Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 299–302

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

An Efficient and Versatile Synthesis of Acylpolyamine Spider Toxins

Ken-ichi Nihei,[a] Massuo J. Kato,[a] Tetsuo Yamane,[b,d] Mario S. Palma[c,d] and Katsuhiro Konno[c,d,*]

[a]*Institute of Chemistry, University of São Paulo, São Paulo, SP 05508-900, Brazil*
[b]*Laboratory of Molecular Toxinology, Institute Butantan, São Paulo, SP 05503-900, Brazil*
[c]*Center of Study of Social Insects, Department of Biology, Institute of Biosciences of Rio Claro, São Paulo State University, Rio Claro, SP 13506-900, Brazil*
[d]*Center for Applied Toxinology, CEPID/FAPESP, São Paulo, SP 05468-901, Brazil*

Received 10 September 2001; accepted 29 October 2001

Abstract—An efficient and versatile synthesis of acylpolyamine spider toxins was developed based on the structural classification of the *Nephila* and *Nephilengys* spider toxins using the 2-nitrobenzenesulfonamide protecting group. The naturally occurring toxins **1**–**5** representing each structural type have been efficiently synthesized by this method in a high overall yield with few steps.

Acylpolyamine toxins are a class of compounds isolated from the venoms of spiders and wasps. The novel structural features and remarkable inhibitory effect on glutamate receptors have prompted much interest in the chemical and pharmacological studies of the acylpolyamine toxins.[1] Much effort has been devoted to synthesizing these toxins and their analogues in order to elucidate the structure–activity relationships (SAR) and mode of action,[2,3] which would lead to useful analogues for neurobiological research and developing therapeutic agents and insecticides.

Recent studies using new, highly sensitive analytical methods with LC–MS and MS/MS revealed that the *Nephila* and *Nephilengys* spider venom glands contain a complex mixture of up to 40 closely related acylpolyamine toxins.[4] The majority of these toxins have not been previously detected by the classical analytical method, which prompted renewed interest in the SAR and mode of action for the acylpolyamine spider toxins. With this interest in mind, we now report a novel synthetic method for the acylpolyamine spider toxins **1**–**5** which is highly efficient and versatile for all structural types of naturally occurring toxins and their analogues.

The structures of the *Nephila* and *Nephilengys* spider toxins consist of three elements: a lipophilic head, a polyamine backbone, and a polyamine chain terminal, and they are linearly connected in this order. There are a variety of each element and the combination of each element results in a complex mixture of the venom gland constituents. These toxin structures are classified into the generalized structures, Types A–E, based on the distinct polyamine backbone structure (Fig. 1).[4] Figure 2 shows the representative toxins for each type. This classification is suitable for a synthetic strategy, that is, construction of the polyamine backbone, followed by successive connection of the lipophilic head and polyamine chain terminal would afford a variety of natural toxins as well as their analogues. On the other hand, Fukuyama et al. reported that using the 2-nitrobenzenesulfonamide (Ns) group as both a protecting and activating group (the Ns-strategy) is exceptionally versatile for the preparation of a variety of secondary amines,[5] and utilized this protocol for the synthesis of

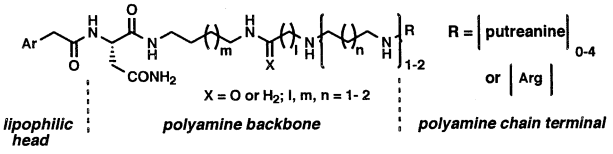


Figure 1. Generalized structure of acylpolyamine spider toxins.

*Corresponding author. Tel.: +55-19-534-8523; fax: +55-19-534-0009; e-mail: kk-gon@rc.unesp.br

0960-894X/02/$ - see front matter
PII: S0960-894X(01)00733-8

the spider toxins HO-416b and Agel-489, demonstrating its utility for the synthesis of acylpolyamine toxins.[6] Our synthetic plan is based on the structural classification of the *Nephila* and *Nephilengys* spider toxins by applying Fukuyama's protocol, aimed at the efficient and versatile synthesis of all the structural types of the naturally occurring toxins and their analogues.

