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# An Efficient Synthesis of JSTX-3, a Potent Neurotoxin of Joro Spider (*Nephila clavata*)

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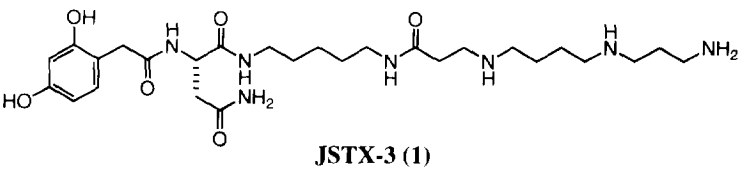
***Abstract:*** JSTX-3 (Joro spider toxin-3) (**1**), a potent neurotoxin isolated from Joro spider (*Nephila clavata*), has been efficiently synthesized starting from 5-azido-1-*N*-Boc-pentylamine (**11**) via the convergent synthetic strategy in which the characteristic polyamine components of the toxin were effectively constructed by the use of two key azide intermediates.

Joro spider toxin (JSTX)[1] and Nephilatoxin (NPTX)[2] isolated from the Joro spider (*Nephila clavata*) have been demonstrated to be potent antagonists of excitatory synaptic neurotransmission and glutamate-mediated currents in various types of neurons.[3-8] Particularly these toxins are known as one of the most potent antagonists of non-NMDA type glutamatergic receptor channels.[5] The common structural feature of these toxins is that they possess an aromatic core and a polycationic side chain composed of polyamine(s) and basic amino acids. A series of JSTXs (JSTX-1~4) have a 2,4-dihydroxybenzene ring as the aromatic component[1] while all NPTXs (NPTX-1~12) possess an indole nucleus as the aromatic moiety.[2] It has been reported that both the aromatic and the polyamine moieties are essential for the binding of the toxins to glutamate receptors.[7-9] Recently Konnerth and co-workers have demonstrated that JSTX-3 (**1**) acts as a potent and subunit-specific blocking agent of AMPA / KA (α-amino-3-hydroxy-5-methylisoxazole-4-propionate / kainate) receptor channels.[10] Thus Joro spider toxins are emerging as unique tools for understanding excitatory amino acid transmission and related pharmacology. Because of extremely limited quantities of the toxins in nature, however, their chemical synthesis has been required for pharmacological evaluation and ongoing biological studies.[11]

We have studied the chemical synthesis of spider toxins, *inter alia* NPTXs, and so far developed practical synthetic methodologies for NPTX-9 and 11,[12] NPTX-10 and 12,[13]

NPTX-8,[14] and NPTX-7[15] based on the azide strategy.

Although JSTX-3 (**1**) has been synthesized so far by two groups,[16] we envisaged a more convenient and straightforward synthetic method for polyamine chains in view of the chemical synthesis of the toxin. We now wish to report a highly efficient synthesis of JSTX-3 (**1**) starting from 5-azido-1-*N*-*t*-butoxycarbonylpentylamine (**11**) via the convergent strategy in which two key azide intermediates were designed for the construction of the characteristic polyamine components.

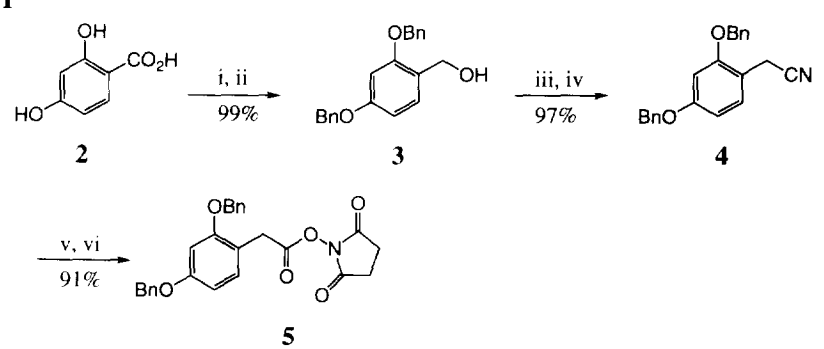


**JSTX-3 (1)**

JSTX-3 (**1**) consists of five components, 2,4-dihydroxyphenylacetic acid, asparagine, cadaverine (1,5-diaminopentane), propionic acid, and spermidine. Crucial points in the synthesis of **1** are construction of the two polyamine segments, cadaverine and spermidine, as well as the order of coupling of five components. In order to solve these problems and to provide an efficient synthetic method for the toxin we designed a synthetic route starting from the central cadaverine segment as shown in Scheme **3** (*vide post*).

