorial chemistry, and as such the complete library of toxins would count 378 members based on the known components and the division into the four groups described above.^[4c] In turn, this means that the majority of the biosynthesized molecules may not have been identified yet or that spiders may, through evolution have discarded non-optimal library members over time. One can only guess, however, given that the biosynthetic mechanisms for production of these toxins in the spiders are poorly understood, as to whether the spiders are capable of adapting their biosynthesis depending upon external stimuli to produce variable acylpolyamine mixtures.

A third class of acylpolyamines has been isolated from funnel web spiders (see Scheme 5 for selected structures). Examples of these toxins were first isolated, characterized, and identified as acylpolyamines in studies of the venom mixture from the American funnel web spider *Agelenopsis aperta* in 1989.^[62] A highly polar fraction of this peptide-rich venom contained toxins that noncompetitively antagonized glutamate receptors in a use-dependent manner. By adopting the Greek letter terminology originally developed by Olivera



Scheme 4. Classes of the nephilatoxins (NPTX) labeled types A–F.^[60a] In addition to the shown classes, a type-G has been suggested which has the cadaverine polyamine backbone as in type D.^[4c] The vast majority of the characterized nephilatoxins are structurally very similar to the known argiotoxins, that is, the head groups containing an asparaginyl residue connected to one of the three arylacetyl groups present in the argiotoxins are highly abundant in the nephilatoxins as well. The high occurrence of the putreanyl unit (shown in blue in the bottom panel of this scheme), however, seems to be rather unique for the nephilatoxins.



Scheme 5. Examples of α agatoxins/curtatoxins/agelenotoxins. Several of these toxins were isolated from different Agelenid spiders, which gave rise to more than one name for a single structure. The Agel terminology is used here to refer to the Agelenidae spider family.

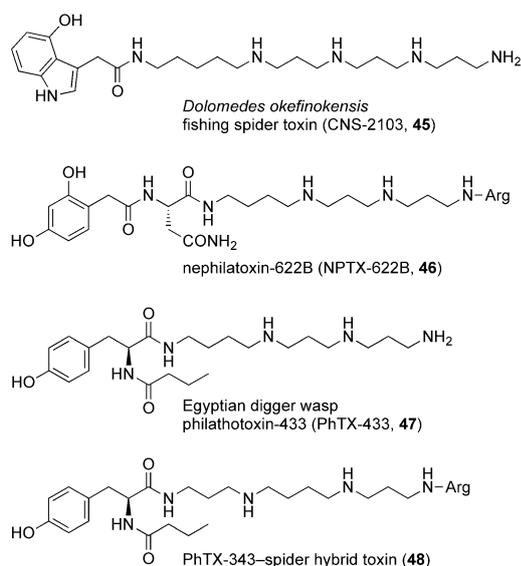
for the classification of conotoxins,^[63] these toxins were classified as α agatoxins.^[62] The preliminary characterization by ultraviolet (UV) spectroscopy and mass spectrometry revealed structural similarities to the argiotoxins and these compounds were also named according to their molecular mass (e.g., the α agatoxin with a molecular mass of 489 Da was named AG₄₈₉ or Agel-489). The structural elucidations of a number of toxins including the four most abundant constituents (Agel-489, Agel-489a, Agel-505, and Agel-505a) were published the following year.^[64] The structures of **41** and **42** were confirmed by total synthesis,^[64a] but the originally proposed structures of Agel-489a and Agel-505a,^[64b] were later revised and shown, by total synthesis, to contain the quaternary amino functionality as shown in the structures of **43** and **44** in Scheme 5.^[65]

Not long after the structural elucidation of the first members of the α agatoxins, ten structurally similar acylpolyamine toxins were isolated from the venom of another funnel web spider, *Hololena curta*.^[66] Several of these compounds were identical to the toxins found in the venom of *A. aperta* (Scheme 5).

Such an example is the shorter Agel-416 toxin **40**, for which the *Hololena curta* venom revealed two different isomers of the polyamine chain (i.e., a 3343 version as shown but also a 4333 version). Several different isomers including analogues containing a 4-hydroxybenzoyl or a 2,5-dihydroxybenzoyl group in place of the indolylacetyl group were later identified in the *A. aperta* venom, but not all the possible combinations were found.^[67] In a remarkable study, Bienz and co-workers demonstrated that total synthesis efforts are not just useful for confirming isolated structures, but also for revealing the existence of minor components in highly complex venom mixtures. By using a combination of parallel synthesis and extensive LC-MS/MS efforts they showed that all the 12 possible combinations of the polyamine structure and head group are present in the natural venom.^[67]

Although the toxins from *Hololena curta* have several structural similarities to the acylpolyamines isolated from the spiders of the Araneidae family (argio- and nephilatoxins) some fundamental differences apply. Most significantly, the latter described do not contain amino acids and may have N-hydroxylated amino groups in the polyamine backbone. The indolyl and 4-hydroxyindolyl arylacetyl head groups are present in toxins from both families, but instead of the 2,3-dihydroxyphenyl acetic acid present in the Araneidae toxins, the Agelenid toxins may contain either a 4-hydroxybenzoyl or a 2,5-dihydroxybenzoyl group (not shown).^[9e]

Compound **45** shown at the top of Scheme 6 is a structurally similar toxin isolated from a spider of a com-



Scheme 6. Examples of miscellaneous acylpolyamine toxins. The numerals in the wasp toxin refer to the number of methylene groups between each amine, counting from left to right.

pletely different family, and was shown to reversibly block voltage-gated calcium channels.^[68]

Philanthotoxin-433 (PhTX-433; **47**) is an acylpolyamine toxin isolated from the Egyptian digger wasp, *Philanthus triangulum*, and its structure elucidation and synthesis was accomplished by Nakanishi, Usherwood, and co-workers.^[69] A comparison of its structure with NPTX-622B (**46**) and the synthetic (“spider-wasp hybrid”) analogue (**48**)^[70] clearly shows a structural resemblance to the spider acylpolyamine toxins.

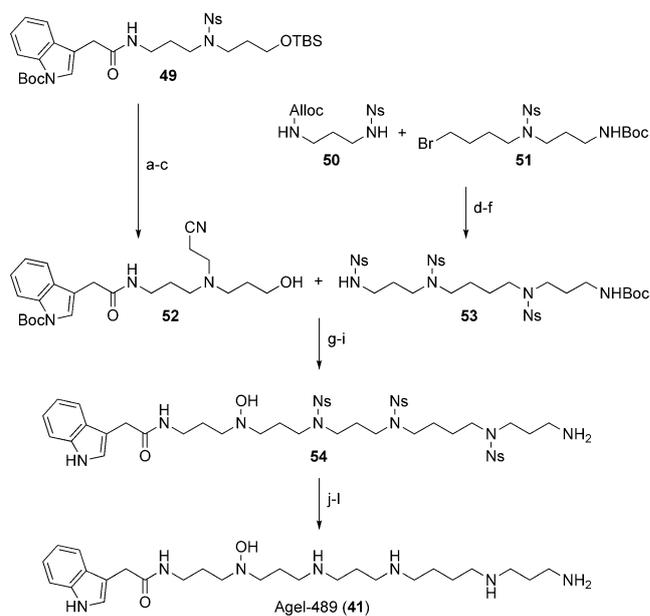
Thus, a wide range of acylpolyamine toxins from the venom of spiders have been isolated and structurally characterized, thereby revealing some remarkable biological properties, the most important of which is their perturbation of glutamate receptors. Moreover, exhaustive structural analyses, primarily using MS, have established that spiders combine a few common building blocks to biosynthesize these toxins in a combinatorial manner. This also suggests that several new acylpolyamine toxins await discovery and that spiders still constitute fertile pools for discovering biological tools.

