Pergamon

Tetrahedron Letters 39 (1998) 6479–6482

TETRAHEDRON LETTERS

Total Synthesis of NPTX-5 and NPTX-6, Joro Spider (*Nephila clavata*) Toxins Having a Unique Putreanine Trimer and Putreanine Tetramer Acylpolyamine Chain

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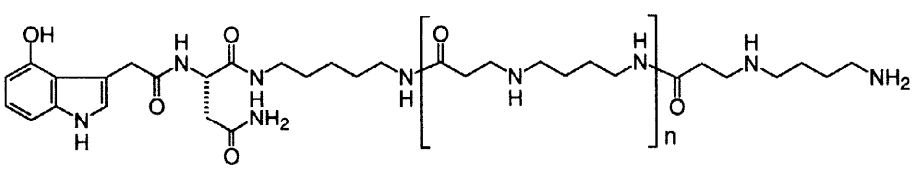
Received 22 May 1998; revised 29 June 1998; accepted 30 June 1998

Abstract: The first total synthesis of NPTX-5 and NPTX-6, Joro spider (*Nephila clavata*) toxins having a 4-hydroxyindole nucleus and a unique putreanine trimer and tetramer acylpolyamine chain, respectively, has been achieved by the iterative use of a key azide compound.

Spider toxins such as NSTX-3,[1] JSTX-3,[1] and Nephilatoxins (NPTX-1~12)[2] are known as potent and specific blockers of glutaminergic neurotransmission and are emerging as unique tools for understanding excitatory amino acid neurotransmission and related pharmacology.[2] Because of the availability of the natural product, however, the chemical synthesis of spider toxins has been required to carry out further pharmacological evaluation and ongoing biological studies.

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 practical synthetic routes for these spider toxins.[8,9] NPTXs are structurally classified into two groups based on the structure of a terminal aromatic moiety, i.e., NPTX-1~6 possess a 4-hydroxyindole nucleus, while NPTX-7~12 have a normal indole core. 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.[10] Very recently, we succeeded in the chemical synthesis of NPTX-1 having a 4-hydroxyindole nucleus and synthetically confirmed its structure.[11] We wish to report herein the first total synthesis of NPTX-5 (**1**) and NPTX-6 (**2**), Joro spider toxins having a 4-hydroxyindole nucleus and a unique putreanine trimer and putreanine tetramer acylpolyamine chain, respectively. A crucial point in the synthesis of **1** and **2** is the construction of the unique acylpolyamine chains involving a putreanine trimer and tetramer linked to cadaverine. Among



n=2 NPTX-5 (**1**)
n=3 NPTX-6 (**2**)

Figure 1

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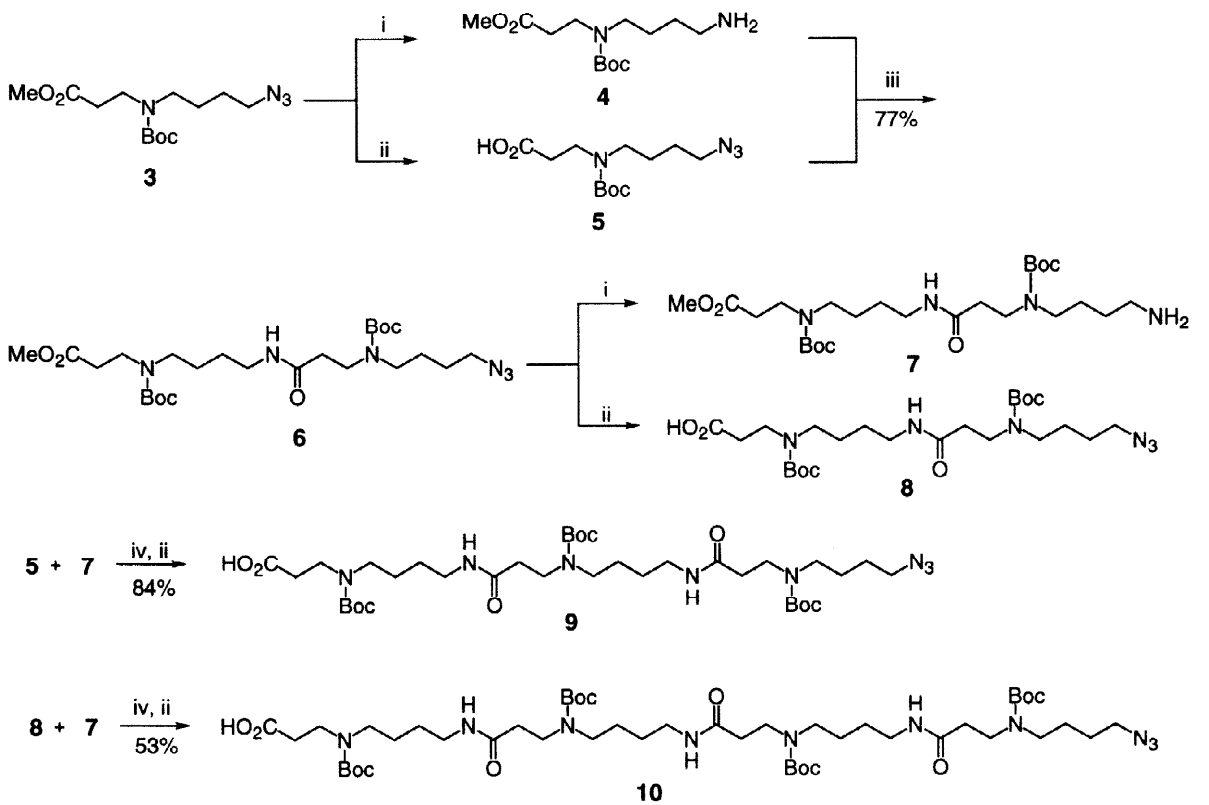
PII: S0040-4039(98)01379-3