First, the aromatic component 2,4-dihydroxyphenylacetic acid was prepared from commercially available 2,4-dihydroxybenzoic acid (**2**) (Scheme **1**). The treatment of **2** with NaH and benzyl bromide in DMF gave benzyl 2,4-dibenzyloxybenzoate which was reduced with $LiAlH_4$ in $Et_2O$ to afford benzyl alcohol **3** (mp 92-93 °C) in nearly quantitative yield. The benzyl alcohol **3** thus obtained was converted to 2,4-dibenzyloxyphenylacetonitrile (**4**)

**Scheme 1**

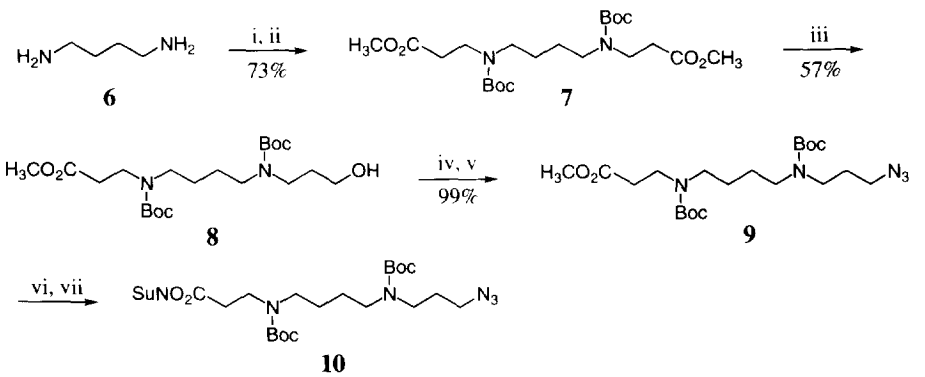


*Reagents:* i. BnBr, NaH, DMF; ii. $LiAlH_4$, $Et_2O$; iii. $SOCl_2$, $C_6H_6$; iv. NaCN, DMF; v. 1N NaOH, EtOH; vi. HONSu, DCC, AcOEt.

(mp 95-96 °C) by a two-step reaction sequence; chlorination with thionyl chloride in benzene followed by substitution with NaCN in DMF (97% for the two steps). Alkaline hydrolysis of the nitrile **4** furnished the desired 2,4-dibenzyloxyphenylacetic acid, which was then transformed into the active ester, *N*-hydroxysuccinimide ester **5** (mp 139-140 °C), by treatment with *N*-hydroxysuccinimide (HONSu) and dicyclohexylcarbodiimide (DCC) in AcOEt in 91% overall yield. In this way, the aromatic component was highly efficiently synthesized.

On the other hand, for the construction of the terminal two segments consisting of propionic acid and spermidine, we designed methyl 4,9-diaza-12-azidododecanoate, a key azide intermediate in the present synthesis. The protected methyl 4,9-diaza-12-azidododecanoate **9** was synthesized according to Scheme 2. Michael addition of putrescine (**6**) to methyl acrylate in EtOH followed by protection of the two amino groups of the resulting bis-adduct with $(Boc)_2O$ gave rise to **7** in 73% yield. One of the ester groups of **7** was then reduced with $LiBH_4$ in aq. THF to afford **8** in 57% yield (86% based on the consumed starting material). Although a wide variety of reducing agents were examined for this transformation, only the above conditions gave the desired product **8** in acceptable yield. The alcohol **8** obtained was converted to azide **9** by the following two-step reaction sequence; 1) mesylation, 2) substitution with $NaN_3$ in DMF (99% for the two steps). Thus the crucial polyamine segment **9** was straightforwardly synthesized starting from putrescine (**6**). Alkaline hydrolysis of the methyl ester **9** furnished the corresponding acid, which was quantitatively transformed into the active ester **10** by treatment with HONSu and DCC in AcOEt.

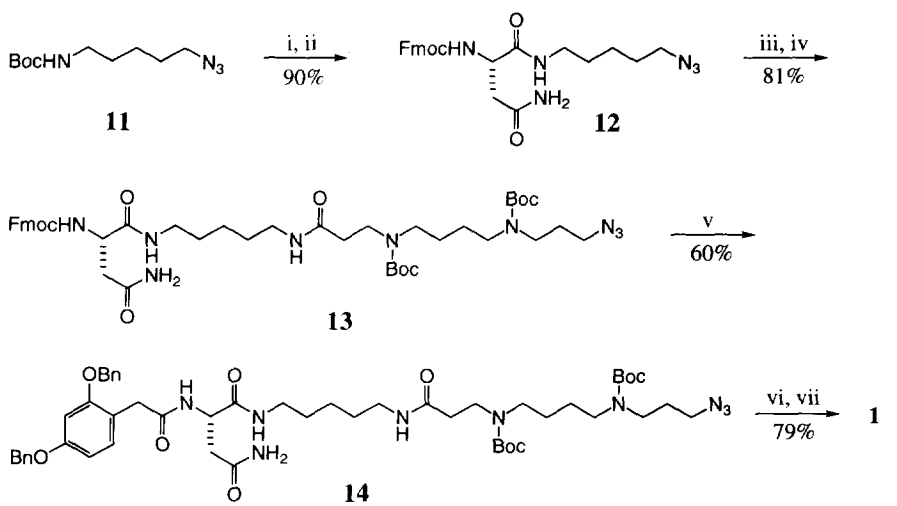
**Scheme 2**




*Reagents:* i. $CH_2$=$CHCO_2CH_3$, EtOH; ii. $(Boc)_2O$, aq. $Na_2CO_3$; iii. $LiBH_4$, THF-$H_2O$ (20:1 v/v); iv. MsCl, pyridine, $CH_2Cl_2$; v. $NaN_3$, DMF; vi. 1N NaOH, EtOH; vii. HONSu, DCC, AcOEt.