3.2. Chemical Synthesis

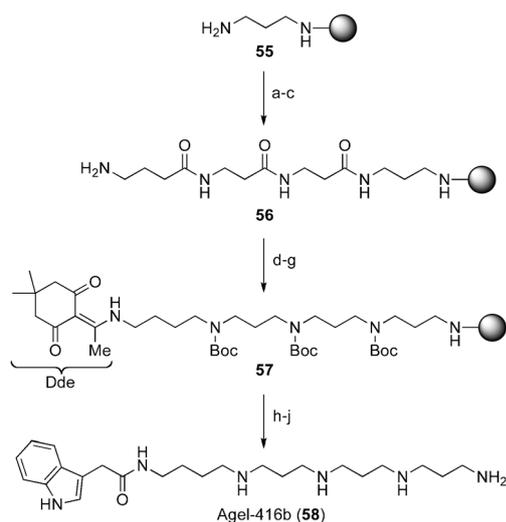
The chemical synthesis of polyamines and polyamine derivatives were for the most part performed in solution until the late nineties,^[71] though the first solid-phase syntheses of spider toxins containing a diamine backbone (NPTX-9 and NPTX-11) were reported in 1994.^[72] Since then a wide variety of procedures have been introduced for the solid-phase synthesis (SPS) of polyamine moieties.^[9b,d,73] As a result of the existing exhaustive reviewing of these subjects, the following section will only highlight a few selected examples including novel approaches not included in those previous review articles.^[9b,d,71,73]

Whereas synthesis of acylpolyamine toxins in solution may be particularly well suited for large-scale preparation of toxins that contain relatively simple polyamine moieties,^[74] SPS technology provides a highly efficient means for obtaining collections of toxin analogues for screening purposes and initial biological characterization (10–30 mg scale).^[75] The Fukuyama–Mitsunobu amination protocol,^[76] in particular, has found use for SPS of acylpolyamine toxin analogues,^[75b–d,77] although yields tend to drop significantly per amination step performed on the solid support.^[78] In contrast, the simple, straightforward, and efficient washing procedures between steps on solid support are highly desirable for purification of these compounds and their intermediates, as strongly polar amino-containing compounds are notoriously tedious to handle. In an impressive effort, Fukuyama and co-workers devised a convergent and high-yielding total synthesis of Agel-489 (Scheme 7), in which excellent yields were obtained for Fukuyama amination steps in solution, and the final removal of all the 2-nitrobenzenesulfonyl (Ns) groups was performed on a solid support to furnish the polar natural product in 31% overall yield.^[79]

The first total synthesis of a spider toxin involving full construction of the polyamine moiety on a solid support was achieved by applying borane reduction of a trityl resin-bound tripeptide (**56**, Scheme 8).^[80] Reduction of secondary amides to give secondary amines with borane^[81] was first adapted to solid-phase synthesis by Schultz and co-workers,^[82] and has



Scheme 7. Convergent total synthesis of Agel-489 (31% overall yield), taking advantage of both solution-phase and solid-phase synthesis procedures: a) PhSH, Cs₂CO₃, CH₃CN; b) acrylonitrile, EtOH, 60 °C; c) HF-NH₃, CH₃CN, 60 °C; d) *n*Bu₄NI, Cs₂CO₃, CH₃CN, 60 °C; e) [Pd(PPh₃)₄], PPh₃, pyrrolidine, CH₂Cl₂; f) NsCl, Et₃N, CH₂Cl₂; g) DEAD, PPh₃, benzene/CH₂Cl₂ (4:1); h) *m*CPBA, CH₂Cl₂, –10 °C; i) SO₂Cl, MeOH; j) excess trityl chloride solid support, *i*Pr₂EtN, CH₂Cl₂; k) 2-mercaptoethanol, DBU, DMF; l) TFA, CH₂Cl₂. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, DEAD = diethyl azodicarboxylate, DMF = *N,N'*-dimethylformamide, *m*CPBA = *meta*-chloroperbenzoic acid, TBS = *tert*-butyldimethylsilyl, TFA = trifluoroacetic acid.



Scheme 8. Solid-phase synthesis of Agel-416 by applying BH_3 -THF amide reduction: a) Fmoc- β -Ala-OH, HBTU, HOBT, $i\text{Pr}_2\text{EtN}$, DMF; b) piperidine/DMF (1:4); c) repeat steps (a) and (b), then (a) using Fmoc- γ -Abu-OH and (b); d) BH_3 -THF, 60°C, 48 h; e) I_2 , THF/HOAc/ $i\text{Pr}_2\text{EtN}$ (7:2:1); f) 2-Ac-dimedone, DMF; g) Boc_2O , $i\text{Pr}_2\text{EtN}$, CH_2Cl_2 ; h) DMF/ NH_2NH_2 (98:2); i) 2-(1*H*-indol-3-yl)acetic (*tert*-butyl carbonic anhydride, Et_3N , DMAP, DMF); j) TFA/ $\text{H}_2\text{O}/i\text{Pr}_3\text{SiH}$ (95:2.5:2.5). Boc = *tert*-butoxycarbonyl, DMAP = 4-dimethylaminopyridine, Fmoc = 9-fluorenylmethoxycarbonyl, HBTU = *O*-(1*H*-benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate, HOBT = 1-hydroxybenzotriazole, THF = tetrahydrofuran.

since been used extensively by Houghten and others.^[73] The challenge when using this method in connection with acylpolyamine SPS is the somewhat limited functional group tolerance, and therefore the method requires suitable linker and protecting group strategies.^[80,83] Hall and co-workers solved this by using the *N*-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde) protecting group, which is selectively introduced to primary amines and base labile, and subsequent *N*-*tert*-butoxycarbonyl (Boc) protection of the internal secondary amines (**57**). After deprotection, acylation, cleavage from the resin with concomitant Boc group removal, and HPLC purification, the title toxin was obtained in an impressive 37% overall yield.^[80]

Also, bidirectional SPS of the polyamines of α agatoxins by attachment of an internal secondary amino group to the solid support has been reported,^[84] and recently this methodology was extended to include preparation of *N*-hydroxylated polyamine units by an oxidative cleavage procedure using a custom-made linker.^[85]

Because of the presence of amino acid tails that are often present in toxins from the Araneidae family, efficient bidirectional SPS strategies are warranted for preparation of these chemotypes. A solution involving loading of aspartic acid onto a Rink resin to give rise to the asparagine amino acid linker moiety upon cleavage from the support was described by Bycroft and co-workers.^[72] The recently reported total solid-phase syntheses of ArgTX-636 and analogues thereof were performed on a backbone amide linker (BAL) resin,^[86] which also enables bidirectional functionalization of the polyamine as described for hybrid spider-wasp toxin ana-

logues.^[70] Such hybrids have furthermore been prepared by anchoring of the tyrosine phenol group through a tritylbromide linker.^[87]

