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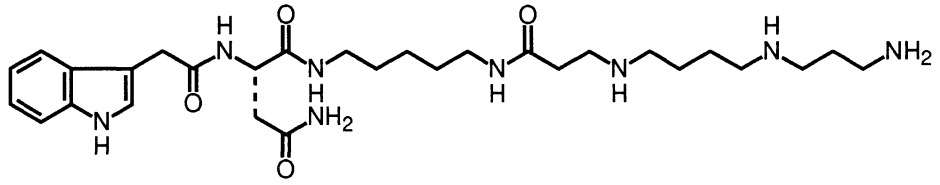
Total Synthesis of Nephilatoxin-8 (NPTX-8), a New Neurotoxin of Joro Spider (*Nephila clavata*)

Masaaki MIYASHITA,* Masayuki MATSUSHITA, Hideaki SATO,
Takashi TOKI,† Terumi NAKAJIMA,†† and Hiroshi IRIE
Faculty of Pharmaceutical Sciences, Nagasaki University, Nagasaki 852
†Research Center, Daicel Chemical Industries, LTD., Himeji 671-12
††Faculty of Pharmaceutical Sciences, The University of Tokyo, Tokyo 113

The first synthesis of Nephilatoxin-8 (NPTX-8), a new neurotoxin of Joro spider (*Nephila clavata*), has been achieved by employing two key azide intermediates for the incorporation of the characteristic polyamines of the toxin, cadaverine and spermidine.

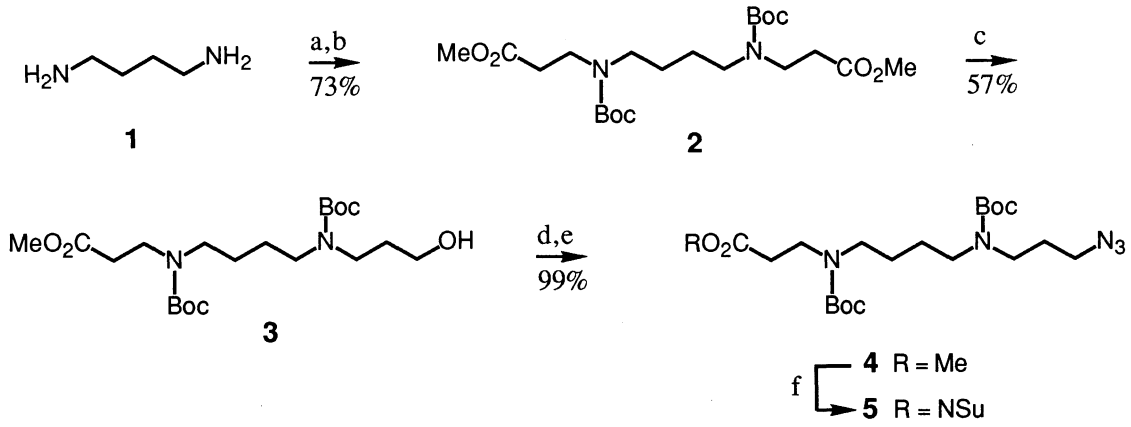
Spider toxins such as NSTX-3,[1] JSTX-3,[1] and Nephilatoxins (NPTXs) [2] have been demonstrated to be potent and specific blockers of glutaminergic neuromuscular transmission and are rapidly emerging as unique tools for understanding excitatory amino acid transmission and related pharmacology.[2-4] Limited quantities have impeded, however, their pharmacological evaluation and ongoing biological studies. We recently reported the first and efficient synthesis of NPTXs, NPTX-9 and 11,[5] and NPTX-10 and 12 [6] by the use of key azide intermediates enabling the effective incorporation of the characteristic polyamine units, cadaverine and putreanine, and opened the practical synthetic routes for these spider toxins.[7] We now wish to report the first synthesis of NPTX-8, which is structurally the third type of Nephilatoxin possessing cadaverine (1,5-diaminopentane) and spermidine (N-(3-aminopropyl)-1,4-diaminobutane) as the polyamine units, based on the extended *azide strategy*. NPTX-8 is structurally similar to JSTX-3 [1] and consists of four components, i. e., indole-3-acetic acid, asparagine, cadaverine, and 9-(3-aminopropyl)putreanine, in which the terminating aromatic moiety is different from that of JSTX-3 having a 2,4-dihydroxyphenylacetyl residue. We successfully synthesized NPTX-8 by employing two key azide intermediates for the incorporation of two characteristic polyamines of the toxin, cadaverine and spermidine.