With the two key segments in hand, we set out the synthesis of JSTX-3 (**1**). In order to achieve a convergent synthesis, the synthesis of **1** was started from the central cadaverine moiety as shown in Scheme 3. After elimination of the Boc group of 5-azido-1-*N*-*t*-butoxycarbonylpentylamine (**11**), another key polyamine intermediate readily obtainable from 5-amino-1-pentanol,[12] with HCl generated *in situ* from acetyl chloride and methanol, the resulting hydrochloride salts were condensed with *N*-(9-fluorenylmethoxycarbonyl)-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 give **12** as colorless crystals (mp 169-170 °C) in 90% yield. Catalytic hydrogenation of the azide **12** over Pd-$BaSO_4$ in MeOH followed by condensation of the resulting amine with the *N*-hydroxysuccinimide ester **10** (*vide ante*) in THF furnished **13** (mp 122-123 °C) in 81% yield. Thus the crucial polyamine segments of JSTX-3 (**1**) were efficiently constructed by employing the two key azide intermediates.

**Scheme 3**




*Reagents:* i. AcCl, MeOH; ii. Fmoc-Asn, EDC·HCl, HOBt, $^{i}Pr_2NEt$, DMF; iii. $H_2$, Pd-$BaSO_4$, MeOH; iv. **10**, THF; v. $^{i}Pr_2NEt$, DMF, DMSO, then **5**, HOBt; vi. $H_2$, 10%Pd-C, AcOH; vii. TFA, $HSCH_2CH_2SH$, $CH_2Cl_2$.

The aromatic component was connected as follows. Removal of the Fmoc group in **13** by treatment with $^{i}Pr_2NEt$ in DMF / DMSO afforded the corresponding amine, which was conducted with the aromatic *N*-hydroxysuccinimide ester **5** (*vide ante*) and HOBt to yield the fully protected compound **14** as colorless crystals (mp 156-157 °C) in 60% yield. Finally,

catalytic hydrogenation of the terminal azido group and two benzyl groups on the aromatic ring over 10% Pd-C in AcOH followed by removal of the two Boc groups with trifluoroacetic acid (TFA) in $CH_2Cl_2$ containing 1,2-ethanedithiol produced the target molecule **1**. The crude product obtained as TFA salts was purified by reverse phase flash chromatography ($H_2O$ / $CH_3CN$ / TFA (95:5:1-70:3:1)) to give the pure toxin ($[\alpha]_D^{20}$ -1.70° (*c* 0.07, $H_2O$), FAB-MS 588 ($M^+$+Na)) in 79% yield. The synthetic compound was identified with TFA salts of natural JSTX-3 (**1**) by comparison of its $^1$H-NMR spectra (500 MHz) with those of the latter.

The present synthesis not only provides an efficient synthetic route for JSTX-3 (**1**) but also demonstrates the synthetic potential of the azide strategy in spider toxin synthesis. Extension of the methodology to other biologically active polyamine compounds as well as spider toxins are in progress in our laboratory.

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## Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu IR-408 spectrometer. $^1$H-NMR and $^{13}$C-NMR spectra were recorded on JEOL FX 90Q (90 MHz), VARIAN Gemini-300 (300 MHz), and VARIAN UNITY plus 500 (500 MHz) spectrometers with tetramethylsilane and chloroform as an internal standard, respectively, and chemical shifts are given in δ (ppm). Optical rotations were measured with a JASCO DIP-370 digital polarimeter and high-resolution mass (HR-MS) spectra were taken with a JEOL JMS-DX303 instrument. Merck Kieselgel Art. 7734 was used for flash column chromatography and Tokyo-Kasei Cosmosil 140 C18-PREP was used for reverse phase column chromatography. Sodium sulfate ($Na_2SO_4$) was used as the drying agent.

### 2,4-Dibenzyloxybenzyl alcohol (3)

Benzyl bromide (5.94 ml, 50 mmol) was added dropwise to a mixture of 2,4-dihydroxybenzoic acid (**2**) (1.54 g, 10 mmol) and NaH (60% in oil, 2.0 g, 50 mmol) in DMF (30 ml) at 0 °C under argon. After stirring at room temperature for 12 h, the excess hydrides were

decomposed by addition of a few drops of water. The reaction mixture was then diluted with AcOEt (400 ml) and washed with half-saturated brine, water, and saturated brine. The organic layer was concentrated after drying. The residual solids were washed with hexane to give the benzyl ester as colorless solids.

A solution of the above benzyl ester in dry $Et_2O$ (50 ml) was added dropwise to a suspension of $LiAlH_4$ (410 mg, 10.8 mmol) in $Et_2O$ (150 ml) at room temperature. The resulting mixture was stirred for 1 h and the excess hydrides were decomposed by addition of wet ether followed by several drops of water. Inorganic materials were filtered off through Celite and the filtrate was washed with saturated $NH_4Cl$ and brine, and dried. Removal of the solvent left an oil which was purified by column chromatography (hexane-AcOEt (8:1-5:1)) to give 2,4-dibenzyloxybenzyl alcohol (**3**) (3.16 g, 99%) as colorless crystals: mp 92-93 °C (lit.[16a] 84-85 °C); IR (KBr) 3250 cm-1; $^1$H-NMR (90 MHz, $CDCl_3$) 2.16 (1H, br t), 4.65 (2H, br d), 5.03 (2H, s), 5.06 (2H, s), 6.01-6.42 (2H, m), 7.14-7.37 (11H, m). Anal. Calcd for $C_{21}H_{20}O_3$: C, 78.72; H, 6.30. Found: C, 78.51; H, 6.43.

