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# Synthetic Studies on Spider Neurotoxins (II): Total Synthesis of Nephilatoxins (NPTX-10 and NPTX-12), New Neurotoxins of Joro Spider (*Nephila clavata*)

**Masaaki Miyashita,*[a] Hideaki Sato,[b] Masayuki Matsushita,[a] Youko Kusumegi,[a] Takashi Toki,[c] Akira Yoshikoshi,[b] Tetsuji Yanami,[c] Yasuo Kikuchi,[d] Chikahisa Takasaki,[d] Terumi Nakajima,[e] and Hiroshi Irie[a]**

[a]Faculty of Pharmaceutical Sciences, Nagasaki University, Nagasaki 852, Japan; [b]Chemical Research Institute of Non-Aqueous Solutions, Tohoku University, Sendai 980, Japan; [c]Research Center, Daicel Chemical Industries, LTD., Himeji 671-12, Japan; [d]Department of Chemistry, Tohoku University, Sendai 980, Japan; [e]Faculty of Pharmaceutical Sciences, University of Tokyo, Tokyo 113, Japan

***Abstract***: The first and highly efficient synthesis of NPTX-10 and NPTX-12, new neurotoxins of Joro spider (*Nephila clavata*), utilizing key azide intermediates is described.

Spider toxins have been recognized to be potent blocking agents of excitatory amino acid neurotransmission and indispensable tools in the field of pharmacological and physiological research.[1,2] Nephilatoxins (NPTX-1~12), new neurotoxins recently isolated from the Joro spider (*Nephila clavata*),[3] have been expected not only as specific blockers of glutaminergic neuromuscular transmission but also as lead compounds for pharmaceutical and pesticide research,[3b] though the quantitative supply remains to be solved.

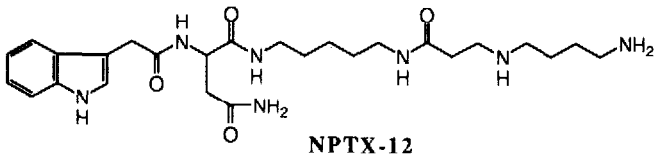
In the preceding paper, we reported the first synthesis of Nephilatoxins, NPTX-9 and NPTX-11, in which the azido group was utilized as key functionality for the incorporation of a polyamine unit.[4] In this communication, we describe the highly efficient synthesis of NPTX-10 and NPTX-12 which are composed of different structural units from the formers[3] based on the similar azide strategy, wherein two azide intermediates play crucial roles in the construction of the characteristic polyamines of the toxins.

NPTX-10, the most potent glutamate blocker among NPTXs,[3b] is structurally similar to NSTX-3[5] and consists of five components containing two characteristic polyamines, cadaverine (1,5-diaminopentane) and putreanine (8-amino-4-azaoctanoic acid),[6] as shown in Fig. 1. NPTX-10 also has an indole-3-acetyl-asparaginyl-cadaverine structure which is common to NPTXs-9 and 11,[3] and putreanine and arginine are linked to this subunit. NPTX-12 has the same structural units with NPTX-10 except a terminating arginine residue.[3]

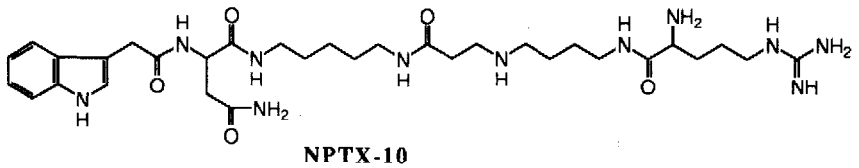
Methyl 8-azido-N-Boc-4-azaoctanoate (**3**), the key putreanine equivalent, was straightforwardly synthesized from 4-amino-1-butanol (**1**) by a four-step reaction sequence in 70% overall yield (Scheme 1)[7]: 1) conjugate addition to methyl acrylate; 2) protection of the amino group; 3) mesylation; 4) substitution with $NaN_3$. The corresponding *p*-nitrophenyl ester **4** was readily derived from **3** by alkaline hydrolysis and

subsequent esterification with *p*-nitrophenol and DCC in 78% overall yield.