twelve NPTXs, NPTX-5 (**1**) has been reported to exhibit the most potent insecticidal activity and histamine release activity from rat peritoneal mast cells.[2]

As shown in Fig. 1, NPTX-5 (**1**) consists of six components, i.e., 4-hydroxyindole-3-acetic acid, asparagine, cadaverine, and three molecules of putreanine (8-amino-4-azaoctanoic acid), while NPTX-6 (**2**) has the same sequence of components with four molecules of putreanine. These origomeric acylpolyamines **9** and **10** were successfully synthesized by the iterative use of a key azide compound, methyl 8-azido-*N*-Boc-4-azaoctanoate (**3**) (Scheme 1), which is readily available from 4-aminobutanol.[4]

First, the putreanine dimer **6** was prepared from **3** as a common intermediate for the synthesis of **9** and **10**. Thus, the azide **3** was reduced to the amine **4** by hydrogenation over PtO_2 in MeOH, while an ester group of **3** was hydrolized with 1M NaOH in EtOH leading to the carboxylic acid **5** (Scheme 1). Both compounds **4** and **5** were then condensed with isobutyl chloroformate in the presence of 4-methylmorpholine to afford the desired putreanine dimer **6** in 77% yield. Similarly, the dimer **6** obtained was quantitatively transformed into the corresponding amine **7** and the carboxylic acid **8** by hydrogenation of an azido group and hydrolysis of a methyl ester, respectively. The key putreanine trimer **9** was synthesized by condensation of the acid **5** and the dimer amine **7** under the same conditions for the coupling of **4** and **5** followed by hydrolysis of the resulting methyl ester with 1M NaOH in 84% overall yield. On the other hand, the other key putreanine tetramer **10** was synthesized similarly by condensation of the dimer acid **8** and the dimer amine **7** followed by hydrolysis in 53% overall yield (Scheme 1). Thus, the two key