**2,4-Dibenzyloxyphenylacetonitrile (4)**

$SOCl_2$ (5 ml) was added to a solution of 2,4-dibenzyloxybenzyl alcohol (**3**) (1.32 g, 4.13 mmol) in benzene (40 ml) and the mixture was heated at gentle reflux for 5 min. After cooling, the solvent and the excess reagent were evaporated in vacuo to give the crude benzyl chloride.

A mixture of the crude benzyl chloride and NaCN (245 mg, 5.0 mmol) in DMF (5 ml) was stirred at room temperature for 1 h. The reaction mixture was diluted with AcOEt and washed with half-saturated brine, water, and saturated brine, and dried. After removal of the solvent, the residue was purified by flash column chromatography (hexane-AcOEt (4:1)) to give the nitrile **4** (1.32 g, 97%) as colorless crystals: mp 95-96 °C; IR (KBr) 3000, 2920, 2260 cm-1; $^1$H-NMR (90 MHz, $CDCl_3$) 3.63 (2H, s), 5.03 (2H, s), 5.05 (2H, s), 6.49-6.60 (2H, m), 7.12-7.45 (11H, m). Anal. Calcd for $C_{22}H_{19}NO_2$: C, 80.21; H, 5.82; N, 4.25. Found: C, 80,24; H, 5.96; N, 4.15.

**2,4-Dibenzyloxyphenylacetic acid *N*-hydroxysuccinimide ester (5)**

A mixture of the nitrile **4** (106.5 mg, 0.32 mmol) and 1N NaOH (6 ml) in EtOH (20 ml) was stirred at 80 °C for 48 h. After removal of the solvent, the residue was poured into 3% HCl and extracted with AcOEt. The extracts were washed with water and saturated brine, dried and concentrated. The residual crystals were dissolved in AcOEt (5 ml) to which HONSu (56.4 mg, 0.49 mmol) and DCC (100 mg, 0.49 mmol) were added and the resulting mixture

was stirred at room temperature for 12 h. After evaporation of the solvent, the residue was purified by flash column chromatography (hexane-AcOEt (8:1-1:1)) to give the active ester **5** (132 mg, 91%) as colorless crystals: mp 139-140 °C; IR (KBr) 3000, 2910, 1730 cm-1; $^1$H-NMR (90 MHz, $CDCl_3$) 2.80 (4H, s), 3.91 (2H, s), 5.00 (2H, s), 5.09 (2H, s), 6.48-6.59 (2H, m), 7.13-7.44 (11H, m). Anal. Calcd for $C_{26}H_{23}NO_6$: C, 70.09; H, 5.21; N, 3.15. Found: C, 69.77; H, 5.27; N, 3.18.

**Dimethyl 4,9-diaza(*N,N'*-bis-*tert*-butoxycarbonyl)dodecandioate (7)**

To a solution of putrescine (**6**) (880 mg, 10.0 mmol) in EtOH (10 ml) was added dropwise a solution of methyl acrylate (1.89 ml, 21.0 mmol) in EtOH (10 ml) at 0 °C over 30 min, then the mixture was stirred at room temperature for 2 h. Concentration of the reaction mixture under reduced pressure left the bis-Michael adduct as an oil.

A mixture of the above bis-adduct, $Na_2CO_3$ (3.18 g, 30.0 mmol), and di-*tert*-butyl dicarbonate (6.54 g, 30.0 mmol) in water (20 ml) was stirred at room temperature for 12 h. The reaction mixture was acidified with 10% citric acid, and extracted with AcOEt. The extracts were washed twice with 10% citric acid and three times with saturated brine, and dried. Evaporation of the solvent left an oily residue, which was purified by flash column chromatography (hexane-AcOEt (4:1-2:1)) to give the diester **7** (3.37 g, 73 %) as a colorless oil: IR ($CHCl_3$): 2980, 1735, 1685 cm-1; $^1$H-NMR (90 MHz, $CDCl_3$) 1.24-1.56 (4H, m), 1.45 (18H, s), 2.55 (4H, t, *J*=7.0 Hz), 3.20 (4H, m), 3.45 (4H, t, *J*=7.0 Hz), 3.67 (6H, s); $^{13}$C-NMR (22.5 MHz, $CDCl_3$) 25.90 (t), 28.44 (q), 33.59 (t), 43.37 (t), 47.43 (t), 51.63 (q), 79.65 (s), 155.28 (s), 172.26 (s). HR-MS *m/z*: Calcd for $C_{22}H_{40}N_2O_8$ ($M^+$): 460.2784. Found 460.2793.