Scheme 1 shows the synthesis of JSTX-3 (**1**), a Type A toxin and the most well-documented among the acylpolyamine toxins.[7] Condensation of the mono-Ns-cadaverine HCl salt **6**[6] and *N*-^{t}Boc-L-asparagine *p*-nitrophenyl ester, followed by removal of the Ns group with our newly developed procedure[8] gave the left-hand segment **8** in high yield.[9] The right-hand segment **9** was prepared from mono-Ns-putrescine **7**[6] in excellent yield through sequential reactions by Michael addition to methyl acrylate, protection of the secondary amine by the Cbz group and hydrolysis of the methyl ester. Coupling of the amine **8** with the succinimide ester prepared from **9** afforded the polyamine backbone precursor **10** in 90% yield. Alkylation of **10** with *N*-Cbz-3-bromopropyl amine in the presence of Cs_2CO_3 furnished the desired polyamine backbone **11** in 92% yield. After removal of the Boc protective group of **11** by TFA, the resultant amine was condensed with dibenzyl-2,4-dihydroxyphenylacetic acid *N*-hydroxysuccinimide ester[10] to give the fully protected JSTX-3 **12** in 87% yield. Successive removal of the Ns and Cbz protective groups by 2-mercaptoethanol and hydrogenation, respectively, afforded **1** in high yield. Thus, JSTX-3 (**1**)[11] was synthesized from mono-Ns-cadaverine **6** in a 39% overall yield via nine steps.

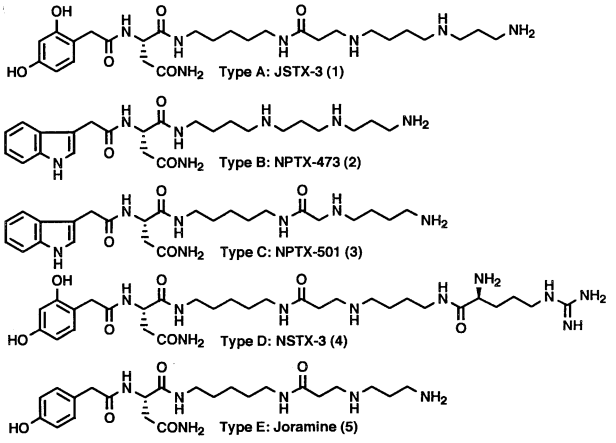
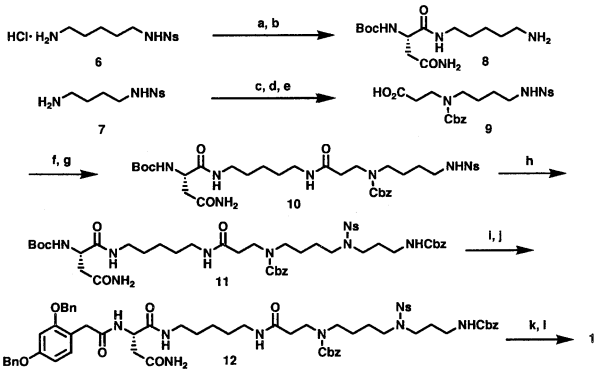


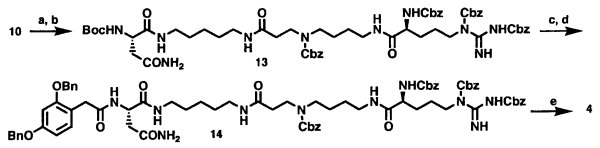
Figure 2. Structures of acylpolyamine spider toxins.



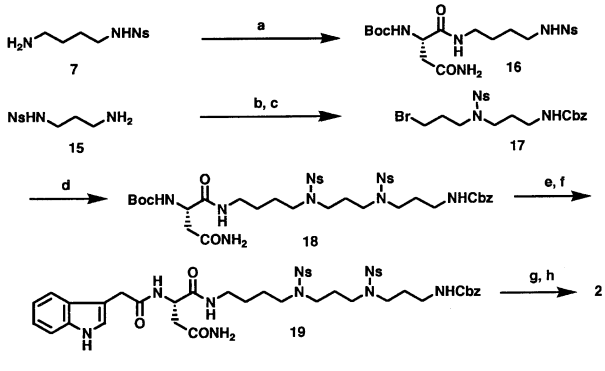
Scheme 1. Synthesis of JSTX-3 (**1**). Reagents and conditions: (a) *N*-^{t}Boc-L-Asn-ONp, Et_3N/DMF, 0 °C to rt, 0.5 h, 97%; (b) 2-mercaptoethanol, DBU/DMF, rt, 0.5 h, 85%; (c) methyl acrylate/EtOH, rt, 5 h; (d) CbzCl, Et_3N/CH_2Cl_2, 0 °C to rt, 2 h, 86% (two steps); (e) NaOH/H_2O–MeOH, 0 °C to rt, 1 h, 96%; (f) HOSu, DCC/CH_2Cl_2, 0 °C, 5 h; (g) **8**, DMF, rt, 0.5 h, 90% (two steps); (h) *N*-Cbz-3-bromopropylamine, Cs_2CO_3/DMF, 50 °C, 1 h, 92%; (i) TFA/$CHCl_3$, 0 °C to rt, 1 h; (j) dibenzyl-2,4-dihydroxybenzoic acid–OSu, Et_3N/DMF, rt, 2 h, 87% (two steps); (k) 2-mercaptoethanol, DBU/DMF, rt, 0.5 h; (l) H_2–Pd(OH)$_2$/AcOH, rt, 3 h, 66% (two steps).

