# Total Syntheses of Spidamine and Joramine, Polyamine Toxins from the Joro Spider, *Nephila clavata*

Tadashige CHIBA,[a] Toshifumi AKIZAWA,[a] Motomi MATSUKAWA,[a] Masatoshi NISHI,[a] Nobufumi KAWAI,[b] and Masanori YOSHIOKA*,[a]

*Faculty of Pharmaceutical Sciences, Setsunan University,[a] 45–1, Nagaotoge-cho, Hirakata, Osaka 573–01, Japan and Department of Physiology, Jichi Ika University,[b] Kawachi-gun, Tochigi 329–04, Japan.*
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**In order to confirm the structures of spidamine and joramine, identified from the venom of a spider (*Nephila clavata*), we convergently synthesized both compounds, which have a common side chain of *N*-(L-asparaginyl)-*N'*-(3-aminopropyl-*β*-alanyl)-1,5-pentanediamine. A synthon of the common side chain was synthesized by starting with *n*-propanolamine which was converted to 7-azido-*N*-benzyloxycarbonyl-4-azaheptanoic acid *N*-hydroxysuccinimidyl ester. The ester was coupled with *tert*-butyloxycarbonyl-L-asparaginyl-1,5-pentanediamine to give the synthon of the common side chain. In the final synthesis of spidamine, the synthon of the common side chain was reacted with 2,4-dibenzyloxyphenylacetic acid *N*-hydroxysuccinimidyl ester, obtained by Willgerodt–Kindler reaction of 2,4-dihydroxyacetophenone. The protected spidamine was catalytically hydrogenated to remove protective groups, affording spidamine itself in 13% yield from *n*-propanolamine. In the final synthesis of joramine, the synthon of the common side chain was reacted with 4-benzyloxyphenyl acetic acid *N*-hydroxysuccinimidyl ester. The protected joramine was also catalytically hydrogenated to produce joramine itself in 11% yield.**

**The conformations of both synthesized compounds were analyzed by $^1$H-NMR and $^{13}$C-NMR. Their blocking activities on glutamate receptors were examined using lobster neuromuscular synapses.**

**Key words** spidamine; joramine; excitatory post synaptic potential; NMR; spider toxin

The polyamine neurotoxins of Joro spider, *Nephila clavata*,[1] have been found to be useful as specific blockers of glutamatergic neuromuscular transmission, particularly in the brain, where the glutamatergic neurons play a very important role in the brain function.[2] A major toxic component of the venom of *N. clavata*, called JSTX-3, has been purified and identified as a blocker of the glutamate receptors inducing the excitatory post synaptic potential (EPSP) of the lobster.[3]

In an earlier paper,[4] we identified two additional neurotoxins from venom glands of *N. clavata*. These compounds were purified and their structures were confirmed by chemical analysis and NMR as spidamine, *N*-(2,4-dihydroxyphenylacetyl-L-asparaginyl)-*N'*-(3-aminopropyl-*β*-alanyl)-1,5-pentanediamine, and joramine, *N*-(4-hydroxyphenylacetyl-L-asparaginyl)-*N'*-(3-aminopropyl-*β*-alanyl)-1,5-pentanediamine. The blocking intensities of both compounds on EPSP were about one-tenth that of JSTX-3. Interestingly, spidamine, possessing a 2,4-dihydroxyphenylacetyl (2,4-DHPA) group, caused an irreversible block of EPSP, whereas joramine, possessing a 4-hydroxyphenylacetyl (4-HPA) group, caused a reversible block of EPSP. Since the stereo-structure of spidamine appeared different from that of joramine, it was assumed that the irreversible action of joramine and of JSTX-3 was due to the 2,4-DHPA group.

In the present paper, we describe the synthesis of spidamine and of joramine. We were able to obtain sufficient quantities of both compounds to permit us to analyze their conformations by NMR and to study the differences in their blocking activity.

**Convergent Syntheses of Spidamine and Joramine** Spidamine and joramine are characterized by the presence of a common side chain consisting of L-asparagine (Asn), 1,5-pentanediamine (Cad), and aminopropyl-*β*-alanine (Ampa). The head of spidamine is a 2,4-DHPA group while the head of joramine is a 4-HPA group.

For the purpose of synthesis, the chemical structures of the target toxins are divided into three regions: Ampa (I), Asn-Cad (II), and the 4-HPA or 2,4-DHPA group (III), as illustrated in Fig. 1.

**Synthon of Region I** Synthesis of a synthon of region I for spidamine and joramine was performed by modifying the method of Miyashita *et al.*[5–7] for the synthesis of nephilatoxins; they converted alkyl azide intermediates[8–10] into various polyamines. The major modification in our approach was a change of the *tert*-butyloxycarbonyl (Boc) protective group for the amine to a benzyloxycarbonyl (Cbz) group. The other modification was the use of the *N*-hydroxysuccinimidyl ester (ONSu) of the carboxylic group in place of the *p*-nitrophenyl ester (ONp).