**Fig. 1**



**NPTX-12**

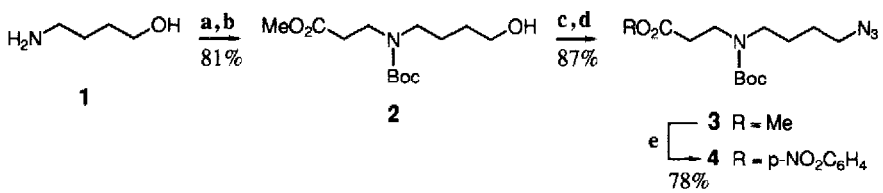


**NPTX-10**

Synthesis of NPTX-12 was carried out by coupling of the common left half-segment **5**, which was used for the synthesis of NPTXs-9 and 11,[4] with the key intermediate **4** (Scheme 2). Thus catalytic hydrogenation of the azide **5** into amine followed by coupling with the ester **4** in DMF in the presence of TEA gave the protected NPTX-12 (**6**) as amorphous solids in 76% yield. Subsequent removal of the Boc group with TFA followed by catalytic hydrogenation of the azido group furnished NPTX-12. 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). All the data of the synthetic compound (HPLC, 400 MHz $^1$H-NMR, $^{13}$C-NMR, FD-MS 516 ($M^+$)) was agreement with the proposed structure.[3b]

On the other hand, hydrogenation of the azide **6** and subsequent coupling of the resulting amine with tri-Z-L-arginine N-hydroxysuccinimide ester in DMF containing TEA yielded the fully protected NPTX-10 (**7**) in 82% yield (Scheme 2). The product was purified by flash column chromatography ($CH_2Cl_2$ : acetone : EtOH = 20 : 10 : 3) and the structure was confirmed by $^1$H-NMR. Finally, deprotection of the Boc group with TFA

