TOTAL SYNTHESIS OF NPTX-643, A NEUROTOXIN OF THE MADAGASCAR SPIDER (*Nephilengys borbonica*) HAVING A NOVEL ACYLPOLYAMINE STRUCTURE

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Abstract- The first total synthesis of NPTX-643, a neurotoxin of the Madagascar spider (*Nephilengys borbonica*) having a unique cadaverine - nor-putreanine - putreanine acylpolyamine chain, has been achieved by using three key azide intermediates.

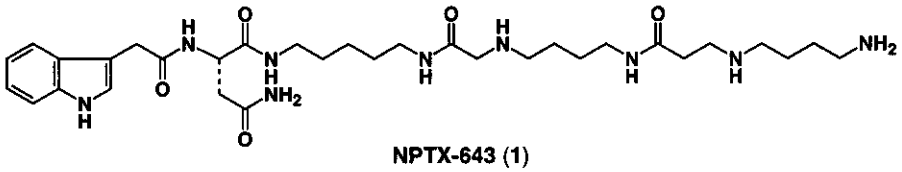
Spider toxins such as NSTX-3,[1] the toxin of the Papua New Guinea spider (*Nephila maculata*), JSTX-3[1] and Nephilatoxins (NPTX-1~12),[2] the toxins of the Joro spider (*Nephila clavata*), are known as potent and specific blockers of glutaminergic neurotransmission and are emerging as unique tools for understanding excitatory amino acid neurotransmission and related pharmacology.[3] Because of limited quantities, however, the chemical synthesis of spider toxins has been required for further pharmacological evaluation and ongoing biological studies.

We have achieved so far the chemical synthesis of Joro spider toxins such as NPTX-9 and 11,[4] NPTX-10 and 12,[5] NPTX-8,[6] NPTX-7,[7] and JSTX-3,[8] by using *the azide strategy* and developed the practical synthetic routes for these spider toxins.[9,10]

A spider venom gland produces various low molecular weight neurotoxins composed of amino acids, acylpolyamines, and a terminus arylacetic acid besides polypeptides and proteins. Since the venom constituents are extremely small quantities and quite complex mixtures, their characterizations have been limited only to the major fractions separable by high performance liquid chromatography. Very recently the authors (Y. I. and T. N.) have developed highly efficient techniques for the characterization of spider toxins using HPLC-FAB/MS and MS/MS detection systems which can analyze a minor level of toxic substances.[11] By the use of the new techniques, spider toxins possessing novel acylpolyamine structures have been discovered.[11]

We wish to report the first total synthesis of NPTX-643 (**1**), a neurotoxin recently discovered in the venom of the Madagascar spider (*Nephilengys borbonica*) by such analytical systems.[11] **1** is a new type of spider

toxin having a unique nor-putreanine component containing a glycine structure in the polyamine chain.



NPTX-643 (1)

The crucial point in the synthesis of **1** is the construction of the unique acylpolyamine moiety consisting of three different diamine components, particularly the nor-putreanine component containing a glycine structure. In order to assemble the novel polyamine chain we decided to employ *the azide strategy*, as in the previous syntheses of spider toxins,[4-9] in which three key azide intermediates (**2**, **3**, and **4**) were designed. Among them, for the two azide compounds (**2**) and (**4**) corresponding to cadaverine and putreanine, respectively, we have already established their efficient synthetic routes and successfully utilized in the synthesis of Joro spider toxins.[4-9]

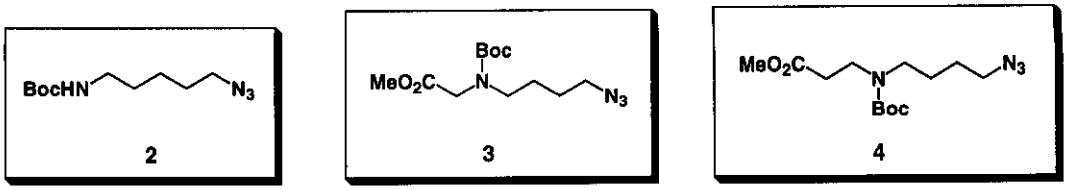
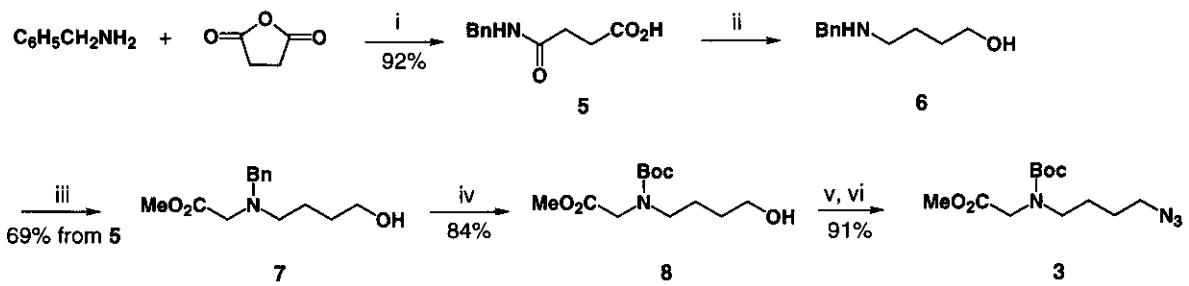