3.3. Biological Effects and Acylpolyamines as Tools

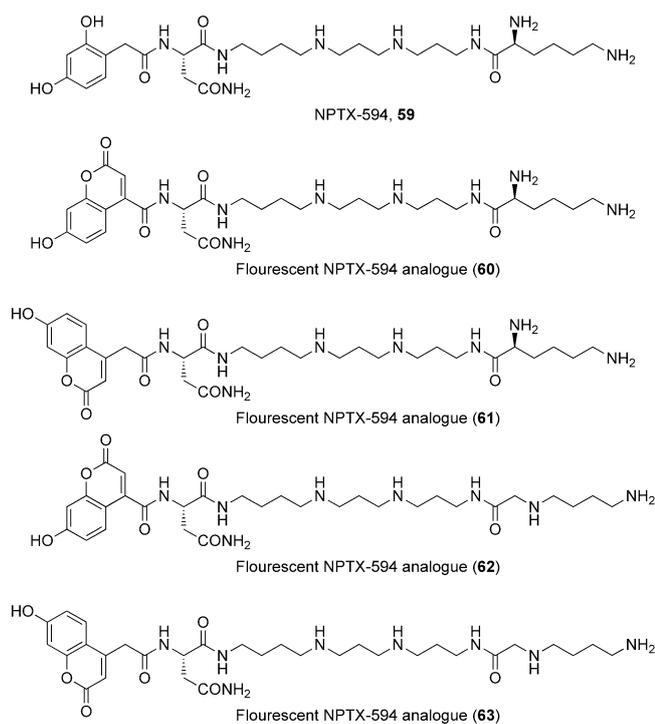
The acylpolyamine toxins are as previously mentioned potent ion-channel blockers of iGlu receptors, that is, they bind to a site inside the ion channel, which is only accessible when the receptor is activated by glutamate (use-dependent inhibitors). A particular hallmark of acylpolyamine toxins is their ability to selectively block Ca^{2+} -permeable iGlu receptors,^[88] which comprises all NMDA receptor combinations as well as certain AMPA and kainate receptors.^[51] In particular, Ca^{2+} -permeable AMPA receptors play key roles in brain development as well as synaptic plasticity, and acylpolyamine toxins have therefore become highly important tools for studies of these receptor subtypes. The Ca^{2+} permeability of iGlu receptors is determined by the so-called Q/R/N site located at the entrance to the ion-channel pore, where the presence of an arginine (R) renders the receptor impermeable to Ca^{2+} ions.^[51] Likewise, this basic arginine residue in the Q/R/N site, most likely denies the terminal amino functionality of acylpolyamine toxins access to the binding site inside the ion channel.

3.3.1. Labeled and Photolabile Cross-linked Analogues

Perhaps in part due to the more complex synthetic routes necessary to prepare spider toxins, synthetic analogues of these have not been explored as widely as for example analogues of the acylpolyamine wasp toxin PhTX-433 (**47**). In contrast, the large amount of work that has been dedicated to total syntheses and characterization of the many natural spider analogues themselves may also have been a contributing factor.

In the late eighties, Kawai, Nakajima and co-workers prepared the first radiolabeled spider toxin analogues based on ^{125}I -containing JSTX-3.^[89] Furthermore, Nakanishi and co-workers synthesized radiolabeled acylpolyamine toxin analogues during the early nineties. These were based on PhTX-433, but also included analogues that were elongated with lysine or arginine, and thus could be regarded as wasp-spider hybrids like **48**. In an initial study, ^{125}I -labeled PhTX-343, PhTX-343-Lys, and PhTX-343-Arg compounds were synthesized and applied to the investigation of rat brain membranes.^[90] Later, analogues containing photolabile cross-linkers in the head group or the tail were prepared for investigation of the binding to ion channels of nicotinic acetylcholine receptors.^[91]

To the best of our knowledge, fluorescently labeled probes based on acylpolyamine spider toxins had not been prepared until recently when Wakamiya and co-workers used NPTX-594 (**59**) as a starting point for the development of such probes. The two initial structures (**60** and **61**), in which one of the hydroxy functionalities of the acyl head group is incorporated into a fluorescent hydroxycoumarin structure (Scheme 9), proved to be 6- to 15-fold less potent than the



Scheme 9. NPTX-594 and examples of fluorescently labeled analogues.

natural product in an assay involving paralysis of crickets (*Gryllus bimaculatus*).^[92] By substituting the lysine tail of the initial compounds with *N*-(4-aminobutyl)-glycine, to give **62** and **63**, a fluorescently labeled analogue of NPTX-594 with relatively good structural similarity and equipotent activity in the assay was observed for **63**.^[92]

3.3.2. Development of Selective Inhibitors

The acylpolyamine spider toxins have attracted the most interest as pharmacological tools for neurobiological studies, especially of the mammalian iGlu receptor systems. Early work indicated substantial differences in the activity of acylamine toxins across subtypes of iGlu receptors both in vertebrate and invertebrate systems, thus raising the possibility that the acylpolyamines can be subtype-selective antagonists for this receptor class.^[93] iGlu receptors are multimeric proteins formed by four subunits that assemble to form a central ion channel with four large extracellular ligand-binding domains. Although some acylpolyamine toxins can act as potentiators of mammalian iGlu receptor mediated currents,^[94] their major effect concerns potent antagonism by binding with nanomolar affinity within the ion-channel domain and thereby blocking ion flow through the ion channel (thus, acting as open-channel blockers). A large amount of experimental and modeling work has been directed at understanding the molecular and structural mechanisms of blocking the iGlu receptor channels.

Thus, although no high-resolution X-ray crystal structures are available of the ion-channel domain of the iGlu receptors, detailed models for polyamine ion-channel blockers, including spider acylpolyamine toxins, have been developed.^[95]

These models propose that the toxins permeate the ion-channel domain, where the polyamine tail penetrates through the narrowest part of the channel, the so-called selectivity filter, also known as the N/Q/R site, into the pore region, whereas the aromatic head group remains locked in the area just above the selectivity filter. The association and dissociation of acylpolyamines with the iGlu receptor ion channel is very sensitive to the cell membrane potential such that the binding rate increases, whereas toxin dissociation slows with increasing negative membrane potential. The inhibitory potencies of most toxins are thus strongly voltage-dependent, and reflect that the migration of the toxin molecules in and out of the channel are influenced by the electrical field by forces similar to those experienced by permeability of cations. In mammals, 18 unique iGlu receptor subunits are expressed, and can combine homo- or heteromerically to form more than 50 known iGlu receptor subtypes with different functional properties and regional expression patterns in the brain.^[51] These subtypes are divided into three major functionally and physiologically distinct subfamilies, designated the NMDA, AMPA, and kainate receptors, as previously described.^[51] It was recognized early that several spider toxins have different affinities for these iGlu subfamilies.^[50c,96] For the non-NMDA type of iGlu receptors, certain acylpolyamine toxins, such as the JSTX analogue, 1-naphthylacetyl spermine (NASP, **64**),^[97] have become an important tool for studying Ca²⁺-permeable AMPA receptors by allowing highly specific blockade compared with Ca²⁺-impermeable receptors. Recently, work in our laboratories has focused on exploring the basis for the iGlu receptor subtype selectivity of acylpolyamine toxins through structure–activity relationship studies of *Argiobe* toxins such as ArgTX-636 and synthetic analogues thereof. The polyamine moiety was identified as a major determinant of selectivity for the NMDA- and AMPA-type subfamilies.^[86] Furthermore, early work on comparing the activity of ArgTX-636 at recombinant NMDA receptors showed that ArgTX-636 targets individual NMDA receptor subtypes with up to 100-fold differences in inhibitory potency.^[88] Characterization of a series of ArgTX-636 analogues across the four major NMDA subtypes, GluN1/N2A, GluN1/N2B, GluN1/N2C, and GluN1/N2D, have so far substantiated these earlier findings, showing that subtle modifications in the polyamine moiety can have major impact on subtype selectivity (see compounds **65** and **66**; Scheme 10). Individual NMDA receptor subtypes are at present poorly distinguished pharmacologically because of a lack of selective antagonists for many subtypes, and the potential of acylpolyamine toxins in this area therefore seems highly promising. More work is needed to realize the potential benefit of acylpolyamines as subtype-selective iGlu receptor antagonists; including the identification of the molecular basis for subtype-selective channel blockade.