7-Azido-*N*-Cbz-4-azaheptanoic acid ONSu (**7**) was synthesized from *n*-propanolamine (**1**) in six steps in a total yield of 41.5%. Michael reaction of *n*-propanolamine with methyl acrylate gave methyl 7-hydroxyl-4-azaheptanoate (**2**) in 97.9% yield; protection of the amino

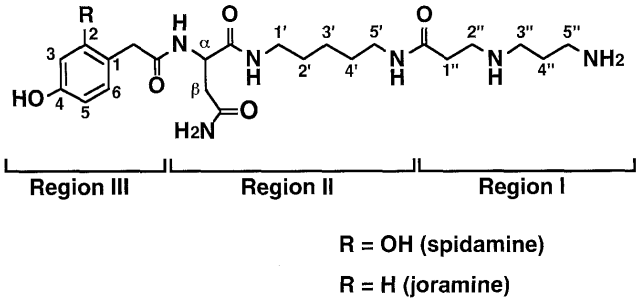


Fig. 1. Structures of Joramine and Spidamine and Their Synthetic Regions

* To whom correspondence should be addressed.

group of **2** with Cbz yielded methyl 7-hydroxyl-*N*-Cbz-4-azaheptanoate (**3**) (85%). The hydroxyl group of **3** was mesylated with methylsulfonyl chloride to give methyl 7-methylsulfonyloxy-*N*-Cbz-4-azaheptanoate (**4**). The methylsulfonyl group of **4** was substituted with an azide group to form methyl 7-azido-*N*-Cbz-4-azaheptanoate (**5**) (63%). The corresponding ONSu was readily derived from 7-azido-*N*-Cbz-4-azaheptanoic acid (**6**), which was obtained by alkaline hydrolysis of **5** and subsequent esterification with *N*-hydroxysuccinimide (HONSu) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) in 80% yield (Chart 1).

**Synthon of Region II** Boc-Asn-Cad (**9**) was synthesized by reacting Boc-Asn-ONp (**8**) with three equivalents of Cad in 35% yield (Chart 2).

**Synthon of Regions I—II** *N*-(Boc-Asn)-*N*′-(7-azido-*N*-Cbz-4-azaheptanoyl)-Cad (**10**) was synthesized by coupling **9** with **7**. Removal of the Boc group of **10** with trifluoroacetic acid (TFA) gave *N*-(Asn)-*N*′-(7-azido-*N*-Cbz-4-azaheptanoyl)-Cad (**11**) in 29.2% yield (Chart 2).

**Synthon of Region III for Joramine** 4-HPA (**12**) was converted to the sodium salt with sodium hydride in methanol (MeOH). The phenolate and carboxylate groups of **13** were benzylated to give 4-benzyloxyphenylacetic acid benzyl ester (**14**) in 77.1% yield. The ester of **14** was hydrolyzed with NaOH in ethanol (EtOH) to give 4-benzyloxyphenylacetic acid (**15**) in 99% yield, and this in turn was esterified with HONSu to give **16** in a total yield of 55.2% (Chart 3).

**Synthon of Region III for Spidamine** The synthesis of 2,4-DHPA according to Kawai *et al.*[11,12] was carried out at high temperature and at high pressure. The method was

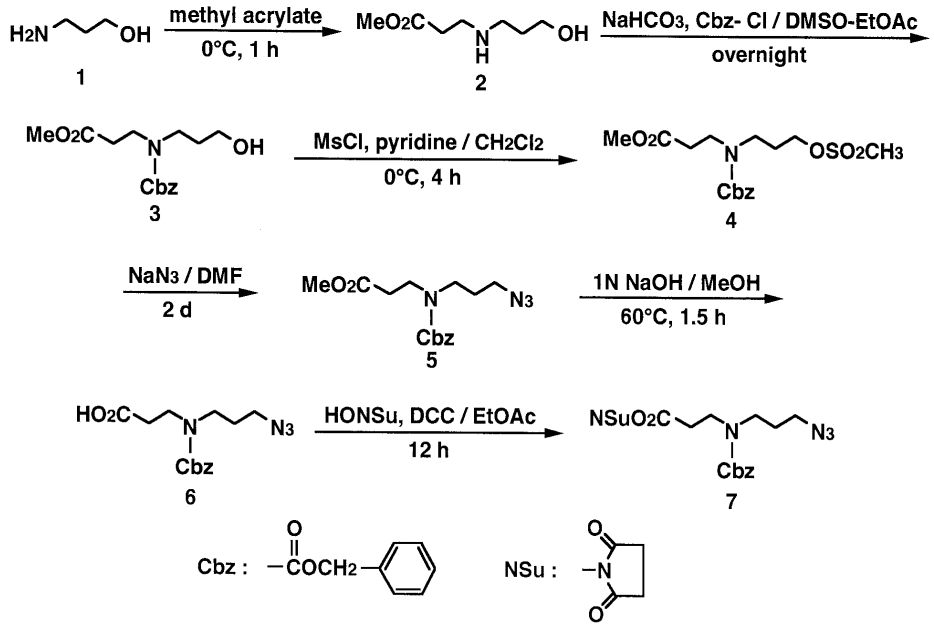


Chart 1. Preparation of Synthon of Region I

Reagents: Cbz-Cl, benzyloxycarbonyl chloride; MsCl, methanesulfonyl chloride; DMF, *N*,*N*′-dimethylformamide; HONSu, *N*-hydroxysuccinimide; DCC, *N*,*N*′-dicyclohexylcarbodiimide.