Figure 1. Key Azide Intermediates

The third key azide compound (**3**) corresponding to the nor-putreanine component was elaborated according to **Scheme 1**. Reaction of benzylamine with succinic anhydride in CH_2Cl_2 containing pyridine gave the crystalline semiacid (**5**) (mp 141 °C) in 92% yield. After reduction of the acid with borane in THF, the resulting *N*-benzyl-4-aminobutanol (**6**) was successively treated with methyl bromoacetate and K_2CO_3 in acetone to produce trialkylamine (**7**) in 69% overall yield. Then the *N*-benzyl protective group of **7** was transformed into a *tert*-butoxycarbonyl (Boc) group by catalytic hydrogenation over 10% Pd-C in EtOH in the presence of di-*tert*-butyl dicarbonate. The amino alcohol (**8**) thus obtained was converted to the desired azide (**3**) in 91 % overall yield by the two-step sequence: (1) mesylation; (2) substitution with NaN_3 in

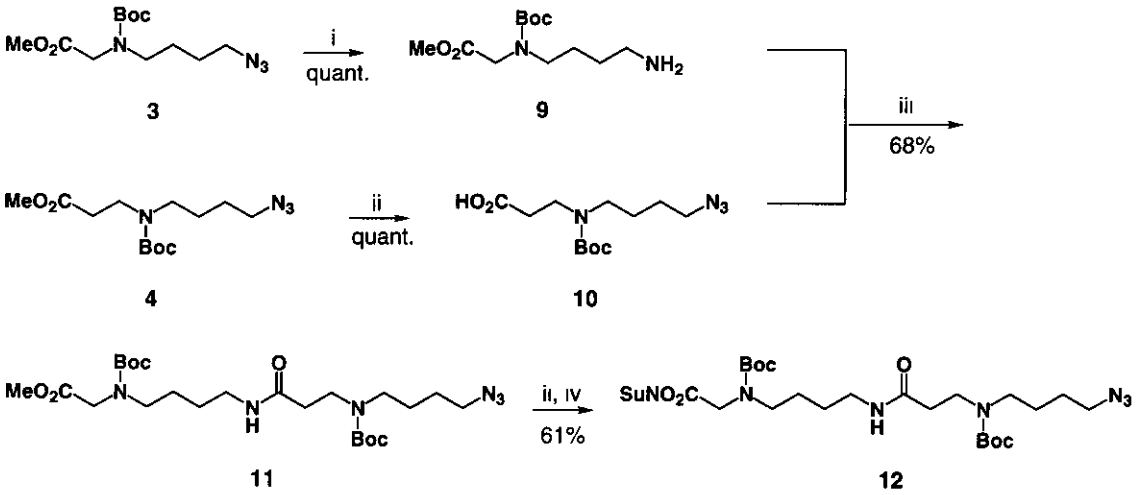


Reagents: i. pyridine, CH_2Cl_2; ii. BH_3, THF, then 1N NaOH, EtOH; iii. $BrCH_2CO_2CH_3$, K_2CO_3, acetone; iv. H_2, 10% Pd-C, $(Boc)_2O$, EtOH; v. MsCl, pyridine, CH_2Cl_2; vi. NaN_3, DMF.

Scheme 1

DMF. Thus the third key azide compound (**3**) could be efficiently synthesized from benzylamine.