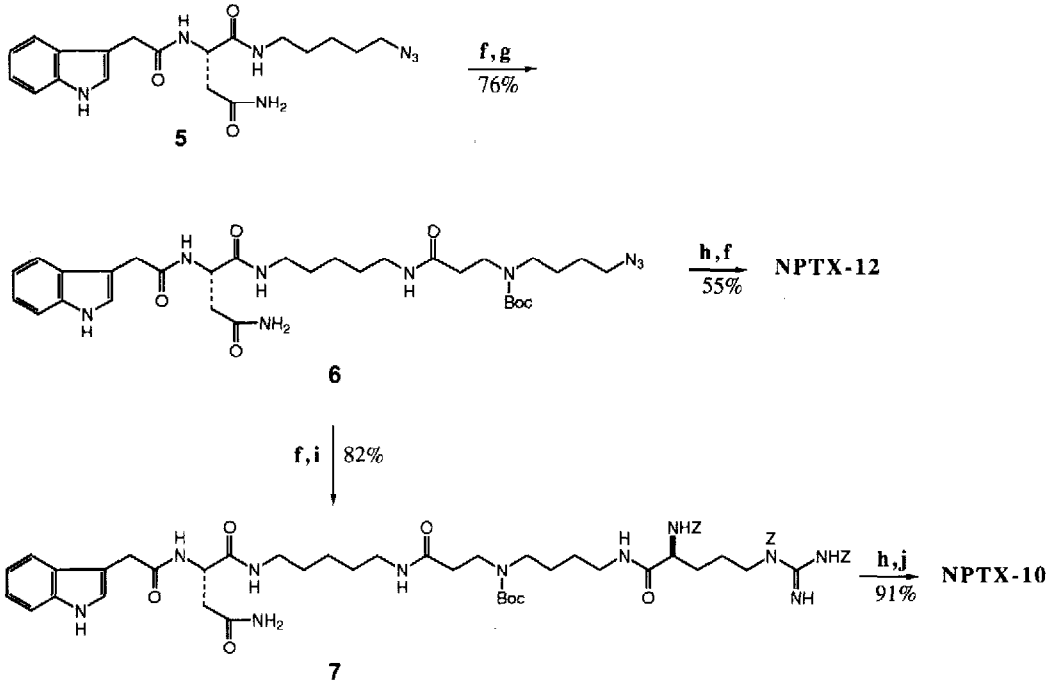
**Scheme 1**



***Reagents***: **a.** $CH_2$=$CHCO_2Me$, 0 °C, 1 h; **b.** Boc-ON, TEA, aq. acetone, r. t. 12 h; **c.** MsCl, pyridine, $CH_2Cl_2$, O °C, 4 h; **d.** $NaN_3$, DMF, r. t., 2 d; **e.** 1N NaOH, MeOH, 60 °C, 1.5 h ; *p*-$NO_2C_6H_4OH$, DCC, AcOEt, r. t. 12 h.

in $CH_2Cl_2$ followed by removal of all the Z groups by catalytic hydrogenation over 10% Pd-C in EtOH cleanly produced NPTX-10. The product was purified by HPLC using the above described conditions and obtained as TFA salts in 91% yield. All the data of the synthetic compound ( HPLC, 400 MHz $^1$H-NMR, $^{13}$C-NMR, FD-MS 672 ($M^+$)) was consistent with the proposed structure.[3b] The biological evaluations of the synthetic compounds by histamine release activity from rat peritoneal mast cells were also conformed to those of natural toxins.

**Scheme 2**




***Reagents***: **f.** 10% Pd-C, $H_2$ (1 atm), EtOH, 3 h; **g.** **4**, TEA, DMF, r. t. 12 h; **h.**TFA, $CH_2Cl_2$, r. t. 3 h; **i.** Z-Arg-($Z_2$)-ONSu, TEA, DMF, r. t. 12 h; **j.** 10% Pd-C, $H_2$ (1 atm), EtOH, 15 h.

The present synthesis emphasizes the usefulness of azide intermediates and their synthetic potential in spider toxins. Further extensions of the methodology to other spider toxins are in progress in our laboratory.[8]

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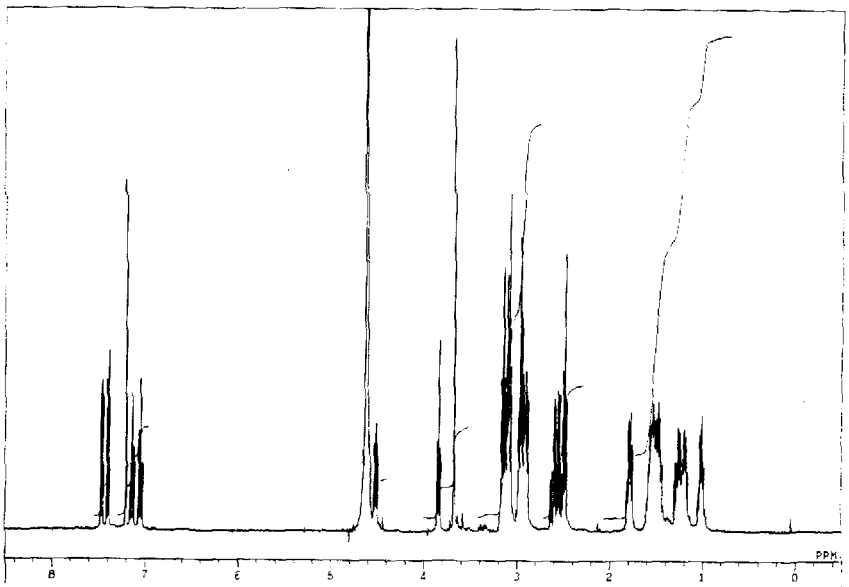


400 MHz $^1$H-NMR spectrum of the synthetic NPTX-10 in $D_2O$ (measured using a JEOL JNM GX-400 spectrometer).

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7) Similarly, methyl 8-azido-4-*N*-Z-azaoctanoate could be prepared from **1** in good overall yield.

8) We have just completed the first synthesis of NPTX-8 based on the similar azide strategy which will be published in near future.

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