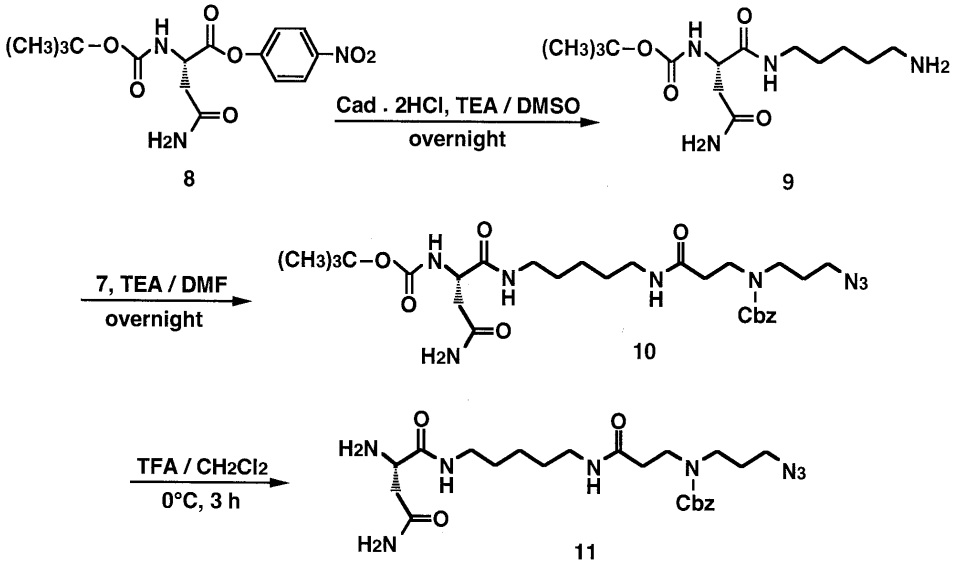


Chart 2. Coupling of Synthons of Regions I and II

Reagents: Cad, 1,5-pentanediamine; TEA, triethylamine; TFA, trifluoroacetic acid.

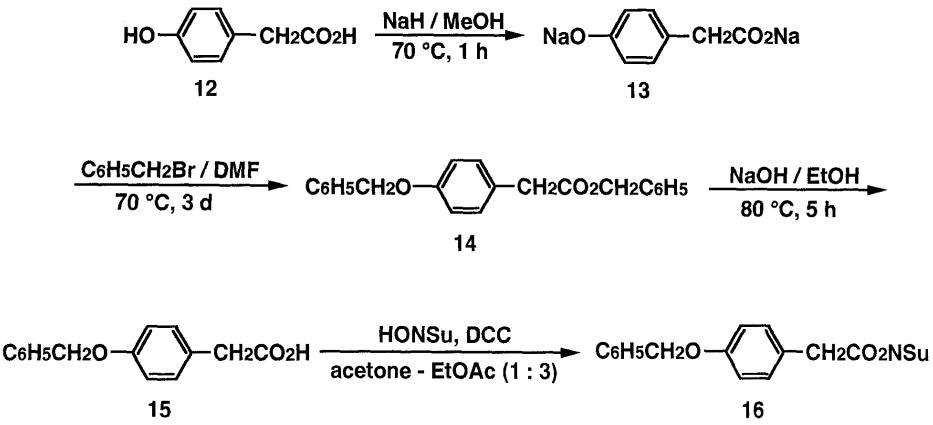


Chart 3. Preparation of Synthon of Region III for Joramine

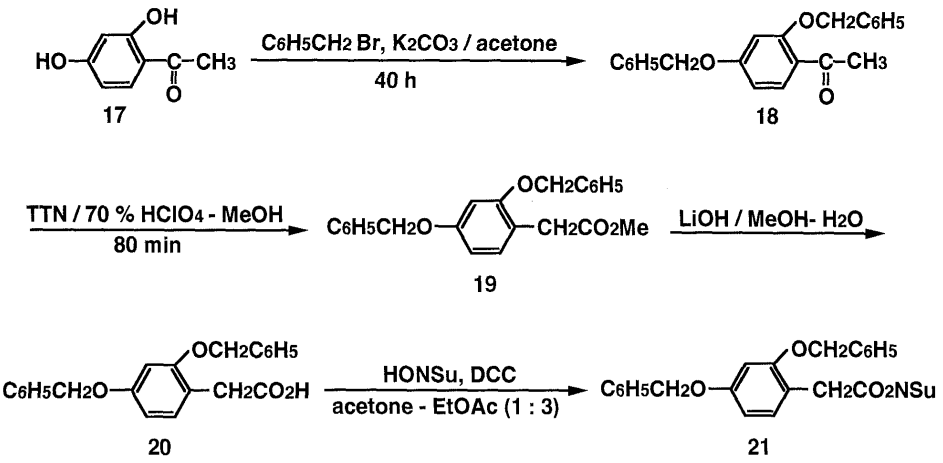


Chart 4. Preparation of Synthon of Region III for Spidamine
Reagents: TTN, thallium(III) nitrate trihydrate.

improved by Nakanishi *et al.*[13] The 2,4-DHPA was prepared as ONSu from a commercially available 2,4-dihydroxyacetophenone by means of the Willgerodt–Kindler reaction.[14] We modified this method in the benzylation of the hydroxyl group and carboxyl group of **12** or of **17**. The benzylation of the hydroxyl groups was performed with benzyl bromide under two conditions, either at 70 °C for 3 d, or in the presence of anhydrous $K_2CO_3$ at room temperature for 40 h. The latter condition was suitable for **13**, whereas the former condition was preferable for **17**.

In fact, the 2,4-dibenzyloxyphenylacetic acid ONSu (**21**) was prepared in four steps starting with 2,4-dihydroxyacetophenone (**17**). After benzylation of the dihydroxyl groups, the acetophenone derivative (**18**) was treated with thallium (III) nitrate trihydrate (TTN) and perchloric acid ($HClO_4$) in MeOH to give the 2,4-dibenzyloxyphenylacetic acid methyl ester (**19**) in 95% yield. After hydrolysis of the methyl ester with LiOH in MeOH, the 2,4-dibenzyloxyphenylacetic acid (**20**) was converted to the ONSu ester (**21**) in an overall yield of 61.5% (Chart 4).