With the three key azide components (**2**, **3**, and **4**) in hand, we focused on the construction of the acylpolyamine moiety of **1** by connection of **3** with **4**. Coupling reaction of both segments was accomplished according to **Scheme 2**. First, the nor-putreanine equivalent (**3**) was subjected to catalytic hydrogenation over platinum(IV) oxide in EtOH to afford the amine (**9**) quantitatively, while the putreanine component (**4**) was hydrolyzed with 1N NaOH in EtOH resulting in formation of the carboxylic acid (**10**) in nearly quantitative yield. Both the diamine segments (**9**) and (**10**) obtained were condensed with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) and 1-hydroxybenzotriazole (HOBt) in DMF to produce the desired acylpolyamine (**11**) in 68% yield. Alkaline hydrolysis of the ester (**11**) followed by esterification of the resulting carboxylic acid with *N*-hydroxysuccinimide and DCC in ethyl acetate furnished the active ester (**12**) in 61% isolated yield.

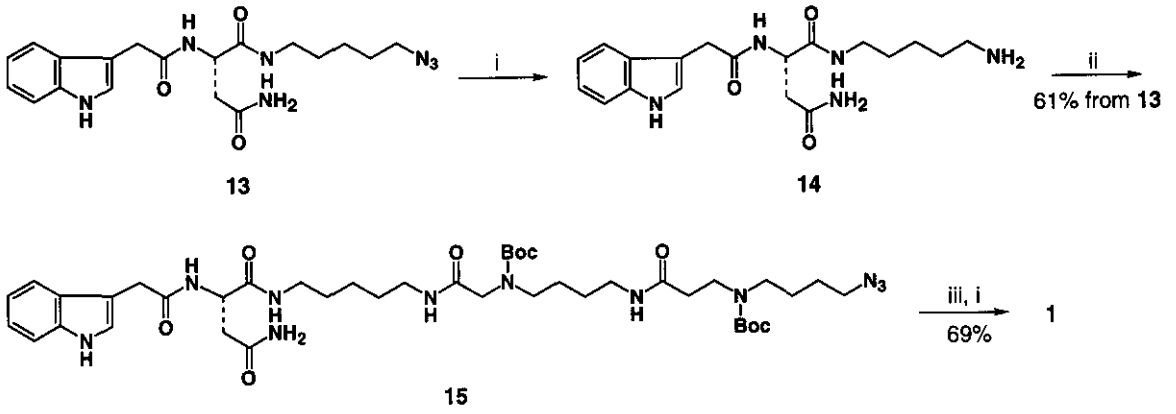


Reagents: i. H_2, PtO_2, EtOH; ii. 1N NaOH, EtOH; iii. EDC HCl, HOBt, DMF; iv. HONSu, DCC, AcOEt.

Scheme 2

The key acylpolyamine chain (**12**) was secure, we set out the synthesis of **1**. The synthesis was carried out by coupling of the left half-segment (**13**), which was used in the previous synthesis of NPTX-8~12,[4-7] with the acylpolyamine (**12**) (**Scheme 3**). The left half-segment (**13**) can be efficiently synthesized from 5-azido-1-*N*-Boc-aminopentane (**2**), i.e. the cadaverine equivalent, by coupling with asparagine, followed by condensation with indole-3-acetic acid.[4] The coupling reaction of both segments was carried out as follows. Catalytic hydrogenation of the azide (**13**) over 10% Pd-C in MeOH yielded the primary amine (**14**) which was condensed with the *N*-hydroxysuccinimide ester (**12**), i.e. the right half-segment, in DMF in the presence of 4-methylmorpholine to give rise to the fully protected compound (**15**) in 61% yield. Finally, deprotection of the Boc groups with TFA in CH_2Cl_2 followed by catalytic hydrogenation of the terminal azido group in MeOH furnished the target compound. The product was purified by a Cosmosil 140 C_{18}-PREP column (20% acetonitrile containing 1% TFA) and the pure toxin (**1**) ($[\alpha]_D^{19}$ -1.87° (*c* 0.15, H_2O)) was obtained as TFA salts in 69% yield. The synthetic compound was identified with the

natural toxin by HPLC-FAB/MS analyses.[11,12]



Reagents: i. H_2, 10% Pd-C, MeOH; ii. **12**, 4-methylmorpholine, DMF; iii. TFA, CH_2Cl_2.

Scheme 3

In summary, we achieved the first synthesis of NPTX-643 (**1**) having a novel acylpolyamine chain by the use of the key azide intermediates. The present synthesis provides an efficient synthetic route for spider toxins containing nor-putreanine and putreanine components and demonstrates the usefulness of *the azide strategy* in spider toxin synthesis.

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12. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were also in agreement with the proposed structure, although those of the natural toxin have not been measured yet owing to its limited quantity.

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