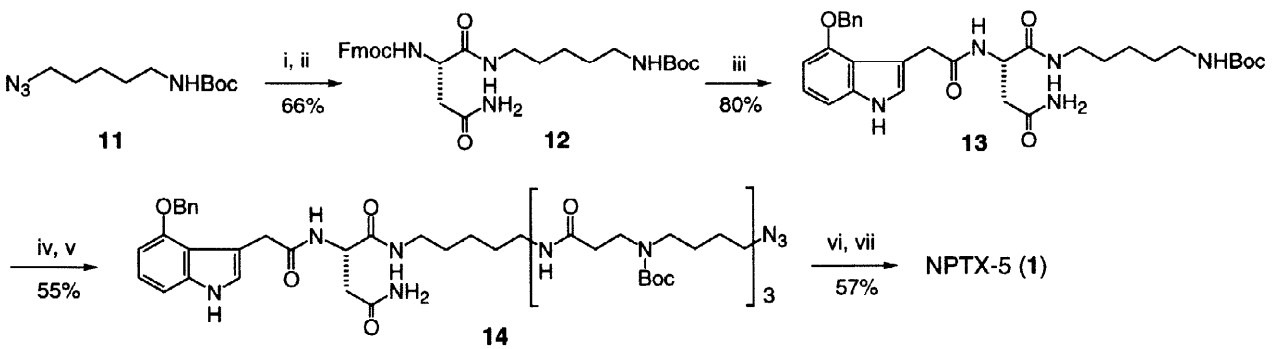


Reagents: i. H_2, PtO_2, MeOH; ii. 1M NaOH, EtOH; iii. IBCF, 4-methylmorpholine, THF, 0 °C, then **4**, 4-methylmorpholine, DMF, 0 °C; iv. IBCF, 4-methylmorpholine, THF, 0 °C, then **7**, 4-methylmorpholine, DMF, 0 °C.

Scheme 1

acylpolyamine chains **9** and **10** were efficiently and straightforwardly synthesized by repeating the same sequence of reactions, respectively, starting from the common azide **3**.

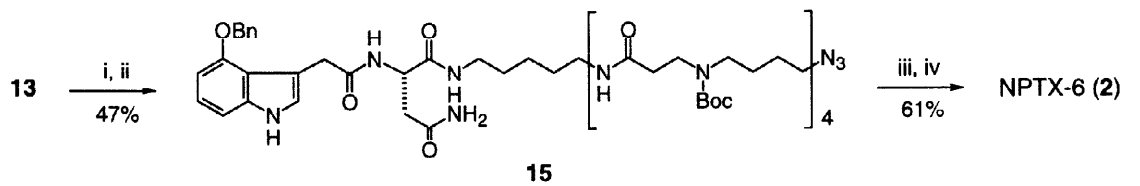
With the requisite polyamine chains **9** and **10** for the synthesis of NPTX-5 (**1**) and NPTX-6 (**2**) in hand, we focused on their total synthesis. The synthesis of **1** was started from 5-azido-1-*N*-Boc-aminopentane (**11**),[3] the same as our recent synthesis of NPTX-1.[11] After hydrogenation of the azide **11** over PtO_2 in ethanol, the resulting amine was condensed with *N*-(9-fluorenylmethoxycarbonyl)-L-asparagine (Fmoc-Asn) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) and 1-hydroxybenzotriazole (HOBt) in DMF to afford **12** in 66% yield (Scheme 2). Removal of the Fmoc group of **12** with $^{i}Pr_2NEt$ in DMF/DMSO followed by coupling with 4-benzyloxyindole-3-acetic acid[12] in the presence of EDC·HCl and HOBt produced the left-half segment **13** in 80% yield. Subsequent treatment of **13** with formic acid[13] and condensation of the resulting amine with the putreanine trimer **9** in the presence of EDC·HCl, HOBt, and $^{i}Pr_2NEt$ in DMF furnished the fully protected compound **14** in 55% yield. Finally, catalytic transfer hydrogenation of the benzyl and the terminal azido groups of **14** with 10% Pd-C and ammonium formate in MeOH followed by removal of the three Boc groups with TFA in CH_2Cl_2 provided NPTX-5 (**1**) as TFA salts in 57% yield.



Reagents: i. H_2, PtO_2, EtOH; ii. Fmoc-Asn, EDC·HCl, HOBt, DMF; iii. $^{i}Pr_2NEt$, DMF-DMSO (1 : 1), then 4-benzyloxyindole-3-acetic acid, EDC·HCl, HOBt; iv. HCO_2H; v. EDC·HCl, HOBt, $^{i}Pr_2NEt$, DMF, **9** in THF ; vi. 10% Pd-C, HCO_2NH_4, MeOH; vii. TFA, CH_2Cl_2.

Scheme 2

Similarly, NPTX-6 (**2**) was successfully synthesized by the coupling of the common left-half segment **13** and the putreanine tetramer **10** employing the same reaction sequence as shown in Scheme 3.



Reagents: i. HCO_2H; ii. EDC·HCl, HOBt, $^{i}Pr_2NEt$, DMF, **10** in THF; iii 10% Pd-C, HCO_2NH_4, MeOH; iv. TFA, CH_2Cl_2.

Scheme 3

The synthetic compounds were identified with natural toxins by the FAB MS/MS analytical method which has recently been developed by the authors (Y. I. and T. N).[14,15] Biological evaluation of the synthetic compounds is in progress in our laboratories.

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15. ^{1}H-NMR (270 MHz) spectra of the synthetic compounds were also in agreement with the proposed structures, although those of the natural toxins have not been measured yet owing to its limited quantity.