NPTX-8

Methyl 12-azido-bis-N-Boc-4,9-diazadodecanoate (**4**), the novel 9-(3-aminopropyl)putreanine equivalent, was elaborated from 1,4-diaminobutane (**1**) by a five-step reaction sequence in 41% overall yield (Scheme 1) :

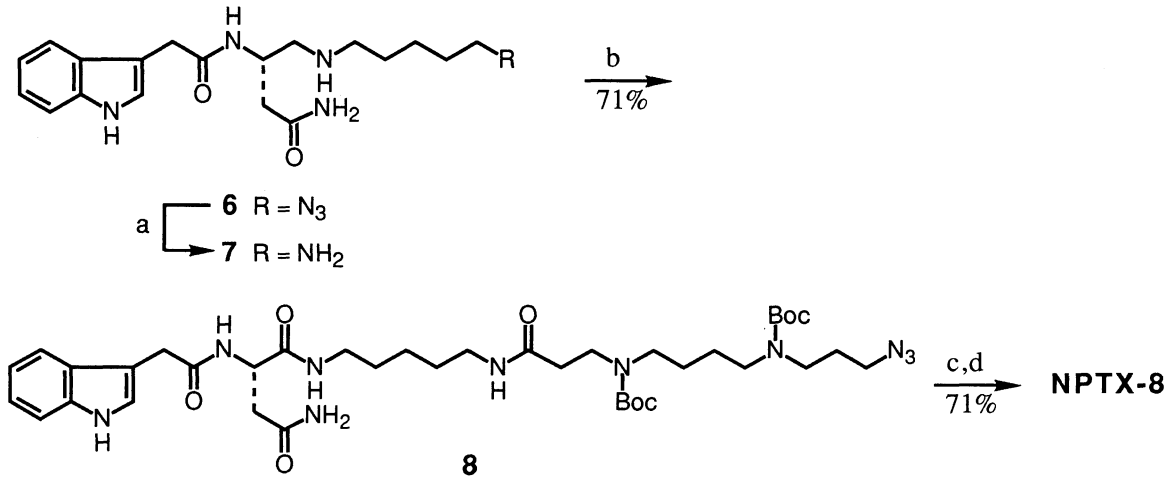
1) conjugate addition of the diamine to methyl acrylate; 2) protection of the amino groups; 3) selective reduction of one ester group; 4) mesylation; 5) substitution with NaN3. The corresponding N-hydroxysuccinimide ester **5** was readily derived from **4** by alkaline hydrolysis and subsequent esterification with N-hydroxysuccinimide and DCC in nearly quantitative yield.



Reagents: a. CH_2=$CHCO_2Me$, 0 °C - r. t., 2 h; b. $(Boc)_2O$, aq. Na_2CO_3, r. t. 12 h; c. $LiBH_4$, aq. THF (THF / H_2O = 50 : 1 ($^v/_v$)), r. t. 6 h; d. MsCl, pyridine, CH_2Cl_2, 0 °C, 12 h; e. NaN_3, DMF, r. t. 12 h; f. 1 mol dm^{-3} NaOH, EtOH, r. t. 2 h ; HONSu, DCC, AcOEt, r. t. 3 h.

Scheme 1.

Synthesis of NPTX-8 was carried out by coupling of the common left half-segment **6**, which was used for the synthesis of NPTXs-9–12,[5,6] with the key 9-(3-aminopropyl)putreanine equivalent **5** (Scheme 2).



Reagents: a. 10% Pd-C, H_2 (1 atm), MeOH, 2 h; b. **5**, TEA, DMF, r. t. 12 h; c. TFA, CH_2Cl_2, r. t. 3 h; d. 10% Pd-C, H_2 (1 atm), EtOH, 2 h.