4. Peptide Toxins

Peptide and protein toxins from spiders, as well as scorpions, cone snails, and snakes have found unparalleled use as pharmacological tools, particularly in studies of ion channels, and have been considered as potential therapeutic

tics.^[98] The biological function of these toxins is most likely to paralyze prey by inhibition of ion channels, thereby perturbing transmission at synapses in the nervous system. Toxins that block voltage-gated K^+ , Na^+ , and Ca^{2+} channels have been isolated from various spider families, although the tarantula spiders, belonging to the *theraphosidae* family, have been studied with particular interest.^[99] Many of these peptide toxins have structural similarities in that they typically consist of 30–40 amino acids and are stabilized by disulfide bridges; the inhibitory cysteine knot (ICK) is a common fold.

The toxins generally work through two modes of action: 1) They block ion channels through open-channel blockade by binding to the outer vestibule of the protein and thereby inhibit flow of ions; or 2) they bind to the region of the channels that control opening and closing (gating) of the ion channel.^[100] The prototypical example of how a peptide toxin can be transformed from an excellent pharmacological tool into a drug is the 25 amino acid ω -conotoxin MVIIA (ziconotide, Prialt Eisai Ltd. UK), which blocks voltage-gated Ca^{2+} channels (CaV2.2 or N-type) and is currently used for the treatment of neuropathic pain. The latter type of toxin has recently been thoroughly exploited in studies of the gating mechanism of K^+ channels, which has been an area of controversy.^[101] For example, Hanatoxin, an amphipathic 35 amino acid (4.1 kDa) peptide toxin (Figure 2a) isolated from *Grammostola spatulata* (bird spider), was shown to inhibit K^+ channels^[5a] and was subsequently used in studies of voltage-sensor paddles of K^+ channels.^[102] Similarly, in studies of Na^+ channels, a range of peptide toxins has been applied. For example SGTx1, which is a 34 amino acid (3.8 kDa) peptide isolated from the tarantula *Scodra griseipes* (Figure 2b).^[6g] In contrast to the mechanism discussed previously, SGTx1 binds to Na^+ channels and obstructs fast inactivation, thus essentially working as a potentiator of Na^+ channels.

Interestingly, tarantula toxins were also identified as agonists of ion channels; specifically, a group of toxins called vanillotoxins function as agonists of the capsaicin (TRPV1) receptor.^[103] Hence, such toxins are very useful tools in studies of this receptor, as well as investigations into structural relationships of transient receptor potential (TRP) channels and voltage-gated ion channels.

Agatoxins are a group of peptide toxins isolated from the American funnel web spider, *Agelenopsis aperta*, which show a broad spectrum of activity: α Agatoxins target iGlu receptors in a similar fashion as observed for the acylpolyamine toxins, that is, they are ion-channel blockers, while μ agatoxins and ω agatoxins target Na^+ and Ca^{2+} channels, respectively (for examples see, Figure 2c and d).^[5b] This multitude of actions most likely works synergistically to ensure fast and efficient paralysis of prey.

5. Summary and Outlook

Natural products in general, have historically had an immense impact on biological studies in general and drug discovery in particular. Here, we have focused on semi-chemicals and acylpolyamine toxins, from spiders, which are emerging as eminent biological probes, but also highlighted

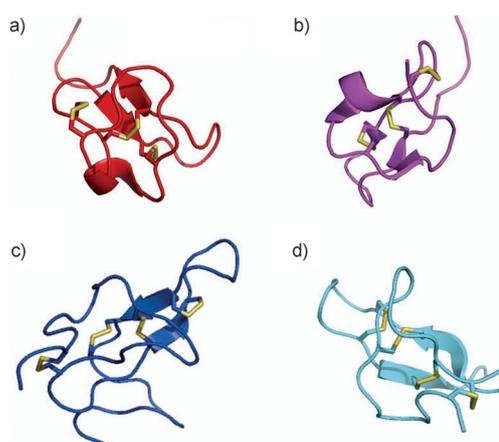
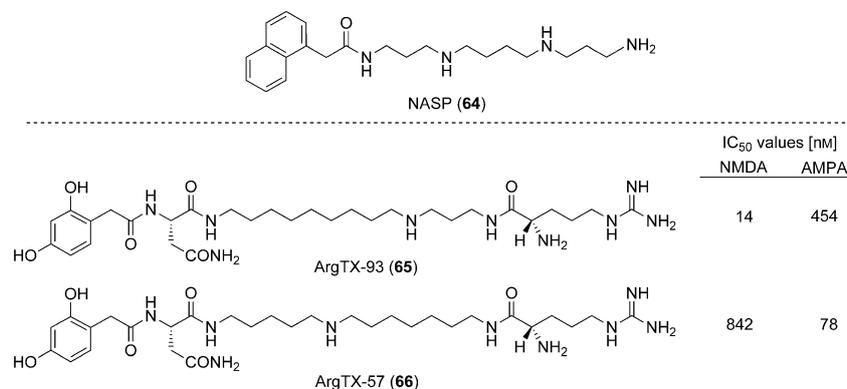


Figure 2. Examples of the peptide spider toxins determined by NMR spectroscopy in solution. a) The potassium channel blocker hanatoxin-1 (PDB code: 1D1H);^[104] b) the sodium channel potentiator SGTx1 (PDB code: 1LA4);^[105] c) the P-type calcium channel antagonist ω -agatoxin-IVB from *Agelenopsis aperta* (PDB code: 1AGG);^[106] d) the sodium channel antagonist μ -agatoxin-I from *Agelenopsis aperta* (PDB code: 1EIT).^[107] Side chains are omitted.



Scheme 10. NASP (64) is a synthetic and structurally simple acylpolyamine toxin, which has been extensively used as a tool in studies of AMPA receptors. ArgTX-93 (65) and ArgTX-57 (66) (numerals denote the methylene spacers in the polyamine moiety) are recently synthesized analogues of ArgTX-636.

are the well-documented properties of peptide and protein toxins from spiders.

The number of semiochemicals isolated and documented in studies of spiders is limited, and although the first pheromones were identified almost 20 years ago, still only eight spider pheromones are known. Notwithstanding are the efforts in identifying these molecules, which are most prominent examples of the power of isolation of natural products, combined with total synthesis and biological evaluation. As more species are investigated, and technologies such as mass spectrometry methods have advanced immensely, we foresee a significant increase in the number of characterized semiochemicals from spiders as well as biological applications of these.

