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Total Synthesis of Nephilatoxin-1 (NPTX-1), a Joro Spider (*Nephila clavata*) Toxin Having a 4-Hydroxyindole Nucleus

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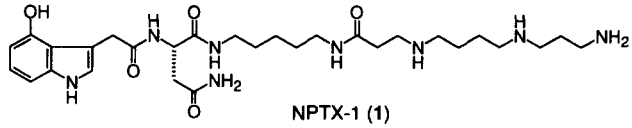
Abstract: The first total synthesis of Nephilatoxin-1 (NPTX-1), a Joro spider (*Nephila clavata*) toxin having a 4-hydroxyindole nucleus, has been achieved by using two key azide intermediates.

Spider toxins such as NSTX-3,[1] JSTX-3,[1] and Nephilatoxins (NPTX-1~12)[2] have been demonstrated to be potent and specific blockers of glutaminergic neurotransmission and are emerging as unique tools for understanding excitatory amino acid neurotransmission and related pharmacology.[2]

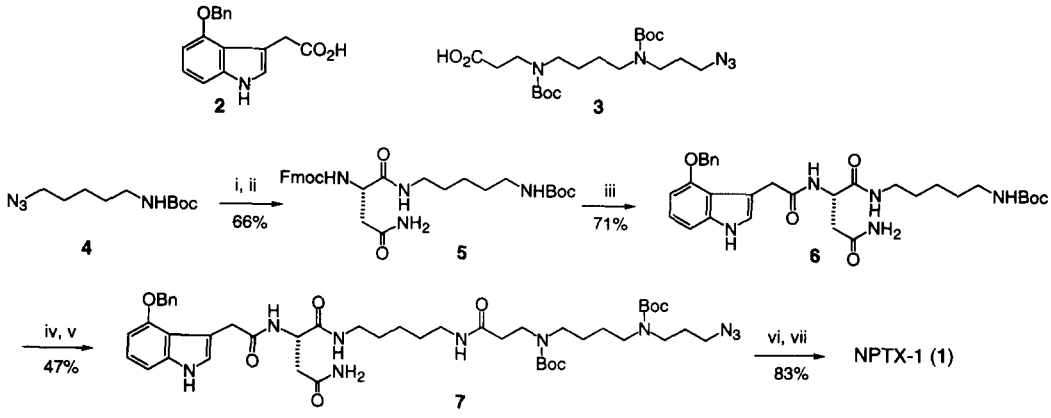
We have achieved so far the chemical synthesis of Joro spider (*Nephila clavata*) toxins, *inter alia* NPTXs having an indole nucleus such as NPTX-9 and 11,[3] NPTX-10 and 12,[4] NPTX-8,[5] NPTX-7,[6] and NPTX-643[7] by using *the azide strategy* and developed the practical synthetic routes for these spider toxins.[8] Nephilatoxins are structurally classified into two groups based on the structure of a terminal aromatic moiety; NPTX-1~6 possess a 4-hydroxyindole nucleus while NPTX-7~12 have an indole core. Very recently the structure of the aromatic moiety of NPTX-1~6 has been revised from the previously proposed 6-hydroxyindole-3-acetic acid to 4-hydroxyindole-3-acetic acid.[9] Although the chemical synthesis of NPTX-7~12 containing an indole nucleus has been established by authors,[8] synthesis of NPTXs having a 4-hydroxyindole nucleus has not been achieved yet. We report here the first total synthesis of NPTX-1 based on *the azide strategy*.



NPTX-1 (**1**)

4-Benzyloxyindole-3-acetic acid (**2**), the aromatic core of NPTX-1, was prepared according to the literatures.[10] The synthesis of **1** started from 5-azido-1-*N*-Boc-aminopentane (**4**), a key azido compound in the synthesis of NPTX-7~12 which is readily obtainable from 5-amino-1-pentanol.[3] After hydrogenation of the azide **4** over PtO_2 in ethanol, the resulting amine was condensed with *N*-(9-fluorenylmethoxy-carbonyl)-L-asparagine (Fmoc-Asn) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl), 1-hydroxybenzotriazole (HOBt), and *N,N*-diisopropylethylamine ($^{i}Pr_2NEt$) in DMF to afford **5** in 66% yield. Removal of the Fmoc group of **5** with $^{i}Pr_2NEt$ in DMF and DMSO followed by treatment with 4-benzyloxyindole-3-acetic acid (**2**) and HOBt furnished the left-half segment **6** composed of 4-hydroxyindole-3-acetyl-asparaginyl-cadaverine units in 71% yield. After removal of the Boc group of **6** in formic acid, the resulting amine was condensed with the spermidine segment **3**[5] in the presence of EDC·HCl, HOBt, and $^{i}Pr_2NEt$ in DMF and DMSO giving rise to the fully protected compound

7 in 47% yield. Finally, catalytic transfer hydrogenation of the benzyl and the terminal azido groups of 7 with 10% Pd-C and ammonium formate in MeOH followed by deprotection of the Boc groups with formic acid in CH_2Cl_2 gave NPTX-1 (1) in 83% yield. The synthetic compound was identified with the natural toxin by HPLC-FAB/MS analyses.[11] Biological evaluation of the synthetic compound and extension of the methodology to other spider toxins are in progress in our laboratories.



Reagents: i. H_2, PtO_2, EtOH; ii. Fmoc-Asn, EDC·HCl, HOBt, $^iPr_2NEt$, DMF; iii. $^iPr_2NEt$, DMF-DMSO (1 : 1), then **2**, EDC·HCl, HOBt; iv. HCO_2H; v. **3**, EDC·HCl, HOBt, $^iPr_2NEt$, DMF-DMSO (1 : 1); vi. 10% Pd-C, HCO_2NH_4, MeOH; vii. HCO_2H, CH_2Cl_2.

Scheme 1

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11 FAB-MS *m/z* 589 $(M+H)^+$; ^1H NMR (270 MHz, CD_3OD) δ: 7.05 (s, 1H), 6.93 (dd, *J*=8.2, 6.5 Hz, 1H), 6.90 (dd, *J*=8.2, 1.9 Hz, 1H), 6.41 (dd, *J*=6.5, 1.9 Hz, 1H), 4.66 (t, *J*=6.3 Hz, 1H), 3.86 (d, *J*=15.3 Hz, 1H), 3.79 (d, *J*=15.3 Hz, 1H), 2.78-3.35 (m, 14 H), 2.68 (d, *J*=6.3 Hz, 2H), 2.52 (t, *J*=6.3 Hz, 2H), 1.98-2.15 (m, 2H), 1.69-1.85 (m, 4H), 1.20-1.43 (m, 4H), 1.10 (qui, *J*=7.1 Hz, 2H).

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