**Joramine and Spidamine** *N*-(4-Benzyloxyphenylacetyl-Asn)-*N'*-(7-azido-*N*-Cbz-4-azaheptanoyl)-Cad (**22**) or *N*-(2,4-dibenzyloxyphenylacetyl-Asn)-*N'*-(7-azido-*N*-Cbz-4-azaheptanoyl)-Cad (**24**) was synthesized by coupling **11** with **16** or **21**, respectively. Reduction of the azide group and removal of the Cbz and benzyl groups of joramine (**23**) or spidamine (**25**) was readily carried out by catalytic hydrogenation over 10% Pd–C with nascent hydrogen generated from ammonium formate in *N,N'*-dimethylformamide (DMF) (Chart 5).

Crude **23** or **25** was purified by HPLC. The retention times of these compounds coincided with those of the natural products. The hydrolysates of joramine and of spidamine yielded aspartic acid, Cad and an *o*-phthalaldehyde-positive component as determined by amino acid-polyamine analysis with HPLC.[4] In FAB-MS, the parent ions of joramine and of spidamine appeared at 478 $(M+1)^+$ and 494 $(M+1)^+$, respectively. UV $\lambda_{max}$ 279.2 nm, $\varepsilon=1690$ and $\lambda_{max}$ 276.4 nm, $\varepsilon=854$, and $[\alpha]_D$ +4.90° and −4.96°, respectively. The IR spectra of joramine and of spidamine showed hydroxyl and amide absorptions. The overall yields of **23** and **25** were 11% and 13%, respectively, from the starting *n*-propanolamine.

**NMR of Spidamine and Joramine** The structures were confirmed by 600 MHz $^1$H-NMR, $^{13}$C-NMR (dimethyl sulfoxide (DMSO) or deuterium oxide ($D_2O$)).

The $^1$H–$^1$H correlation spectroscopy (COSY), $^{13}$C–$^1$H COSY and heteronuclear multiple quantum coherence (HMBC) spectra clarified the structural moieties in spidamine and joramine, while stereo-chemical connectivities were assigned by nuclear Overhauser and exchange spectroscopy (NOESY). The chemical shifts of the protons and carbons of the common side chain were

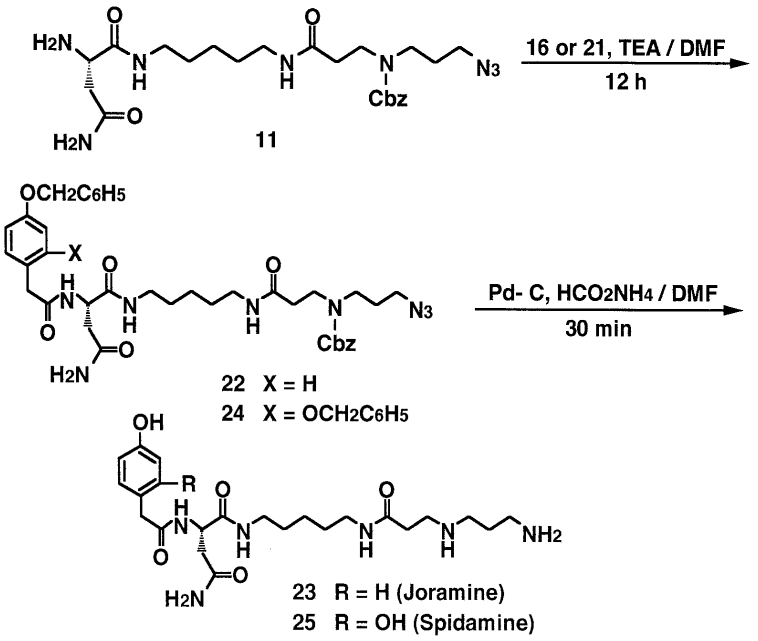


Chart 5. Coupling of Synthon of Region III with Synthon of Regions I—II for Spidamine and Joramine

Reagents: Pd–C, palladium-activated carbon.

Table 1. $^1$H-NMR Data for Synthetic Spidamine and Joramine

| Moiety position | | Spidamine | Joramine |
|---|---|---|---|
| 2,4-DHPA | 3 | 6.29, d (2.3)[a] | |
| | 5 | 6.16, dd (8.3, 2.3) | |
| | 6 | 6.84, d (8.3) | |
| | $CH_2$ | 3.29, d (15.0) | |
| | | 3.32, d (15.0) | |
| 4-HPA | 2 and 6 | | 7.03, d (8.6) |
| | 3 and 5 | | 6.67, d (8.6) |
| | $CH_2$ | | 3.33, s |
| Asn | $\alpha$ | 4.47, dd (12.2, 7.0) | 4.48, dd (11.8, 7.6) |
| | $\beta$ | 2.41, dd (14.1, 7.6) | 2.37 dd (13.2, 7.6) |
| | | 2.46, dd (14.4, 6.2) | 2.46, dd (14.1, 6.1) |
| | NH | 8.00, d (8.1) | 8.10, d (7.9) |
| | $NH_2$ | 6.83, s | 6.83, s |
| | | 7.32, s | 7.29, s |
| Cad | 1′ | 3.03, m | 3.14, m |
| | 2′ | 1.37, m | 1.43, m |
| | 3′ | 1.21, q (7.5) | 1.20, q (7.7) |
| | 4′ | 1.37, m | 1.43, m |
| | 5′ | 3.04, m | 3.14, m |
| | (NH) 1′ | 7.58, t (5.6) | 7.64, t (5.6) |
| | (NH) 5′ | 8.07, t (5.5) | 8.09, t (5.6) |
| Ampa | 1″ | 2.51, br | 2.51, t (7.0) |
| | 2″ | 3.11, br | 3.11, t (7.0) |
| | 3″ | 3.00, m | 3.00, t (7.6) |
| | 4″ | 1.91, q (7.6) | 1.91, q (7.6) |
| | 5″ | 2.88, br | 2.88, t (7.6) |
| | (NH) 2″, 3″ | 8.67, br | 8.67, br |
| | ($NH_2$) 5″ | 7.95, br | 7.99, br |

In DMSO-$d_6$, at 30 °C, 600 MHz, TMS. *a*) (*J*, Hz). The position of each proton is shown in Fig. 1.