The number of acylpolyamine toxins that have been identified to date are far greater, however, for the major part of these, the biological effects have not been examined, and only in one case, ArgTX-636, have medicinal chemistry modification studies been performed. The application of mass spectrometry has already increased the throughput of discovery of new acylpolyamine toxins significantly, and as more species are investigated, a treasure of new compounds should be available. Acylpolyamine toxins are already indispensable tools in the study of iGlu receptors, and with the recent progress in identifying subtype-selective derivatives, a revival of the application of these compounds is imminent. With the emergence of new selective chemotypes, their potential for neuroprotective treatment should increase significantly.

Small-molecule natural products from spiders have so far provided excellent biological probes, and will certainly have the ability to flourish even more in the future.

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- [1] a) F. Vollrath, D. P. Knight, *Nature* **2001**, *410*, 541; b) F. Hagn, L. Eisoldt, J. G. Hardy, C. Vendrely, M. Coles, T. Scheibel, H. Kessler, *Nature* **2010**, *465*, 239; c) G. Askarieh, M. Hedhammar, K. Nordling, A. Saenz, C. Casals, A. Rising, J. Johansson, S. D. Knight, *Nature* **2010**, *465*, 236; d) M. Heim, D. Keerl, T. Scheibel, *Angew. Chem.* **2009**, *121*, 3638; *Angew. Chem. Int. Ed.* **2009**, *48*, 3584; e) M. Heim, L. Romer, T. Scheibel, *Chem. Soc. Rev.* **2010**, *39*, 156.
- [2] J. A. Atkins, C. W. Wingo, W. A. Sodeman, *Science* **1957**, *126*, 73.
- [3] G. P. Cantore, S. Bettini, *Rend. Ist. Super. Sanita* **1958**, *21*, 794.
- [4] a) C. R. Geren, T. K. Chan, B. C. Ward, D. E. Howell, K. Pinkston, G. V. Odell, *Toxicon* **1973**, *11*, 471; b) F. L. Schanbacher, C. K. Lee, J. E. Hall, I. B. Wilson, D. E. Howell, G. V. Odell, *Toxicon* **1973**, *11*, 21; c) M. S. Palma, T. Nakajima, *Toxin Rev.* **2005**, *24*, 209.
- [5] a) K. J. Swartz, R. MacKinnon, *Neuron* **1995**, *15*, 941; b) K. J. Swartz, R. MacKinnon, *Neuron* **1997**, *18*, 665; c) I. M. Mintz, V. J. Venema, K. M. Swiderek, T. D. Lee, B. P. Bean, M. E. Adams, *Nature* **1992**, *355*, 827; d) I. M. Mintz, M. E. Adams, B. P. Bean, *Neuron* **1992**, *9*, 85; e) S. Y. Lee, R. MacKinnon, *Nature* **2004**, *430*, 232; f) M. E. Adams, B. M. Olivera, *Trends Neurosci.* **1994**, *17*, 151; g) B. G. Fry, K. Roelants, D. E. Champagne, H. Scheib, J. D. A. Tyndall, G. F. King, T. J. Nevalainen, J. A. Norman, R. J. Lewis, R. S. Norton, C. Renjifo, R. C. R. de La Vega, *Annu. Rev. Genomics Hum. Genet.* **2009**, *10*, 483; h) M. E. Adams, *Toxicon* **2004**, *43*, 509.
- [6] a) S. Diochot, *Toxin Rev.* **2005**, *24*, 291; b) P. Escoubas, F. Bosmans, *Expert Opin. Drug Discovery* **2007**, *2*, 823; c) G. Estrada, E. Villegas, G. Corzo, *Nat. Prod. Rep.* **2007**, *24*, 145; d) G. F. King, *Toxicon* **2007**, *49*, 513; e) M. R. Mortari, A. O. S. Cunha, L. B. Ferreira, W. Ferreira dos Santos, *Pharmacol. Ther.* **2007**, *114*, 171; f) G. M. Nicholson, *Toxicon* **2007**, *49*, 490; g) F. Bosmans, K. J. Swartz, *Trends Pharmacol. Sci.* **2010**, *31*, 175.
- [7] a) S. Schulz in *Advances in Insect Chemical Ecology* (Eds.: R. T. Cardé, J. G. Millar), Cambridge University Press, Cambridge, **2004**, pp. 110–150; b) A. C. Gaskett, *Biol. Rev. Cambridge Philos. Soc.* **2007**, *82*, 27.
- [8] E. V. Grishin, T. M. Volkova, A. S. Arseniev, O. S. Reshetova, V. V. Onoprienko, L. G. Magazanic, S. M. Antonov, I. M. Fedorova, *Bioorg. Khim.* **1986**, *12*, 1121.
- [9] a) A. L. Mueller, R. Roeloffs, H. Jackson, *Alkaloids* **1995**, *46*, 63; b) K. Strømgaard, K. Andersen, P. Krosgaard-Larsen, J. W. Jaroszewski, *Mini-Rev. Med. Chem.* **2001**, *1*, 317; c) S. Bienz, R. Detterbeck, C. Ensch, A. Guggisberg, U. Hausermann, C. Meisterhans, B. Wendt, C. Werner, M. Hesse, *Alkaloids* **2002**, *58*, 83; d) K. Strømgaard, I. Mellor, *Med. Res. Rev.* **2004**, *24*, 589; e) I. R. Mellor, P. N. R. Usherwood, *Toxicon* **2004**, *43*, 493; f) N. Kawai, *Toxin Rev.* **2005**, *24*, 273; g) K. Strømgaard, L. S. Jensen, S. B. Vogensen, *Toxicon* **2005**, *45*, 249.
- [10] S. Schulz, *Angew. Chem.* **1997**, *109*, 324; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 314.
- [11] J. S. Rovner, F. G. Barth, *Science* **1981**, *214*, 464.
- [12] L. R. Ayyagari, W. J. Tietjen, *J. Chem. Ecol.* **1987**, *13*, 237.
- [13] S. Schulz, S. Toft, *Science* **1993**, *260*, 1635.
- [14] M. K. Stowe, J. H. Tumlinson, R. R. Heath, *Science* **1987**, *236*, 964.
- [15] K. V. Yeargan, *Annu. Rev. Entomol.* **1994**, *39*, 81.
- [16] W. Steck, E. W. Underhill, M. D. Chisholm, *J. Chem. Ecol.* **1982**, *8*, 731.
- [17] a) C. Gemeno, K. V. Yeargan, K. F. Haynes, *J. Chem. Ecol.* **2000**, *26*, 1235; b) K. F. Haynes, C. Gemeno, K. V. Yeargan, J. G. Millar, K. M. Johnson, *Chemoecology* **2002**, *12*, 99.
- [18] L. B. Bjostad, W. L. Roelofs, *Science* **1983**, *220*, 1387.
- [19] K. F. Haynes, *J. Chem. Ecol.* **1990**, *16*, 2615.
- [20] J. Zhu, K. F. Haynes, *J. Chem. Ecol.* **2004**, *30*, 2047.
- [21] A. M. Schaner, R. J. Bartelt, L. L. Jackson, *J. Chem. Ecol.* **1987**, *13*, 1777.
- [22] K. F. Haynes, K. V. Yeargan, J. G. Millar, B. B. Chastain, *J. Chem. Ecol.* **1996**, *22*, 75.
- [23] R. A. Allan, M. A. Elgar, R. J. Capon, *Proc. R. Soc. London Ser. B* **1996**, *263*, 69.
- [24] W. G. Eberhard, *Science* **1977**, *198*, 1173.
- [25] a) S. Schulz, S. Toft, *Tetrahedron* **1993**, *49*, 6805; b) M. Trabalon, A. G. Bagneres, N. Hartmann, A. M. Vallet, *Insect Biochem. Mol. Biol.* **1996**, *26*, 77; c) A. G. Bagneres, M. Trabalon, G. J. Blomquist, S. Schulz, *Arch. Insect Biochem. Physiol.* **1997**, *36*, 295; d) M. Trabalon, A. G. Bagneres, C. Roland, *J. Chem. Ecol.* **1997**, *23*, 747; e) O. Prouvost, M. Trabalon, M. Papke, S. Schulz, *Arch. Insect Biochem. Physiol.* **1999**, *40*, 194.
- [26] a) M. Trabalon, A. M. Bautz, M. Moriniere, P. Porcheron, *Gen. Comp. Endocrinol.* **1992**, *88*, 128; b) G. Pourie, M. Trabalon, *Gen. Comp. Endocrinol.* **2003**, *131*, 250.
- [27] M. Trabalon, G. Pourie, N. Hartmann, *Insect Biochem. Mol. Biol.* **1998**, *28*, 751.