**Methyl 4,9-diaza(*N,N'*-bis-*tert*-butoxycarbonyl)-12-hydroxydodecanoate (8)**

$LiBH_4$ (480 mg, 21.8 mmol) was added in small quantities to a solution of the diester **7** (1.67 g, 3.63 mmol) in THF (700 ml) containing water (14 ml) over 6 h at room temperature. After stirring for an additional hour, 10% citric acid (50 ml) was added and the mixture was concentrated. The residue was extracted with AcOEt and the extracts were washed with saturated brine and dried. Removal of the solvent left an oil which was purified by flash column chromatography (hexane-AcOEt (1:1)) to give the starting material **7** (574 mg, 34%) and the hydroxy ester **8** (889 mg, 57%): IR ($CHCl_3$): 3450, 2970, 1735, 1685 cm-1; $^1$H-NMR (90 MHz, $CDCl_3$): 1.45 (18H, s), 1.24-1.80 (6H, m), 2.56 (2H, t, *J*=7.0 Hz), 2.96-3.68 (11H, m), 3.68 (3H, s); $^{13}$C-NMR (75 MHz, $CDCl_3$): 25.85, 27.58, 28.50, 30.75, 34.03, 42.71, 43.40, 46.78, 46.95, 51.80, 58.37, 79.87, 80.11, 155.40, 177.14. HR-MS *m/z*: Calcd for $C_{21}H_{40}N_2O_7$ ($M^+$): 432.2836. Found 432.2846.

**Methyl 4,9-diaza(*N,N'*-bis-*tert*-butoxycarbonyl)-12-azidododecanoate (9)**

Methanesulfonyl chloride (0.23 ml, 3.0 mmol) was added to a solution of **8** (634 mg, 1.47 mmol) and pyridine (0.48 ml, 6.0 mmol) in $CH_2Cl_2$ (5.0 ml) at 0 °C and the mixture was stirred at the same temperature for 12 h. More of pyridine (0.48 ml, 6.0 mmol) and methanesulfonyl chloride (0.23 ml, 3.0 mmol) were added and the resulting mixture was further stirred for 24 h in the cold. The mixture was then poured onto ice-water and extracted with AcOEt. The AcOEt extracts were washed with water and saturated brine, and concentrated. The crude mesylate obtained was used for the next step without further purification.

A mixture of the above mesylate and $NaN_3$ (195 mg, 3.0 mmol) in DMF (4 ml) was stirred at room temperature for 12 h. The mixture was diluted with AcOEt (200 ml) and washed with half-saturated brine, water, and saturated brine. The organic layer was concentrated after drying. The residue was purified by flash column chromatography (hexane-AcOEt (4:1)) to give the azide **9** (667 mg, 99 %): IR ($CHCl_3$): 2980, 2120, 1735, 1680 cm-1; $^1$H-NMR (300 MHz, $CDCl_3$): 1.46 (18H, s), 1.46-1.63 (4H, m), 1.79 (2H, q, *J*=7 Hz), 2.56 (2H, t, *J*=7 Hz), 3.08-3.30 (6H, m), 3.30 (2H, t, *J*=7 Hz), 3.45 (2H, t, *J*=7 Hz), 3.68 (3H, s); $^{13}$C-NMR (75 MHz, $CDCl_3$): 26.30 (t), 28.10 (t), 28.50 (t), 34.42 (t), 44.00 (t), 45.20 (t), 47.69 (t), 47.77 (t), 49.77 (t), 52.39 (q), 80.27 (s), 156.00 (s), 156.19 (s), 173.18 (s). HR-MS *m/z*: Calcd for $C_{21}H_{39}N_5O_6$ ($M^+$): 457.2900. Found 457.2908.

**4,9-Diaza(*N,N'*-bis-*tert*-butoxycarbonyl)-12-azidododecanoic acid *N*-hydroxysuccinimide ester (10)**

To a solution of the methyl ester **9** (1.47 g, 3.2 mmol) in EtOH (50 ml) was added 1N NaOH (10 ml) and the mixture was stirred at room temperature for 2 h. After removal of the solvent, the residue was poured into 10% citric acid and extracted with AcOEt. The extracts were washed with water and saturated brine, dried, and concentrated. The crude carboxylic acid obtained was used for the next step without further purification.

A mixture of the crude carboxylic acid, HONSu (460 mg, 4.0 mmol), and DCC (824 mg, 4.0 mmol) in AcOEt (100 ml) was stirred at room temperature for 12 h. After concentration to the half-volume, the remaining mixture was passed through a short silica gel column by the aid of hexane-AcOEt (1:1) to remove ureas. The filtrate was concentrated to give the succinimide ester **10** (1.54 g) which was immediately used for the next reaction.

