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Synthetic Studies on Spider Neurotoxins (I): Total Synthesis of Nephilatoxins (NPTX-9 and NPTX-11), New Neurotoxins of Joro Spider (*Nephila clavata*)

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Abstract: The first and efficient synthesis of Nephilatoxins (NPTX-9 and NPTX-11), new neurotoxins of Joro spider (*Nephila clavata*), has been achieved by employing a key azide intermediate as the polyamine unit.

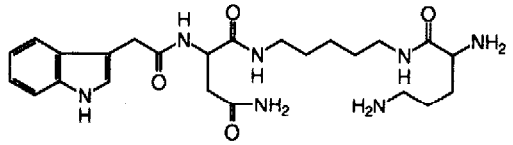
Spider toxins such as NSTX-3, isolated from the Papua New Guinean spider (*Nephila maculata*),[1] and JSTX-3, isolated from the Joro spider (*Nephila clavata*),[1] have been demonstrated to be potent blockers of glutaminergic neuromuscular transmission in the mammalian synapse[2] as well as invertebrates.[3] Nephilatoxins (NPTX-1~12), new neurotoxins recently isolated from the Joro spider (*Nephila clavata*),[4] have also been shown to have potent activities on the mast cell degranulation as well as specific interruption of glutaminergic neuromuscular transmission.[4b] In particular NPTXs have been shown to have more potent histamine release activities from rat peritoneal mast cells than NSTX-3 and JSTX-3.[4b] These toxins are structurally similar to NSTX and JSTX in that they possess a polycationic straight chain composed of basic amino acids and polyamines. However, in contrast to the latters, an indole-3-acetyl moiety instead of a 2,4-dihydroxyphenylacetyl moiety is connected at the N-termini of the polycationic chain of NPTXs, and the half of NPTXs contain ornithine as an amino acid unit which has not been found in other spider toxins.[5]

Although spider toxins are rapidly emerging as unique tools for understanding excitatory amino acid transmission and related pharmacology,[2,3] limited quantities have impeded their pharmacological evaluation and ongoing biological studies. We report here the first and efficient (practical) synthesis of NPTXs, NPTX-9 and NPTX-11, in which the azido group is employed as key functionality enabling the effective incorporation of the polyamine units.[6,7]

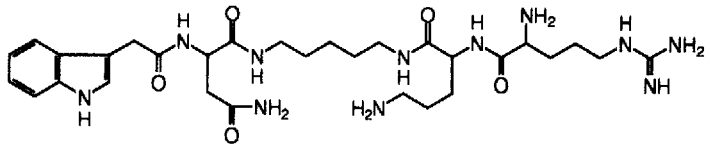
NPTX-9 and NPTX-11 have been assigned to the structures shown in Fig. 1,[4] though the stereochemistry of amino acids has not been defined. NPTX-9, one of the most potent glutamate blockers among NPTXs,[4b] consists of five components, i.e., an indole-3-acetyl-asparaginyl-cadaverine-ornithine-

arginine structure, while NPTX-11 is composed of the same structural units lacking of a terminating arginine residue of NPTX-9.

Fig. 1



NPTX-11



NPTX-9

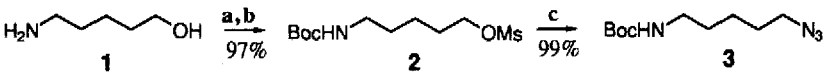
The key point of the synthesis of spider toxins lies in the effective construction of the common polyamine units such as cadaverine (1,5-diaminopentane) and putreanine (8-amino-4-azaoctanoic acid) since each terminus of these α,ω-diamines is linked to a different amino acid, hence the use of the suitably masked α,ω-diamine or its equivalent is needed.[8] In our synthetic strategy azide intermediates were designed as such ideally suited equivalents.

5-Azido-1-N-Boc-aminopentane (**3**), a cadaverine equivalent, was readily prepared starting from commercially available 5-amino-1-pentanol (**1**) in 96% overall yield in three steps[9] (Scheme 1): 1) protection of the amino group with $(Boc)_2O$; 2) mesylation; 3) substitution with NaN_3.

With the cadaverine equivalent **3** in hand, we set out first to synthesize NPTX-11 by incorporating the L-forms of asparagine and ornithine.[10] Removal of a Boc group of **3** with trifluoroacetic acid (TFA) in CH_2Cl_2 followed by treatment with N-Boc-L-asparagine *p*-nitrophenyl ester in DMF containing triethylamine (TEA) gave **4** in 69% yield (Scheme 2). Similar treatment of **4** with TFA in CH_2Cl_2 followed by coupling with indole-3-acetic acid *p*-nitrophenyl ester in DMF in the presence of TEA furnished **5** having an indol-3-acetyl-asparaginyl-cadaverine structure in 76% yield. Both compounds **4** and **5** were easily purified by silica gel column chromatography, and the structures were confirmed by IR and ^{1}H-NMR. Catalytic hydrogenation of the azide **5** over 10% Pd-C in EtOH and subsequent coupling of the resulting amine with N^{α}-Boc-N^{δ}-Z-L-ornithine *p*-nitrophenyl ester in DMF gave the protected NPTX-11 (**6**) as amorphous solids in 89% yield. Removal of the Boc and the Z groups of **6** was readily accomplished under previously described conditions, i.e., TFA in CH_2Cl_2 followed by catalytic hydrogenation, yielding NPTX-11, which was purified by HPLC using a TOSOH TSK-gel ODS-120T column (water : acetonitrile : TFA= 85 : 15 : 0.1). The structure of the toxin, obtained in 51% yield, was confirmed by 270 MHz ^{1}H-NMR (D_2O), FD-MS 488 (M^+), and HPLC.