Scheme 2.

Thus catalytic hydrogenation of the azide **6** gave the amine **7**, which was subjected to the coupling reaction with the active ester **5** in DMF in the presence of triethylamine (TEA) to afford the protected NPTX-8 derivative (**8**) in 71% overall yield after purification by silica gel flash chromatography. Subsequent removal of the two Boc groups with TFA followed by catalytic hydrogenation of the azido group over Pd-C in EtOH furnished NPTX-8 as TFA salts. The product was purified by HPLC using a Tosoh TSK-GEL ODS-120T column (4.6 x 250 mm, 15% acetonitrile containing 0.1% TFA, 1 ml / min, 42 min) whose retention time coincided with that of natural NPTX-8. In addition, all the spectral data of the synthetic compound (400 MHz ^{1}H-NMR, ^{13}C-NMR, FD-MS 572 (M^{+})) were in agreement with the proposed structure.[8] The biological evaluations of the synthetic compound including histamine release activity from rat peritoneal mast cells were also conformed to those of natural toxin.

The present synthesis provides a new and practical route for the synthesis of spider toxins which contain cadaverine and spermidine as polyamine units, and also demonstrates the synthetic potential of *the azide strategy* as well as the previous ones. [5,6] Further extensions of the methodology to other spider toxins are in progress in our laboratory.

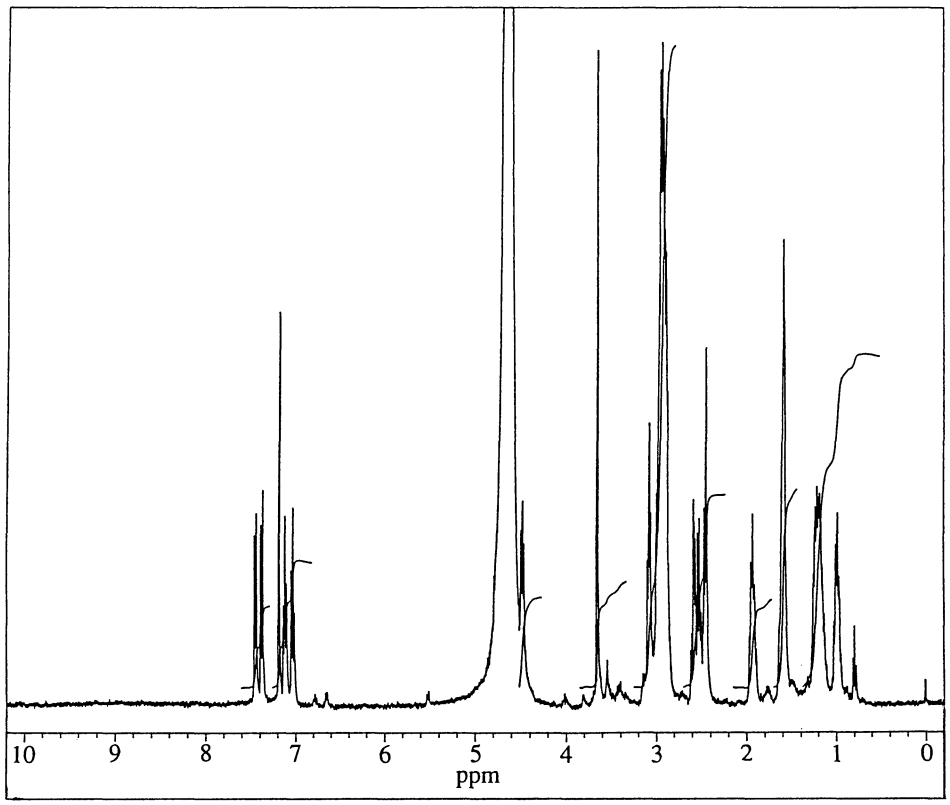


Fig. 1. 400 MHz ^{1}H-NMR spectrum of the synthetic NPTX-8 in D_2O (measured using a JEOL JNM GX-400 spectrometer).

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8) NMR spectrum of natural NPTX-8 has not been measured yet owing to its limited quantity.

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