**Condensation of Fmoc-L-asparagine and 5-azido-1-*N*-*tert*-butoxycarbonylpentylamine (11)**

Acetyl chloride (1 ml) was added dropwise to a solution of **11**[12] (1.25 g, 5.49 mmol) in

MeOH (20 ml) at 0°C and the mixture was stirred at room temperature for 2 h. Removal of the solvent *in vacuo* gave 5-azidopentylamine hydrochloride as colorless amorphous. A mixture of the hydrochloride, Fmoc-L-asparagine (2.13 g, 6.0 mmol), EDC·HCl (1.53 g, 8.0 mmol), HOBt (810 mg, 6.0 mmol), and $^{i}Pr_2NEt$ (0.87 ml, 5.0 mmol) in DMF (40 ml) was stirred at room temperature for 12 h. The reaction mixture was diluted with AcOEt (900 ml) and washed with half-saturated brine, 10% citric acid, water, and saturated brine. The organic layer was concentrated after drying. The residue was dissolved in a small amount of MeOH and the product was precipitated by addition of water. The precipitates were collected to give 2.28 g (90 %) of **12** as colorless crystals: mp 169-170 °C; $[\alpha]_D^{20}$ +5.86° (*c* 0.15, MeOH); IR (KBr): 3280, 2920, 2110, 1690, 1646 cm-1; $^1$H-NMR (300 MHz, $CDCl_3$): 1.25-1.66 (6H, m), 2.51-2.62 (1H, m), 2.92-3.03 (1H, m), 3.23-3.28 (4H, m), 4.22 (1H, t, *J*=6.0 Hz), 4.44 (2H, d, *J*=6.0 Hz), 4.49 (1H, m), 5.43 (1H, m), 5.83 (1H, m), 6.39 (1H, m), 6.86 (1H, m), 7.32 (2H, t, *J*=7.4 Hz), 7.41 (2H, t, *J*=7.4 Hz), 7.60 (2H, d, *J*=7.4 Hz), 7.78 (2H, d, *J*=7.4 Hz); $^{13}$C-NMR (75 MHz, $CDCl_3$): 24.98 (t), 29.52 (s), 29.83 (t), 38.30 (t), 40.29 (t), 48.43 (d), 52.34 (t), 53.46 (d), 68.18 (t), 120.93 (d), 126.18 (d), 128.17 (d), 128.79 (d), 142.60 (s), 145.25 (s), 158.40 (s), 173.48 (s), 174.95 (s). FAB-MS *m/z*: 465 ($M^+$+1), 487 ($M^+$+Na). Anal. Calcd for $C_{24}H_{28}N_6O_4 \cdot 1/6\ H_2O$: C, 61.64; H, 6.11; N, 17.98. Found: C, 61.91; H, 6.02; N, 17.66.

**Condensation of 12 and 10**

A mixture of **12** (696 mg, 1.5 mmol) and Pd-$BaSO_4$ (200 mg) in MeOH (100 ml) was stirred under a hydrogen atmosphere for 2 h. The catalyst was filtered off through a membrane filter. To this filtrate was added a solution of the active ester **10** (1.54 g) in THF (10 ml) and the mixture was stirred for 12 h at room temperature. After removal of the solvent, the residual solids were successively washed with hexane-AcOEt (1:1), water, and $Et_2O$. The remaining solids were dissolved in MeOH and recrystalized by addition of water. By this procedure, 1.06 g (81%) of pure **13** was obtained as colorless crystals: mp 122-123 °C; $[\alpha]_D^{20}$ -5.47° (*c* 0.34, MeOH); IR (KBr): 3280, 2920, 2120, 1690, 1650 cm-$^1$; $^1$H-NMR (300 MHz, $CDCl_3$): 1.20-1.81 (12H, m), 1.44 (9H, s), 1.45 (9H, s), 2.23-2.64 (3H, m), 2.82-3.56 (15H, m), 4.22 (1H, t, *J*=6.3 Hz), 4.44 (2H, d, *J*=6.3 Hz), 4.50 (1H, m), 6.19 (1H, m), 6.44 (2H, m), 6.86 (2H, m), 7.32 (2H, t, *J*=7.4 Hz), 7.41 (2H, t, *J*=7.4 Hz), 7.59 (2H, d, *J*=7.4 Hz), 7.77 (2H, d, *J*=7.4 Hz); $^{13}$C-NMR (75 MHz, $CDCl_3$): 25.35 (t), 26.72 (t), 27.28 (t), 30.20 (t), 36.45 (t), 36.73 (t), 38.59 (t), 40.55 (t), 40.55 (t), 45.33 (t), 46.08 (t), 48.33 (t), 48.64 (d), 48.84 (t), 48.98 (t), 50.38 (t), 53.72 (t), 68.37 (t), 81.29 (s), 81.37 (s), 121.23 (d), 126.50 (s), 128.47 (d), 129.09 (d), 142.85 (s), 145.50 (s), 157.46 (s), 157.55 (s), 158.41 (s), 173.74 (s), 173.90 (s), 175.17 (s). FAB-MS *m/z:* 864 ($M^+$+1), 886 ($M^+$+Na).