- [28] M. Trabalón, J. Niogret, C. Legrand-Frossi, *Gen. Comp. Endocrinol.* **2005**, *144*, 60.
- [29] F. Romer, W. Gnatzy, *Cell Tissue Res.* **1981**, *216*, 449.
- [30] a) A. Butenandt, R. Beckmann, E. Hecker, *Hoppe Seylers Z. Physiol. Chem.* **1961**, *324*, 71; b) A. Butenandt, R. Beckmann, D. Stamm, *Hoppe Seylers Z. Physiol. Chem.* **1961**, *324*, 84.
- [31] a) K. Mori, *Eur. J. Org. Chem.* **1998**, 1479; b) K. Mori, *Acc. Chem. Res.* **2000**, *33*, 102; c) P. H. G. Zarbin, J. A. F. P. Villar, I. Marchi, J. Bergmann, A. R. M. Oliveira, *Curr. Org. Chem.* **2009**, *13*, 299; d) J. Bergmann, J. A. F. P. Villar, M. F. Flores, P. H. G. Zarbin, *Curr. Org. Chem.* **2009**, *13*, 683; e) J. Bergmann, A. Gonzalez, P. H. G. Zarbin, *J. Braz. Chem. Soc.* **2009**, *20*, 1206; f) K. Mori, *Chemical Synthesis of Hormones, Pheromones, and Other Bioregulators*, Wiley, Chichester, **2010**, p. 299.
- [32] P. J. Watson, *Science* **1986**, *233*, 219.
- [33] J. R. Aldrich, T. M. Barros, *J. Arachnol.* **1995**, *23*, 212.
- [34] M. Papke, S. Schulz, H. Tichy, E. Gingl, R. Ehn, *Angew. Chem.* **2000**, *112*, 4517; *Angew. Chem. Int. Ed.* **2000**, *39*, 4339.
- [35] H. Tichy, E. Gingl, R. Ehn, M. Papke, S. Schulz, *J. Comp. Physiol. A* **2001**, *187*, 75.
- [36] M. D. Papke, S. E. Riechert, S. Schulz, *Anim. Behav.* **2001**, *61*, 877.
- [37] Y. Xiao, J. Zhang, S. Li, *J. Chem. Ecol.* **2009**, *35*, 769.
- [38] S. P. Chinta, S. Goller, J. Lux, S. Funke, G. Uhl, S. Schulz, *Angew. Chem.* **2010**, *122*, 2077; *Angew. Chem. Int. Ed.* **2010**, *49*, 2033.
- [39] E. Jerhot, J. A. Stoltz, M. C. B. Andrade, S. Schulz, *Angew. Chem.* **2010**, *122*, 2081; *Angew. Chem. Int. Ed.* **2010**, *49*, 2037.
- [40] a) W. J. Tietjen, *J. Arachnol.* **1978**, *6*, 197; b) C. W. Olive, *J. Arachnol.* **1982**, *10*, 241; c) T. Miyashita, H. Hayashi, *J. Arachnol.* **1996**, *24*, 9; d) L. E. Searcy, A. L. Rypstra, M. H. Persons, *J. Chem. Ecol.* **1999**, *25*, 2527.
- [41] a) W. C. Still, *J. Am. Chem. Soc.* **1979**, *101*, 2493; b) M. A. Adams, K. Nakanishi, W. C. Still, E. V. Arnold, J. Clardy, C. J. Persoons, *J. Am. Chem. Soc.* **1979**, *101*, 2495; c) S. L. Schreiber, C. Santini, *J. Am. Chem. Soc.* **1984**, *106*, 4038.
- [42] a) R. F. Foelix, I. W. Chuwang, *Tissue Cell* **1973**, *5*, 451; b) R. F. Foelix, I. W. Chuwang, *Tissue Cell* **1973**, *5*, 461.
- [43] K. Dumpert, *Experientia* **1978**, *34*, 754.
- [44] E. A. Seyfarth, F. G. Barth, *J. Comp. Physiol.* **1972**, *78*, 176.
- [45] R. Ehn, H. Tichy, *J. Comp. Physiol. A* **1994**, *174*, 345.
- [46] a) K. Sato, M. Pellegrino, T. Nakagawa, L. B. Vosshall, K. Touhara, *Nature* **2008**, *452*, 1002; b) D. Wicher, R. Schafer, R. Bauernfeind, M. C. Stensmyr, R. Heller, S. H. Heinemann, B. S. Hansson, *Nature* **2008**, *452*, 1007; c) R. Benton, K. S. Vannice, C. Gomez-Diaz, L. B. Vosshall, *Cell* **2009**, *136*, 149; d) U. B. Kaupp, *Nat. Rev. Neurosci.* **2010**, *11*, 188.
- [47] F. G. Fischer, H. Bohn, *Hoppe Seylers Z. Physiol. Chem.* **1957**, *306*, 265.
- [48] a) N. Kawai, A. Niwa, T. Abe, *Brain Res.* **1982**, *247*, 169; b) T. Abe, N. Kawai, A. Miwa, *J. Physiol.* **1983**, *339*, 243; c) N. Kawai, S. Yamagishi, M. Saito, K. Furuya, *Brain Res.* **1983**, *278*, 346.
- [49] a) P. N. R. Usherwood, I. R. Duce, P. Boden, *J. Physiol.* **1984**, *79*, 241; b) A. Bateman, P. Boden, A. Dell, I. R. Duce, D. L. J. Quicke, P. N. R. Usherwood, *Brain Res.* **1985**, *339*, 237; c) P. N. R. Usherwood, I. R. Duce, *Neurotoxicology* **1985**, *6*, 239; d) P. B. Usmanov, D. Kalikulov, N. Shadyeva, B. A. Tashmukhamedov, *Dokl. Akad. Nauk SSSR* **1983**, *273*, 1017.
- [50] a) M. Saito, N. Kawai, A. Miwa, H. Pan-Hou, M. Yoshioka, *Brain Res.* **1985**, *346*, 397; b) N. Akaike, N. Kawai, N. I. Kiskin, E. M. Kljuchko, O. A. Krishtal, A. Tsyndrenko, *Neurosci. Lett.* **1987**, *79*, 326; c) T. Priestley, G. N. Woodruff, J. A. Kemp, *Br. J. Pharmacol.* **1989**, *97*, 1315.