The polyamine backbone of Type D is quite similar to that of Type A and missing only the 1,3-diaminopropane unit at the right end.[4a] Therefore, the toxins of these two types can be synthesized from a common polyamine precursor. Thus, the Type D toxin NSTX-3 (**4**) was synthesized in a manner similar to that for JSTX-3 as shown in Scheme 2. Removal of the Ns protective group of **10** in 94% yield,[8] followed by condensation with tri-Cbz-arginine furnished **13** in 81% yield. Deprotection of the Boc protective group of **13** by TFA, and subsequent condensation with the 2,4-dihydroxyphenylacetic acid *N*-hydroxysuccinimide ester afforded the fully protected toxin **14** in 87% yield. Finally, hydrogenation of **14** afforded NSTX-3 (**4**)[11] in a 49% overall yield from **10**.

Scheme 3 shows the synthesis of the Type B toxin NPTX-473 (**2**). This polyamine backbone has unique features such that there is no amide bond and the putrescine (C_4) unit instead of the cadaverine (C_5) unit



Scheme 2. Synthesis of NSTX-3 (**4**). Reagents and conditions; (a) 2-mercaptoethanol, DBU/DMF, rt, 0.5 h; (b) tri-Cbz-arginine-OSu, DMF, rt, 1 h, 76% (two steps); (c) TFA/$CHCl_3$, 0 °C to rt, 1 h; (d) dibenzyl-2,4-dihydroxybenzoinc acid–OSu, Et_3N/DMF, rt, 2 h, 87% (two steps); (e) H_2–Pd(OH)$_2$/AcOH, rt, 4 h, 74%.

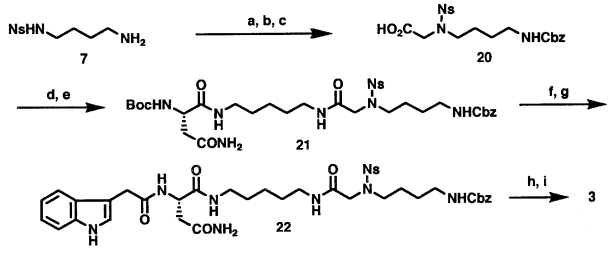


Scheme 3. Synthesis of NPTX-473 (**2**). Reagents and conditions: (a) *N*-^{t}Boc-L-Asn-ONp, DMF, 0 °C to rt, 2 h, 95%; (b) CbzCl, Et_3N/CH_2Cl_2, 0 °C to rt, 1 h, 88%; (c) 1,3-dibromopropane, Cs_2CO_3/DMF, 50 °C, 0.5 h, 91%; (d) **16**, Cs_2CO_3–TBAI/DMF, 70 °C, 1 h, 94%; (e) TFA/$CHCl_3$, 0 °C to rt, 1 h; (f) indoleacetic acid–OSu, Et_3N/DMF, rt, 84% (two steps); (g) 2-mercaptoethanol, DBU/DMF, rt, 0.5 h; (h) H_2–Pd(OH)$_2$/AcOH, rt, 2 h, 60% (two steps).

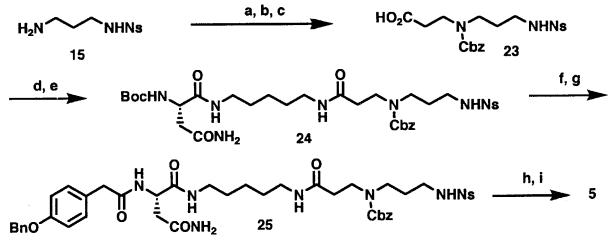
connects to the asparagine moiety. Alkylation of the left-half segment **16**, obtained from coupling of the mono-Ns-putrescine **7**[6] with *N*-^{t}Boc-L-asparagine *p*-nitrophenyl ester in 95% yield, with the right-half segment **17**, produced from the mono-Ns-1,3-diaminopropane **15**[6] by the Cbz protection and subsequent alkylation with 1,3-dibromopropane in high yield, gave the desired polyamine backbone **18** in 94% yield. The Boc deprotection of **18**, followed by condensation with indoleacetic acid *N*-hydroxysuccinimide ester[10] gave the fully protected toxin **19** in 84% yield. Deprotection of **19** in the usual manner finally afforded NPTX-473 (**2**)[11] in a 45% overall yield from **7** via six steps.