**Condensation of 13 and 5**

A solution of **13** (632 mg, 0.73 mmol) and $^{i}Pr_2NEt$ (1.5 ml, 8.61 mmol) in DMF (2 ml) and DMSO (2 ml) was stirred at room temperature for 2 h. To this solution was added the active ester **5** (534 mg, 1.2 mmol) and HOBt (67.5 mg, 0.50 mmol), and the resulting mixture was further stirred for 12 h at room temperature. After dilution with AcOEt (200 ml), the mixture was washed with half-saturated brine, 10% citric acid, water, 5% $NaHCO_3$, and saturated brine. The organic layer was dried and concentrated to leave a residue, which was purified by flash column chromatography ($CH_2Cl_2$-acetone-EtOH (10:1:0.5-3:1:1)) to give **14** (424 mg, 60%) as colorless crystals: mp 156-157 °C; $[\alpha]_D^{20}$ -1.86° (*c* 0.15, MeOH); IR (KBr): 3250, 2100, 1690, 1640 cm-1; $^1$H-NMR (500 MHz, $CDCl_3$): 0.85-1.61 (12H, m), 1.58 (18H, s), 2.26-2.52 (3H, m), 2.80-2.89 (1H, m), 3.06-3.27 (10H, m), 3.27-3.33 (2H, m), 3.33-3.48 (2H, m), 3.59 (2H, s), 4.63 (1H, m), 5.02 (2H, s), 5.09 (2H, s), 6.17 (1H, m), 6.24 (1H, m), 6.56-6.60 (1H, m), 6.65-6.67 (1H, m), 6.72 (1H, m), 6.91 (1H, m), 7.12-7.44 (11H, m); $^{13}$C-NMR (125 MHz, $CDCl_3$): 24.99 (t), 26.72 (t), 27.28 (t), 28.75 (s), 29.84 (t), 29.87 (t), 36.45 (t), 6.73 (t), 37.60 (t), 38.39 (t), 40.24 (t), 40.24 (t), 45.16 (t), 45.82 (t), 48.32 (t), 48.42 (t), 48.70 (t), 48.98 (d), 51.64 (t), 71.13 (t), 71.25 (t), 81.03 (t), 81.11 (t), 101.97 (d), 107.54 (d), 128.37 (d), 128.42 (s), 128.59 (d), 128.92 (d), 129.06 (s), 129.23 (s), 129.35 (d), 129.51 (d), 129.64 (d), 132.68 (d), 157.20 (s), 157.29 (s), 158.77 (s), 160.94 (s), 172.85 (s), 173.64 (s), 174.36 (s) 174.64 (s), 174.92 (s). FAB-MS *m/z*: 972 ($M^+$+1), 994 ($M^+$+Na).

**JSTX-3 (1)**

A mixture of **14** (23.9 mg, 0.025 mmol) and 10% Pd-C (15 mg) in AcOH (5 ml) was stirred under a hydrogen atmosphere for 1 h. After filtration of the catalyst, the filtrate was subjected to freeze-drying to leave powders. The powders were dissolved in $CH_2Cl_2$ (0.1 ml) containing TFA (0.2 ml) and 1,2-ethanedithiol (0.05 ml, 0.60 mmol) and the whole was stirred at room temperature for 2h. Then the mixture was concentrated *in vacuo* and the residue was purified by reverse phase flash column chromatography ($H_2O$ / $CH_3CN$ / TFA (95:5:1-70:3:1)) to give JSTX-3 (**1**) as TFA salts (17.6 mg, 79%); $[\alpha]_D^{20}$ -1.70° (*c* 0.07, $H_2O$); $^1$H-NMR (500 MHz, $CDCl_3$): 1.04-1.54 (8H, m), 1.66-1.80 (4H, m), 1.96-2.08 (1H, m), 2.60 (2H, t, *J*=6.6 Hz), 2.69 (1H, dd, *J*=5.5, 13.7 Hz), 2.93-3.20 (12H, m), 3.24 (2H, t, *J*=6.6 Hz), 3.44 (1H, d, *J*=15.6 Hz), 3.51 (1H, d, *J*=15.6 Hz), 4.55 (1H, d, *J*=15.6 Hz), 4.55 (1H, m), 6.40 (2H, m), 7.03 (1H, d, *J*=8.9 Hz); $^{13}$C-NMR (125 MHz, $CDCl_3$): 22.86 (t), 22.98 (t), 23.44 (t), 25.99 (t), 28.02 (t), 28.11 (t), 31.16 (t), 36.36 (t), 36.84 (t), 37.21 (t), 39.48 (t), 39.53 (t), 43.82 (t), 44.80 (t), 47.02 (t), 47.25 (t), 51.12 (d), 103.19 (d), 107.83 (d), 114.02 (s), 115.74 (s), 118.07 (s), 132.68 (d), 155.46 (s), 156.48 (s), 162.85 (s), 163.13 (s), 163.41 (s), 163.69 (s), 171.80 (s), 172.53 (s), 174.94 (s), 175.12 (s). FAB-MS *m/z*: 588 ($M^+$+Na).

**References**


1 Y. Aramaki, T. Yasuhara, T. Higashijima, M. Yoshioka, A. Miwa, N. Kawai, and T. Nakajima, *Proc. Jpn. Acad.*, 1986, **62 (B)**, 359.

2 T. Toki, T. Yasuhara, Y. Aramaki, N. Kawai, and T. Nakajima, *Biomedical Res.*, 1988, **9**, 75; T. Toki, T. Yasuhara, Y. Aramaki, K. Osawa, A. Miwa, N. Kawai, and T. Nakajima, *ibid.*, 1988, **9**, 421.

3 N. Kawai, A. Miwa, M. Saito, H. Pan-Hou, and M. Yoshioka, *J. Phsiol., Paris*, 1984, **79**, 228; H. Pan-Hou and Y. Suda, *Brain Res.*, 1987, **418**, 198; T. Nishizaki and Y. Okada, *ibid.*, 1988, **452**, 11; H. Jackson and P. N. R. Usherwood, *Trends Neurosci.*, 1988, **11**, 278.