Table 2. $^{13}$C-NMR Data for Synthetic Spidamine and Joramine

| Moiety position | | Spidamine | Joramine |
|---|---|---|---|
| 2,4-DHPA | 1 | 112.8 | |
| | 2 | 155.9 | |
| | 3 | 102.6 | |
| | 4 | 157.1 | |
| | 5 | 106.2 | |
| | 6 | 131.0 | |
| | $CH_2$ | 36.7 | |
| | CO | 171.3 | |
| 4-HPA | 1 | | 126.2 |
| | 2 and 6 | | 129.9 |
| | 3 and 5 | | 114.9 |
| | 4 | | 155.8 |
| | $CH_2$ | | 41.2 |
| | CO | | 168.8 |
| Asn | $\alpha$ | 49.8 | 49.8 |
| | $\beta$ | 37.1 | 37.3 |
| | CONH | 170.6 | 170.6 |
| | $CONH_2$ | 171.6 | 171.4 |
| Cad | 1′ | 38.4 | 38.39 |
| | 2′ | 28.46 | 28.48 |
| | 3′ | 23.5 | 23.5 |
| | 4′ | 28.50 | 28.51 |
| | 5′ | 38.4 | 38.44 |
| Ampa | 1″ | 30.7 | 30.7 |
| | 2″ | 43.0 | 43.0 |
| | 3″ | 43.9 | 43.9 |
| | 4″ | 23.5 | 23.6 |
| | 5″ | 36.1 | 36.1 |
| | CO | 168.8 | 168.8 |

In DMSO-$d_6$, at 30 °C, 150 MHz, TMS. The position of each carbon is shown in Fig. 1.

almost identical. The spectral data for spidamine and joramine are summarized in Tables 1, 2.

In the $^1$H-NMR of spidamine, the methylene protons at 3.29 ppm (d, $J$=15.0 Hz, 1H) and 3.32 ppm (d, $J$=15.0 Hz, 1H) were assigned to the geminal protons of the methylene in the 2,4-DHPA moiety. On the other hand, the signals of the methylene protons of joramine appeared at 3.33 ppm as a singlet. Presumably rotation of the C-phenyl bond was restricted by the hydroxyl group at the 2-position of 2,4-DHPA in spidamine, whereas this was not the case for joramine, as shown in Fig. 2. NOESY correlations confirmed the immobilization of the phenyl ring (data not shown). In particular, nuclear Overhauser

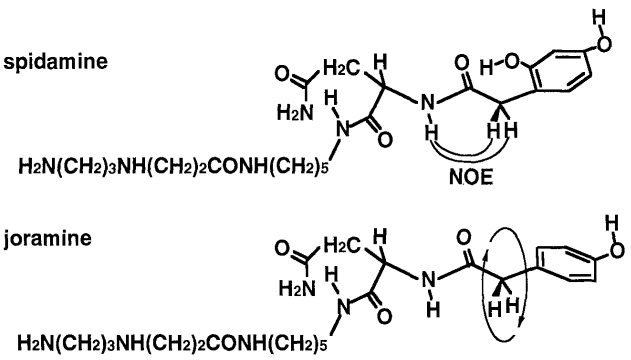


Fig. 2. Probable Conformations of Joramine and Spidamine Based Observed NOE

Thin lines show NOEs in spidamine. A solid arrow indicates free rotation of the phenyl ring of joramine.

effect (NOE) correlations were observed between the methylene protons of 2,4-DHPA and the amide proton between the Asn and the 2,4-DHPA residue in spidamine.

**Glutamate Receptor Blocking Activity** Blocking activity was assayed in terms of by inhibition of EPSP in a lobster neuromuscular preparation as described previously.[3] The EPSP blocking activities of both synthetic toxins were about one-tenth that of JSTX-3. Spidamine, possessing a 2,4-DHPA moiety, showed an irreversible block, whereas joramine, possessing a 4-HPA moiety, exhibited a reversible block in the same manner as the purified compounds from the venom of the spider.[4]

## Experimental

All chemicals were of the highest grade commercially available. HPLC purifications were carried out on a Capcell pak C18 (10 × 250 mm, Shiseido Corporation, Ltd., Tokyo) column at 40 °C with an indicated eluent at a flow rate of 3.0 ml/min, and target fractions were lyophilized. Melting points were measured with a Yanagimoto micro melting point (MP-S3) apparatus. UV spectra were measured with a JASCO UVDEC-610C. Optical rotations were measured with a JASCO DIP-140 digital polarimeter. IR spectra were measured with a Janssen MFT-2000. FAB-MS (glycerin matrix) and electron impact-mass spectra (EI-MS) (high-resolution; HR) were measured with a JEOL JMX-DX300 mass spectrometer (JEOL, Tokyo). $^1$H-NMR and $^{13}$C-NMR (100 MHz for carbon NMR resonance) were measured with a JEOL JNM-GSX 400 (JEOL, Tokyo) in either of the following deuterated solvents: DMSO-$d_6$ with tetramethylsilane (TMS) and $D_2O$ with 3-(trimethylsilyl)propionic acid sodium salt-$d_4$. Proton and carbon chemical shifts were reported in parts per million (ppm) down field from TMS or 3-(trimethylsilyl)propionic acid sodium salt-$d_4$ with the appropriate reference for each synthetic compound.