The β-alanine unit in Type D is replaced by the glycine unit in Type C.[4c] Therefore, starting with methyl bromoacetate and basically the same method as that for Type D would afford Type C toxins. Thus, as shown in Scheme 4, the requisite right-hand segment **20** was obtained from **7** by a series of the reactions of Cbz protection, alkylation with methyl bromoacetate and hydrolysis in a high overall yield. The treatment of **20** with a similar process as that used for **18** led the Type C toxin NPTX-501 (**3**)[11] in a 33% overall yield from **7** via nine steps.

Similar to Type C, the Type E polyamine backbone is only slightly different from Type D, that is, the 1,3-diaminopropane (C_3) unit is on the right end instead of the putrescine (C_4) unit.[4d] Accordingly, starting with the mono-Ns-1,3-diaminopropane **15** and using the same method as that for the Type D would give the Type E toxin. Scheme 5 shows the synthesis of joramine (**5**). As anticipated, the polyamine backbone **24** was smoothly obtained from **15** through the acid **23**, and subsequent treatment in the usual manner gave the Type E toxin joramine (**5**)[11] in a 36% overall yield from **15** via nine steps.



Scheme 4. Synthesis of NPTX-501 (**3**). Reagents and conditions: (a) CbzCl, Et_3N/CH_2Cl_2, 0 °C to rt, 1 h, 95%; (b) methyl bromoacetate, Cs_2CO_3/DMF, 50 °C, 0.5 h, 86%; (c) $NaOH/H_2O$–MeOH, 0 °C to rt, 2 h, 86%; (d) HOSu, DCC/CH_2Cl_2, 0 °C, 4 h; (e) **8**, DMF, rt, 0.5 h, 83% (two steps); (f) $TFA/CHCl_3$, 0 °C to rt, 1 h; (g) indoleacetic acid–OSu, Et_3N/DMF, rt, 2 h, 88% (two steps); (h) 2-mercaptoethanol, DBU/DMF, rt, 0.5 h; (i) H_2–$Pd(OH)_2$/AcOH, rt, 2 h, 65% (two steps).



Scheme 5. Synthesis of joramine (**5**). Reagents and conditions: (a) methyl acrylate/EtOH, rt, 5 h; (b) CbzCl, Et_3N/CH_2Cl_2, 0 °C to rt, 2 h, 86% (two steps); (c) $NaOH/H_2O$–MeOH, 0 °C to rt, 1 h, 85%; (d) HOSu, DCC/CH_2Cl_2, 0 °C, 5 h; (e) **8**, DMF, rt, 0.5 h, 82% (two steps); (f) $TFA/CHCl_3$, 0 °C to rt, 1 h; (g) *O*-benzyl-*p*-hydroxybenzoic acid–OSu, Et_3N/DMF, rt, 2 h, 88% (two steps); (h) 2-mercaptoethanol, DBU/DMF, rt, 0.5 h; (i) H_2–$Pd(OH)_2$/AcOH, rt, 3 h, 69% (two steps).

Thus, we have developed an efficient and versatile synthesis of acylpolyamine spider toxins based on the structural classification of the *Nephila* and *Nephilengys* spider toxins using the 2-nitrobenzenesulfonamide group (the Ns-strategy). The naturally occurring toxins **1**–**5** representing each structural type have been efficiently synthesized by this method in a high overall yield with few steps. This method is so versatile that it would allow us to synthesize a variety of analogues as well as naturally occurring toxins. Therefore, it would be highly useful for SAR and mode of action studies of the acylpolyamine toxins in more detail. Studies along this line are currently underway in this laboratory.

Acknowledgements

We are grateful to Drs. Toshiyuki Kan (The University of Tokyo) and Shojiro Maki (The University of Electro-Communications) for their helpful discussions and technical assistance. Thanks are also due to Dr. H. Naoki (Suntory Institute for Bioorganic Research) for the measurement of the MS spectra. This work was supported by the grant from the State of São Paulo Research Foundation (FAPESP). K.K. is a fellow from FAPESP (proc. 1998/11693-5). M.S.P. is a researcher for the Brazilian Council for Scientific and Technological Development (CNPq, 500079/90-0).