4 N. Kawai, A. Miwa, and T. Abe, *Brain Res.*, 1982, **247**, 169; T. Abe, N. Kawai, and A. Miwa, *J. Physiol.*, 1983, **339**, 243; N. Kawai, S. Yamagishi, M. Saito, and K. Furuya, *Brain Res.*, 1983, **278**, 346; A. Miwa, N. Kawai, M. Saito, H. Pan-Hou, and M. Yoshioka, *J. Neurophsiol.*, 1987, **58**, 319; M. Saito, Y. Sahara, Y. Miwa, K. Shimazaki, T. Nakajima, and N. Kawai, *Brain Res.*, 1989, **481**, 16.

5 N. Kawai, *J. Toxicol.* 1991, **10**, 131.

6 A. Ajima, T. Hensch, R. T. Kado, M. Ito, *Neurosci. Res.*, 1991, **12**, 281.

7 P. N. R. Usherwood, in *Excitatory Amino Acids*, eds. B. S. Meldrum, F. Moroni, R. P. Simon, and J. H. Wood, Raven, New York, 1991, pp 379-396.

8 H. Jackson and P. N. R. Usherwood, *Trends Neurosci.* 1988, **11**, 278.

9 T. Teshima, T. Matsumoto, T. Wakamiya, T. Shiba, T. Nakajima, and N. Kawai, *Tetrahedron*, 1990, **46**, 3813.

10 M. Blaschke, B. U. Keller, R. Rivosecchi, M. Hollmann, S. Heinemann, and A Konnerth, *Proc. Natl. Acad. Sci. USA*, 1993, **90**, 6258.

11 Syntheses of spider toxins, see: **NSTX-3:** T. Teshima, T. Wakamiya, Y. Aramaki, T. Nakajima, N. Kawai, and T. Shiba, *Tetrahedron Lett.*, 1987, **28**, 3509; D. M. Nason, V. J. Jasys, P. R. Kelbaugh, D. Phillips, N. A. Saccomano, and R. A. Volkman, *ibid.*, 1989, **30**, 2337; T. Teshima, T. Matsumoto, M. Miyagawa, T. Wakamiya, T. Shiba, N. Narai, M. Yoshioka, and T. Nakajima, *Tetrahedron*, 1990, **46**, 3819; **Argiotoxin:** T. L. Shih, J. R-Sanchez, and H. Mrozik, *ibid.*, 1987, **28**, 6015; M. E. Adams, R. L. Carney, F. E. Enderlin, E. T. Fu, M. A. Jarema, J. P. Li, C. A. Miller, D. A. Schooley, M. J. Shapiro, and V. J. Venema, *Biochem. Biophys. Res. Commun.*, 1987, **148**, 678; V. J. Jasys, P. R. Kelbaugh, D. M. Nason, D. Phillips, N. A. Saccomano, and R. A. Volkamann, *Tetrahedron Lett.*, 1988, **29**, 6223; **Clavamine:** T. Teshima, T. Matsumoto, M. Miyagawa, T. Wakamiya, T. Shiba, N. Narai, M. Yoshioka, T. Nakajima, and N. Kawai, *ibid.*, 1990, **46**, 3819; **Agelenopsis**

**aperta:** V. J. Jasys, P. R. Kelbaugh, D. M. Nason, D. Phillips, K. J. Rosnack, N. A. Saccomano, J. G. Stroh, and R. A. Volkamann, *J. Am. Chem. Soc.*, 1990, **112**, 6696; **NPTX-9 and 11:** B. W. Bycroft, W. C. Chan, N. D. Hone, S. Millington, and I. A. Nash, *J. Am. Chem. Soc.*, 1994, **116**, 7415; **FTX**: I. S. Blagbrough and E. Moya, *Tetrahedron Lett.*, 1994, **35**, 2057.

12 M. Miyashita, H. Sato, A. Yoshikoshi, T. Toki, M. Matsushita, H. Irie, T. Yanami, Y. Kikuchi, C. Takasaki, and T. Nakajima, *Tetrahedron Lett.*, 1992, **33**, 2833.

13 M. Miyashita, H. Sato, M. Matsushita, Y. Kusumegi, T. Toki, A. Yoshikoshi, T. Yanami, Y. Kikuchi, C. Takasaki, T. Nakajima, and H. Irie, *Tetrahedron Lett.*, 1992, **33**, 2837.

14 M. Miyashita, M. Matsushita, H. Sato, T. Toki, T. Nakajima, and H. Irie, *Chem. Lett.*, 1993, 929.

15 M. Matsushita, T. Kanemura, S. Hatakeyama, H. Irie, T. Toki, and M. Miyashita, *Tetrahedron Lett.*, 1995, **36**, 5231.

16 a) Y. Hashimoto, Y. Endo, K. Shudo, Y. Aramaki, N. Kawai, and T. Nakajima, *Tetrahedron Lett.*, 1987, **28**, 3511; b) D. M. Nason, V. J. Jasys, P. R. Kelbaugh, D. Phillips, N. A. Saccomano, and R. A. Volkman, *ibid.*, 1989, **30**, 2337.

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