For spidamine and joramine, $^1$H-NMR spectra and $^{13}$C-NMR spectra (150 MHz for carbon NMR resonance) were measured with a GE Omega 600 MHz (Shimadzu, Kyoto) and assignments were made on the basis of $^1$H–$^1$H COSY, $^{13}$C–$^1$H COSY, $^1$H-detected multiple-bond HMBC, and NOESY experiments. Coupling constants ($J$ values) are given in hertz (Hz). Data processing was usually carried out on a SUN 4 workstation (SPARC Station 330, Itoh Technoh Science Co., Ltd., Tokyo).

**Methyl 7-Hydroxyl-4-azaheptanoate (2)** Methyl acrylate (9.7 ml, 0.1 mol) was added to *n*-propanolamine (**1**) (7.5 ml, 0.1 mol). The mixture was placed in an ice bath at 0 °C for 1 h and then allowed to warm to room temperature. Methyl 7-hydroxyl-4-azaheptanoate (**2**) was obtained as a clear oil in 97.9% yield (15.8 g, 0.98 mol). FAB-MS $m/z$: 162 $(M+1)^+$. High-resolution EI-MS $m/z$: Calcd for $C_7H_{15}NO_3$: 161.1053. Found: 161.1059. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 1.59 (q, $J$=6.4 Hz, 2H), 2.45 (t, $J$=6.4 Hz, 2H), 2.61 (t, $J$=6.4 Hz, 2H), 2.77 (t, $J$=6.4 Hz, 2H), 3.50 (t, $J$=6.4 Hz, 2H), 3.62 (s, 3H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 32.8, 34.6, 45.3, 47.1, 51.4, 60.2, 173.0.

**Methyl 7-Hydroxyl-*N*-Cbz-4-azaheptanoate (3)** Methyl 7-hydroxyl-4-azaheptanoate (**2**) (20 mmol, 3.22 g) was dissolved in 50 ml of benzyloxycarbonyl chloride (20 mmol, 2.83 ml) and $NaHCO_3$ (100 mmol, 8.3 g) in a mixed solvent of DMSO (20 ml) and ethyl acetate (EtOAc) (30 ml). The mixture was stirred at room temperature overnight, then concentrated *in vacuo* to give an oily residue. The residue was dissolved in EtOAc (100 ml), and the organic solution was washed with 100 ml of water three times. The washed solution was dried over anhydrous $Na_2SO_4$, and concentrated *in vacuo* to give crude methyl 7-hydroxyl-*N*-Cbz-4-azaheptanoate (**3**) as a yellow oily residue. The residue was dissolved in acetonitrile (MeCN) (10 ml) and purified by HPLC with a linear gradient from water containing 0.1% TFA to 70% MeCN containing 0.1% TFA. The product (**3**) was eluted at 23.9 min and was obtained as a clear oil in 85% yield (5.0 g, 17 mmol). FAB-MS $m/z$: 296 $(M+1)^+$. High-resolution EI-MS $m/z$: Calcd for $C_{15}H_{21}NO_5$: 295.1420. Found: 295.1415. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 1.66 (q, $J$=6.4 Hz, 2H), 2.56 (t, $J$=4.0 Hz, 2H), 3.30 (t, $J$=7.3 Hz, 2H), 3.42 (t, $J$=6.4 Hz, 2H), 3.49 (t, $J$=7.0 Hz, 2H), 3.59 (s, 3H), 5.08 (s, 2H), 7.30—7.39 (m, 5H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 31.3, 32.9, 43.2, 44.5, 51.2, 58.2, 66.0, 127.3, 127.6, 128.3, 136.9, 155.1, 171.5.

**Methyl 7-Methylsulfonyloxy-*N*-Cbz-4-azaheptanoate (4)** Methyl 7-hydroxyl-*N*-Cbz-4-azaheptanoate (**3**) (20 mmol, 5.9 g) was treated with methanesulfonyl chloride (40 mmol, 3.2 ml) and pyridine (40 mmol, 3.2 ml) in 20 ml of $CH_2Cl_2$. The mixture was stirred in an ice bath at 0 °C for 4 h to give a white precipitate. After removal of the precipitate on filter paper (Toyo Roshi Kaisha, Ltd., Tokyo), the filtrate was concentrated *in vacuo* to give a yellow oily residue. The residue was dissolved in EtOAc (200 ml), and the organic solution was washed with 100 ml of water three times. The washed solution was dried over anhydrous $Na_2SO_4$, and concentrated *in vacuo* to give crude methyl 7-methylsulfonyloxy-*N*-Cbz-4-azaheptanoate (**4**) as a yellow oily residue. The product (**4**) was used without further purification. FAB-MS $m/z$: 374 $(M+1)^+$. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 1.90 (m, 2H), 2.57 (td, $J$=2.2, 7.1 Hz, 2H), 3.12 (s, 3H), 3.33 (t, $J$=7.0 Hz, 2H), 3.48 (t, $J$=7.0 Hz, 2H), 3.57 (s, 3H), 4.19 (t, $J$=6.3 Hz, 2H), 5.08 (s, 2H), 7.31—7.39 (m, 5H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 21.8, 31.6, 36.6, 43.8, 45.1, 51.3, 66.2, 127.3, 127.7, 128.3, 136.8, 155.1, 171.5.