- [51] S. F. Traynelis, L. P. Wollmuth, C. J. McBain, F. S. Menniti, K. M. Vance, K. K. Ogden, K. B. Hansen, H. Yuan, S. J. Myers, R. Dingledine, *Pharmacol. Rev.* **2010**, *62*, 405.
- [52] a) S. A. Lipton, *Nat. Rev. Neurosci.* **2007**, *8*, 803; b) K. M. Cosman, L. L. Boyle, A. P. Porsteinsson, *Expert Opin. Pharmacother.* **2007**, *8*, 203.
- [53] M. E. Adams, R. L. Carney, F. E. Enderlin, E. T. Fu, M. A. Jarema, J. P. Li, C. A. Miller, D. A. Schooley, M. J. Shapiro, V. J. Venema, *Biochem. Biophys. Res. Commun.* **1987**, *148*, 678.
- [54] E. V. Grishin, T. M. Volkova, A. S. Arseniev, *Toxicon* **1989**, *27*, 541.
- [55] Y. Aramaki, T. Yasuhara, T. Higashijima, A. Miwa, N. Kawai, T. Nakajima, *Biomed. Res.* **1987**, *8*, 167.
- [56] Y. Aramaki, T. Yasuhara, K. Shimazaki, N. Kawai, T. Nakajima, *Biomed. Res.* **1987**, *8*, 241.
- [57] a) T. Teshima, T. Wakamiya, Y. Aramaki, T. Nakajima, N. Kawai, T. Shiba, *Tetrahedron Lett.* **1987**, *28*, 3509; b) Y. Hashimoto, Y. Endo, K. Shudo, Y. Aramaki, N. Kawai, T. Nakajima, *Tetrahedron Lett.* **1987**, *28*, 3511.
- [58] T. Chiba, T. Akizawa, M. Matsukawa, H. Panhou, M. Yoshioka, *Chem. Pharm. Bull.* **1994**, *42*, 1864.
- [59] a) T. Fujita, Y. Itagaki, H. Naoki, T. Nakajima, K. Hagiwara, *Rapid Commun. Mass Spectrom.* **1995**, *9*, 365; b) T. Fujita, Y. Itagaki, M. Hisaka, H. Naoki, T. Nakajima, M. Andriantsiferana, *Rapid Commun. Mass Spectrom.* **1997**, *11*, 1115; c) Y. Itagaki, H. Naoki, T. Fujita, M. Hisada, T. Nakajima, *J. Pharm. Soc. Jpn.* **1997**, *117*, 715.
- [60] a) M. Hisada, T. Fujita, H. Naoki, Y. Itagaki, H. Irie, M. Miyashita, T. Nakajima, *Toxicon* **1998**, *36*, 1115; b) M. S. Palma, Y. Itagaki, T. Fujita, H. Naoki, T. Nakajima, *Toxicon* **1998**, *36*, 485.
- [61] Y. Itagaki, T. Nakajima, *J. Toxicol. Toxin Rev.* **2000**, *19*, 23.
- [62] a) W. S. Skinner, M. E. Adams, G. B. Quistad, H. Kataoka, B. J. Cesarin, F. E. Enderlin, D. A. Schooley, *J. Biol. Chem.* **1989**, *264*, 2150; b) M. E. Adams, E. E. Herold, V. J. Venema, *J. Comp. Physiol. A* **1989**, *164*, 333.
- [63] B. M. Olivera, J. Rivier, J. K. Scott, D. R. Hillyard, L. J. Cruz, *J. Biol. Chem.* **1991**, *266*, 22067.
- [64] a) V. J. Jasys, P. R. Kelbaugh, D. M. Nason, D. Phillips, K. J. Rosnack, N. A. Saccomano, J. G. Stroh, R. A. Volkmann, *J. Am. Chem. Soc.* **1990**, *112*, 6696; b) G. B. Quistad, S. Suwanrumpha, M. A. Jarema, M. J. Shapiro, W. S. Skinner, G. C. Jamieson, A. Lui, E. W. Fu, *Biochem. Biophys. Res. Commun.* **1990**, *169*, 51.
- [65] V. J. Jasys, P. R. Kelbaugh, D. M. Nason, D. Phillips, K. J. Rosnack, J. T. Forman, N. A. Saccomano, J. G. Stroh, R. A. Volkmann, *J. Org. Chem.* **1992**, *57*, 1814.
- [66] G. B. Quistad, C. C. Reuter, W. S. Skinner, P. A. Dennis, S. Suwanrumpha, E. W. Fu, *Toxicon* **1991**, *29*, 329.
- [67] N. Manov, M. Tzouros, S. Chesnov, L. Bigler, S. Bienz, *Helv. Chim. Acta* **2002**, *85*, 2827.
- [68] K. D. McCormick, K. Kobayashi, S. M. Goldin, N. L. Reddy, J. Meinwald, *Tetrahedron* **1993**, *49*, 11155.
- [69] A. T. Eldefrawi, M. E. Eldefrawi, K. Konno, N. A. Mansour, K. Nakanishi, E. Oltz, P. N. R. Usherwood, *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 4910.
- [70] C. A. Olsen, M. Witt, H. Franzyk, J. W. Jaroszewski, *Tetrahedron Lett.* **2007**, *48*, 405.
- [71] a) V. Kuksa, R. Buchan, P. K. T. Lin, *Synthesis* **2000**, 1189; b) G. Karigiannis, D. Papaioannou, *Eur. J. Org. Chem.* **2000**, 1841.
- [72] B. W. Bycroft, W. C. Chan, N. D. Hone, S. Millington, I. A. Nash, *J. Am. Chem. Soc.* **1994**, *116*, 7415.
- [73] C. A. Olsen, H. Franzyk, J. W. Jaroszewski, *Synthesis* **2005**, 2631.
- [74] a) P. Wellendorph, J. W. Jaroszewski, S. H. Hansen, H. Franzyk, *Eur. J. Med. Chem.* **2003**, *38*, 117; b) A. Nilsen, P. M. England, *J. Am. Chem. Soc.* **2007**, *129*, 4902.
- [75] a) K. Strømgaard, T. J. Brier, K. Andersen, I. R. Mellor, A. Saghyan, D. Tikhonov, P. N. R. Usherwood, P. Krogsgaard-Larsen, J. W. Jaroszewski, *J. Med. Chem.* **2000**, *43*, 4526; b) H.