References and Notes

1. (a) For reviews, see: McCormick, K. D.; Meinwald, J. *J. Chem. Ecol.* **1993**, *19*, 2411. (b) Schäfer, A.; Benz, H.; Fiedler, W.; Guggisberg, A.; Bienz, S.; Hesse, M. In *The Alkaloids: Chemistry and Pharmacology*, Cordell, G. A., Brossi, A., Eds.; Academic: San Diego, 1994; Vol. 45, p 1. (c) Mueller, A. L.; Roeloffs, R.; Jackson, H. In *The Alkaloids: Chemistry and Pharmacology*, Cordell, G. A., Brossi, A., Eds.; Academic: San Diego, 1995; Vol. 46, p 65.
2. For leading references on the synthesis, see: (a) Saito, H.; Yuri, E.; Miyazawa, M.; Itagaki, Y.; Nakajima, T.; Miyashita, M. *Tetrahedron Lett.* **1998**, *39*, 6479. (b) Wang, F.; Manku, S.; Hall, D. G. *Org. Lett.* **2000**, *2*, 1581.
3. For leading references on the SAR studies, see (a) Moe, S. T.; Smith, D. L.; Chien, Y.; Raszkiewicz, J. L.; Artman, L.; Mueller, A. L. *Pharm. Res.* **1998**, *15*, 31. (b) Strømgaard, K.; Brierly, M. J.; Andersen, K.; Sløk, F. A.; Mellor, I. R.; Usherwood, P. N. R.; Krogsgaard-Larsen, P.; Jaroszewski, J. W. *J. Med. Chem.* **1999**, *42*, 5224. (c) Bixel, M. G.; Krauss, M.; Liu, Y.; Bolognesi, M. L.; Rosini, M.; Mellor, I. S.; Usherwood, P. N. R.; Melchiorre, C.; Nakanishi, K.; Hucho, F. *Eur. J. Biochem.* **2000**, *267*, 110.
4. (a) Itagaki, Y.; Fujita, T.; Naoki, H.; Yasuhara, T.; Andriantsiferana, M.; Nakajima, T. *Nat. Toxins* **1997**, *5*, 1. (b)

Palma, M. S.; Itagaki, Y.; Fujita, T.; Hisada, M.; Naoki, H.; Nakajima, T. *Nat. Toxins* **1997**, *5*, 47. (c) Palma, M. S.; Itagaki, Y.; Fujita, T.; Naoki, H.; Nakajima, T. *Toxicon* **1998**, *36*, 485. (d) Hisada, M.; Fujita, T.; Naoki, H.; Itagaki, Y.; Irie, H.; Miyashita, M.; Nakajima, T. *Toxicon* **1998**, *36*, 1115. For a review, see: Itagaki, Y.; Nakajima, T. *J. Toxicol.-Toxin Rev.* **2000**, *19*, 23

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

6. (a) Hidai, Y.; Kan, T.; Fukuyama, T. *Tetrahedron Lett.* **1999**, *40*, 4711. (b) Hidai, Y.; Kan, T.; Fukuyama, T. *Chem. Pharm. Bull.* **2000**, *48*, 1570.

7. Aramaki, Y.; Yasuhara, T.; Higashijima, T.; Yoshioka, M.; Miwa, A.; Kawai, N.; Nakajima, T. *Proc. Japan Acad.* **1986**, *62B*, 259. For a review, see: Kawai, N. *J. Toxicol.-Toxin Rev.* **1991**, *10*, 131

8. Nihei, K.; Kato, M. J.; Yamane, T.; Palma, M. S.; Konno, K. *Synlett* **2001**, 1167.

9. The spectral data of this compound were consistent with those previously reported: Chiba, T.; Akizawa, T.; Matsukawa, M.; Nishi, M.; Kawai, N.; Yoshioka, M. *Chem. Pharm. Bull.* **1996**, *44*, 972.

10. Matsushita, M.; Kanemasa, T.; Hatakeyama, S.; Irie, H.; Toki, T.; Miyashita, M. *Tetrahedron* **1995**, *51*, 10687.

11. Thus synthesized JSTX-3 (**1**) was identical with authentic specimen in HPLC co-elution and the spectroscopic data were consistent with those previously reported.[10] The toxins **2**–**5** were fully characterized by the spectroscopic analyses.