**Methyl 7-Azido-*N*-Cbz-4-azaheptanoate (5)** Crude methyl 7-methylsulfonyloxy-*N*-Cbz-4-azaheptanoate (**4**) (10 mmol, 3.7 g) was treated with sodium azide (20 mmol, 1.3 g) in 20 ml of DMF. The mixture was stirred at room temperature for 2 d, then concentrated *in vacuo* to give an oily residue. The residue was dissolved in EtOAc (200 ml), and the organic solution was washed with 100 ml of water three times. The washed solution was dried over anhydrous $Na_2SO_4$, and concentrated *in vacuo* to give crude methyl 7-azido-*N*-Cbz-4-azaheptanoate (**5**) as a yellow oily residue. The residue was dissolved in MeCN (5 ml) and purified by HPLC with a linear gradient from 30% MeCN containing 0.1% TFA to 70% MeCN containing 0.1% TFA. The product (**5**) was eluted at 31.1 min and was obtained as a clear oil in 63% yield (2.0 g, 6.2 mmol). FAB-MS $m/z$: 321 $(M+1)^+$. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 1.74 (q, $J$=6.9 Hz, 2H), 2.56 (t, $J$=7.1 Hz, 2H), 3.25—3.33 (m, 4H), 3.48 (t, $J$=7.1 Hz, 2H), 3.57 (s, 3H), 5.08 (s, 2H), 7.30—7.39 (m, 5H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 27.3, 32.9, 43.3, 44.7, 48.4, 51.3, 66.3, 127.4, 127.7, 128.3, 137.0, 155.2, 171.6.

**7-Azido-*N*-Cbz-4-azaheptanoic Acid (6)** Methyl 7-azido-*N*-Cbz-4-azaheptanoate (**5**) (10 mmol, 3.2 g) was dissolved in 1.0 N NaOH in 20 ml of MeOH. The mixture was allowed to stand at 60 °C for 1.5 h, then brought to pH 7.0 by the addition of 10 ml of 1.0 N HCl, and was concentrated *in vacuo* to give crude 7-azido-*N*-Cbz-4-azaheptanoic acid (**6**) as an oily residue. The residue was dissolved in MeCN (10 ml) and purified by HPLC with a linear gradient from 30% MeCN containing 0.1% TFA to 70% MeCN containing 0.1% TFA. The product (**6**) was eluted at 16.4 min and was obtained as a clear oil in 99% yield (3.0 g, 9.8 mmol). FAB-MS $m/z$: 307 $(M+1)^+$, 305 $(M-1)^-$. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 1.74 (q, $J$=6.9 Hz, 2H), 2.47 (t, $J$=7.2 Hz, 2H), 3.28—3.33 (m, 4H), 3.44 (t, $J$=7.2 Hz, 2H), 5.08 (s, 2H), 7.29—7.39 (m, 5H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 27.3, 33.3, 43.3, 44.7, 48.4, 66.2, 127.3, 127.7, 128.3, 136.9, 155.1, 172.7.

**7-Azido-*N*-Cbz-4-azaheptanoic Acid *N*-Hydroxysuccinimidyl Ester (7)** 7-Azido-*N*-Cbz-4-azaheptanoic acid (**6**) (10 mmol, 3.0 g) was treated with DCC (11 mmol, 2.3 g) and HONSu (11 mmol, 1.3 g) in a mixed solvent of acetone (7.5 ml) and EtOAc (22.5 ml). The mixture was stirred in an ice bath at 0 °C for 12 h to give a white precipitate, and then was allowed to warm to room temperature. The precipitate was filtered off, and the filtrate was concentrated *in vacuo* to give crude 7-azido-*N*-Cbz-4-

azaheptanoic acid ONSu (**7**) as a yellow oily residue. The residue was dissolved in MeCN (10 ml) and purified by HPLC with a linear gradient from 30% MeCN to 95% MeCN. The product (**7**) was eluted at 17.9 min and was obtained as a clear oil in 80% yield (3.2 g, 8.0 mmol). FAB-MS *m/z*: 404 $(M+1)^+$, 402 $(M-1)^-$. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 1.74 (q, $J=6.9$ Hz, 2H), 2.81 (s, 4H), 2.96 (t, $J=6.7$ Hz, 2H), 3.30—3.35 (m, 4H), 3.57 (t, $J=6.7$ Hz, 2H), 5.10 (s, 2H), 7.31—7.37 (m, 5H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 25.4, 27.4, 29.6, 43.0, 44.8, 48.3, 66.3, 127.3, 127.7, 128.3, 136.7, 155.1, 167.3, 170.0.

**Boc-L-asparaginyl-1,5-pentanediamine (9)** Boc-Asn-ONp (**8**) (10 mmol, 3.5 g) was treated with various concentrations of 1,5-pentanediamine dihydrochloride (Cad–2HCl), *i.e.*, 1.8 g/10 mmol, 5.3 g/30 mmol, and 8.8 g/50 mmol, and various amounts of triethylamine (TEA), *i.e.*, 1.4 ml/10 mmol, 4.2 ml/30 mmol, and 7.0 ml/50 mmol, in 200 ml of DMSO. Each mixture was stirred at room temperature overnight, then concentrated *in vacuo* to give crude Boc-Asn-Cad (**9**) as a yellow powder residue. The yields ranged from less than 10% (first concentrations above) to more than 35% (third concentrations). The residue was dissolved in water (50 ml) to give a white precipitate. This was filtered off and the filtrate was purified by HPLC with a linear gradient from water containing 0.1% TFA to 50% MeCN containing 0.1% TFA. The product (**9**) was eluted at 10.5 min and was obtained as a white solid in 35% maximum yield (1.1 g, 3.5 mmol). mp 141—143 °C. FAB-MS *m/z*: 317 $(M+1)^+$, 315 $(M-1)^-$. High-resolution EI-MS *m/z*: Calcd for $C_{14}H_{28}N_4O_4$: 316.2112. Found: 316.2105. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 1.29 (m, 2H), 1.38 (m, 2H), 1.38 (s, 9H), 1.55 (m, 2H), 2.39 (m, 2H), 2.73 (t, $J=7.6$ Hz, 2H), 3.04 (m, 2H), 4.17 (m, 1H), 6.80 (d, $J=7.9$ Hz, 1H), 6.87 (s, 1H), 7.32 (s, 1H), 7.74 (s, 1H), 7.99 (s, 2H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 22.9, 26.5, 28.1, 28.3, 37.5, 38.2, 38.6, 51.5, 78.1, 155.0, 171.2, 171.6.