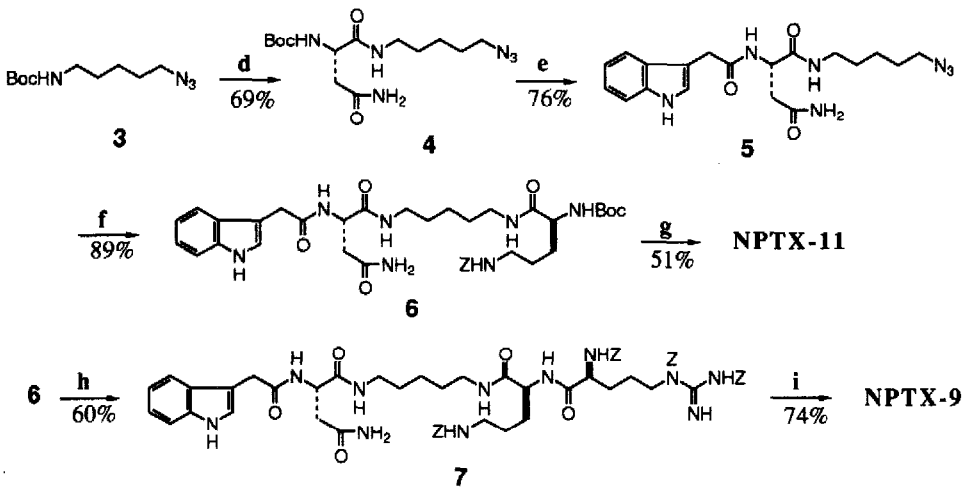
On the other hand, treatment of **6** with TFA in CH_2Cl_2 followed by coupling of the resulting amine with tri-Z-L-arginine N-hydroxysuccinimide ester in DMF containing TEA yielded the fully protected NPTX-9 (**7**) in 60% yield (Scheme 2). The product could be purified by recrystallization from aqueous AcOH and the structure was confirmed by ^{1}H-NMR. Finally, deprotection of all the Z groups was readily accomplished by catalytic hydrogenation over 10% Pd-C in AcOH containing TFA to afford NPTX-9. The product was purified by HPLC using a JASCO Megapak SIL-C_{18} column (gradient: A. 0.1% aq. TFA; B. 50% aq. acetonitrile containing 0.1% TFA) and obtained as TFA salt in 74% yield. All the data of the synthetic

Scheme 1



***Reagents*: a.** $(Boc)_2O$, aq. Na_2CO_3, r. t. 12 h, 99%; **b.** MsCl, pyridine, CH_2Cl_2, O °C, 1 h; **c.** NaN_3, DMF, r. t. 12 h, 97% for the 2 steps.

Scheme 2



***Reagents*: d.** TFA, CH_2Cl_2, r. t. 3 h; Boc-Asn-ONp, TEA, DMF, r. t. 12 h, 69%; **e.** TFA, CH_2Cl_2, r. t. 3 h; Indole-3-acetic acid *p*-nitrophenyl ester, TEA, DMF, r. t. 12 h, 76%; **f.** 10% Pd-C, H_2 (1 atm), EtOH, 3.5 h; Boc-Orn (Z)-ONp, TEA, DMF, r. t. 17 h, 89%; **g.** TFA, CH_2Cl_2, r. t. 3 h; 10% Pd-C, H_2 (1 atm), EtOH, 5 h, 51%; **h.** TFA, CH_2Cl_2, r. t. 4 h; Z-Arg(Z_2)-ONSu, TEA, DMF, r. t. 12 h, 60%; **i.** 10% Pd-C, H_2 (1 atm), TFA, AcOH, 5 h, 74%.

compound (HPLC, 270 MHz ^{1}H-NMR, ^{13}C-NMR, FD-MS 644 (M^{+}), FAB-MS 645 (M+1)) was agreement with the proposed structure. The biological evaluation of the synthetic NPTX-9 and NPTX-11 by histamine release activity from rat peritoneal mast cells was also conformed to those of natural toxins.

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8) In general preparation of monocarbamates from α,ω-diamines is not satisfied owing to low yields and tedious manipulations.

9) During our study similar strategy for the preparation of N^{α}-Boc α,ω–diamines from aminoalcohols has been reported. P. W. Mattingly, *Synthesis*, **1990**, 366.

10) The structures of NSTX and JSTX have been established to be composed of L-amino acids.[1,7)]

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