- Kromann, S. Krikstolaityte, A. J. Andersen, K. Andersen, P. Krogsgaard-Larsen, J. W. Jaroszewski, J. Egebjerg, K. Strømgaard, *J. Med. Chem.* **2002**, *45*, 5745; c) T. F. Andersen, D. B. Tikhonov, U. Bølcho, K. Bolshakov, J. K. Nelson, F. Pluteanu, I. R. Mellor, J. Egebjerg, K. Strømgaard, *J. Med. Chem.* **2006**, *49*, 5414; d) L. S. Jensen, U. Bølcho, J. Egebjerg, K. Strømgaard, *ChemMedChem* **2006**, *1*, 419; e) M. R. Jørgensen, C. A. Olsen, I. R. Mellor, P. N. Usherwood, M. Witt, H. Franzyk, J. W. Jaroszewski, *J. Med. Chem.* **2005**, *48*, 56; f) S. Frølund, A. Bella, A. S. Kristensen, H. L. Ziegler, M. Witt, C. A. Olsen, K. Strømgaard, H. Franzyk, J. W. Jaroszewski, *J. Med. Chem.* **2010**, *53*, 7441.
- [76] a) T. Fukuyama, C. K. Jow, M. Cheung, *Tetrahedron Lett.* **1995**, *36*, 6373; b) T. Fukuyama, M. Cheung, C. K. Jow, Y. Hidai, T. Kan, *Tetrahedron Lett.* **1997**, *38*, 5831.
- [77] a) K. Strømgaard, K. Andersen, T. Ruhland, P. Krogsgaard-Larsen, J. W. Jaroszewski, *Synthesis* **2001**, 877; b) C. A. Olsen, M. Witt, J. W. Jaroszewski, H. Franzyk, *Synlett* **2004**, 473; c) S. R. Chhabra, A. N. Khan, B. W. Bycroft, *Tetrahedron Lett.* **2000**, *41*, 1099; d) N. D. Hone, L. J. Payne, *Tetrahedron Lett.* **2000**, *41*, 6149.
- [78] C. A. Olsen, M. Witt, S. H. Hansen, J. W. Jaroszewski, H. Franzyk, *Tetrahedron* **2005**, *61*, 6046.
- [79] Y. Hidai, T. Kan, T. Fukuyama, *Chem. Pharm. Bull.* **2000**, *48*, 1570.
- [80] F. Wang, S. Manku, D. G. Hall, *Org. Lett.* **2000**, *2*, 1581.
- [81] H. C. Brown, P. Heim, *J. Am. Chem. Soc.* **1964**, *86*, 3566.
- [82] S. J. Paikoff, T. E. Wilson, C. Y. Cho, P. G. Schultz, *Tetrahedron Lett.* **1996**, *37*, 5653.
- [83] C. A. Olsen, M. Witt, J. W. Jaroszewski, H. Franzyk, *J. Org. Chem.* **2004**, *69*, 6149.
- [84] N. Manov, S. Bienz, *Tetrahedron* **2001**, *57*, 7893.
- [85] M. Meret, S. Bienz, *Eur. J. Org. Chem.* **2008**, 5518.
- [86] J. K. Nelson, S. U. Frølund, D. B. Tikhonov, A. S. Kristensen, K. Strømgaard, *Angew. Chem.* **2009**, *121*, 3133; *Angew. Chem. Int. Ed.* **2009**, *48*, 3087.
- [87] C. A. Olsen, M. R. Jørgensen, S. H. Hansen, M. Witt, J. W. Jaroszewski, H. Franzyk, *Org. Lett.* **2005**, *7*, 1703.
- [88] M. Raditsch, J. P. Ruppertsberg, T. Kuner, W. Gunther, R. Schoepfer, P. H. Seeburg, W. Jahn, V. Witzemann, *FEBS Lett.* **1993**, *324*, 63.
- [89] a) K. Hagiwara, Y. Aramaki, K. Shimazaki, N. Kawai, T. Nakajima, *Chem. Pharm. Bull.* **1988**, *36*, 1233; b) K. Shimazaki, K. Hagiwara, Y. Hirata, T. Nakajima, N. Kawai, *Neurosci. Lett.* **1988**, *84*, 173.
- [90] R. A. Goodnow, R. Bukownik, K. Nakanishi, P. N. R. Usherwood, A. T. Eldefrawi, N. A. Anis, M. E. Eldefrawi, *J. Med. Chem.* **1991**, *34*, 2389.
- [91] a) S.-K. Choi, A. G. Kalivretenos, P. N. R. Usherwood, K. Nakanishi, *Chem. Biol.* **1995**, *2*, 23; b) K. Fang, M. Hashimoto, S. Jockusch, N. J. Turro, K. Nakanishi, *J. Am. Chem. Soc.* **1998**, *120*, 8543.
- [92] T. Nishimaru, M. Sano, Y. Yamaguchi, T. Wakamiya, *Bioorg. Med. Chem.* **2009**, *17*, 57.
- [93] a) H. Jackson, P. N. Usherwood, *Trends Neurosci.* **1988**, *11*, 278; b) H. Jackson, T. N. Parks, *Annu. Rev. Neurosci.* **1989**, *12*, 405; c) N. Kawai, A. Miwa, K. Shimazaki, Y. Sahara, H. P. Robinson, T. Nakajima, *Comp. Biochem. Physiol. Part C* **1991**, *98*, 87.
- [94] S. D. Donevan, M. A. Rogawski, *Neuroscience* **1996**, *70*, 361.
- [95] D. B. Tikhonov, *Mol. Membr. Biol.* **2007**, *24*, 135.
- [96] a) A. Draguhn, W. Jahn, V. Witzemann, *Neurosci. Lett.* **1991**, *132*, 187; b) A. L. Mueller, B. C. Albensi, A. H. Ganong, L. S. Reynolds, H. Jackson, *Synapse* **1991**, *9*, 244; c) T. N. Parks, A. L. Mueller, L. D. Artman, B. C. Albensi, E. F. Nemeth, H. Jackson, V. J. Jasys, N. A. Saccomano, R. A. Volkmann, *J. Biol. Chem.* **1991**, *266*, 21523.
- [97] M. Koike, M. Iino, S. Ozawa, *Neurosci. Res.* **1997**, *29*, 27.
- [98] R. J. Lewis, M. L. Garcia, *Nat. Rev. Drug Discovery* **2003**, *2*, 790.
- [99] P. Escoubas, L. Rash, *Toxicol.* **2004**, *43*, 555.
- [100] K. J. Swartz, *Toxicol.* **2007**, *49*, 213.
- [101] K. J. Swartz, *Nature* **2008**, *456*, 891.
- [102] L. R. Phillips, M. Milescu, Y. Li-Smerin, J. A. Mindell, J. I. Kim, K. J. Swartz, *Nature* **2005**, *436*, 857.
- [103] J. Siemens, S. Zhou, R. Piskorowski, T. Nikai, E. A. Lumpkin, A. I. Basbaum, D. King, D. Julius, *Nature* **2006**, *444*, 208.
- [104] H. Takahashi, J. I. Kim, H. J. Min, K. Sato, K. J. Swartz, I. Shimada, *J. Mol. Biol.* **2000**, *297*, 771.
- [105] C. W. Lee, S. Kim, S. H. Roh, H. Endoh, Y. Kodera, T. Maeda, T. Kohno, J. M. Wang, K. J. Swartz, J. I. Kim, *Biochemistry* **2004**, *43*, 890.
- [106] M. D. Reily, V. Thanabal, M. E. Adams, *J. Biomol. NMR* **1995**, *5*, 122.
- [107] D. O. Omecinsky, K. E. Holub, M. E. Adams, M. D. Reily, *Biochemistry* **1996**, *35*, 2836.