***N*-(Boc-L-asparaginyl)-*N'*-(7-azido-*N*-Cbz-4-azaheptanoyl)-1,5-pentanediamine (10)** Boc-Asn-Cad (**9**) (1 mmol, 316 mg) was dissolved in a solution of 7-azido-*N*-Cbz-4-azaheptanoic acid ONSu (**7**) (1 mmol, 403 mg) in 10 ml of DMF. The mixture was stirred at room temperature overnight to give a white precipitate. This was filtered off, and the filtrate was concentrated *in vacuo* to give crude *N*-(Boc-Asn-)-*N'*-(7-azido-*N*-Cbz-4-azaheptanoyl)-Cad (**10**) as a white residue. The residue was dissolved in MeCN (5 ml) and purified by HPLC with a linear gradient from 30% MeCN containing 0.1% TFA to 95% MeCN containing 0.1% TFA. The product (**10**) was eluted at 14.0 min and was obtained as a white solid in 85% (513 mg, 0.85 mmol) yield. mp 78.5—79.5 °C. FAB-MS *m/z*: 605 $(M+1)^+$, 603 $(M-1)^-$. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 1.23 (m, 2H), 1.37 (s, 13H), 1.73 (q, $J=6.9$ Hz, 2H), 2.33 (t, $J=7.0$ Hz, 2H), 2.37 (m, 2H), 2.98—3.05 (m, 4H), 3.26—3.35 (m, 4H), 4.17 (br, 1H), 5.07 (s, 2H), 6.77 (br, 1H), 6.96 (s, 1H), 7.22 (s, 1H), 7.28—7.39 (m, 5H), 7.59 (br, 1H), 7.79 (t, $J=5.2$ Hz, 1H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 23.5, 27.3, 28.0, 28.6, 34.9, 37.4, 38.3, 38.4, 43.9, 44.5, 48.3, 51.4, 66.0, 78.1, 127.2, 127.6, 128.2, 136.9, 154.9, 155.0, 169.8, 171.0, 171.5.

***N*-(L-Asparaginyl)-*N'*-(7-azido-*N*-Cbz-4-azaheptanoyl)-1,5-pentanediamine (11)** *N*-(Boc-Asn)-*N'*-(7-azido-*N*-Cbz-azaheptanoyl)-Cad (**10**) (0.1 mmol, 60.4 mg) was dissolved in a mixture of TFA (2 ml) and dry $CH_2Cl_2$ (3 ml). The mixture was allowed to stand in an ice bath at 0 °C for 3 h and was then concentrated *in vacuo* to give an oily residue. The free TFA was removed by treating the oily residue with 2 ml of MeOH three times. The residue was then concentrated *in vacuo* to give crude *N*-(Asn)-*N'*-(7-azido-*N*-Cbz-4-azaheptanoyl)-Cad (**11**) as a clear oily residue. The product (**11**) was obtained as a clear oil in 98% yield (49.4 mg, 0.098 mmol). FAB-MS *m/z*: 505 $(M+1)^+$. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 1.25 (m, 2H), 1.35—1.45 (m, 4H), 1.73 (q, $J=7.0$ Hz, 2H), 2.33 (t, $J=7.0$ Hz, 2H), 2.58 (dd, $J=7.9$, 16.8 Hz, 1H), 2.66 (dd, $J=5.2$, 16.8 Hz, 1H), 2.99—3.15 (m, 4H), 3.26—3.35 (m, 4H), 3.59 (m, 2H), 3.98 (br, 1H), 5.07 (s, 2H), 7.17 (s, 1H), 7.30—7.39 (m, 5H), 7.60 (s, 1H), 7.83 (t, $J=5.3$ Hz, 1H), 8.05 (br, 2H), 8.26 (t, $J=5.5$ Hz, 1H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 23.5, 24.3, 27.4, 28.3, 28.6, 34.3, 35.5, 38.3, 38.7, 43.5, 44.6, 48.4, 49.2, 66.1, 127.2, 127.7, 128.3, 136.9, 155.1, 167.6, 169.9, 170.5.

**4-Benzyloxyphenylacetic Acid Benzyl Ester (14)** 4-HPA (**12**) (10 mmol, 1.5 g) was dissolved in a solution of sodium hydride (approximately 60% oil suspension; 20 mmol, 800 mg) in 50 ml of MeOH. The mixture was allowed to stand at 70 °C for 1 h, then concentrated *in vacuo* to give a white residue. The residue was dissolved in DMF (50 ml) and the solution was added to benzyl bromide (2.3 ml, 20 mmol). The mixture was stirred at 70 °C for 3 d to give a white precipitate. This was filtered off, and the filtrate was washed with 100 ml of petroleum ether three times. The washed solution was concentrated *in vacuo* to give crude 4-benzyloxyphenylacetic acid benzyl ester (**14**) as a brown residue. The residue was dissolved in benzene (100 ml) and the organic solution was washed with 100 ml of 1% NaOH three times and with water (100 ml) once. The washed solution was dried over anhydrous $Na_2SO_4$, and concentrated *in vacuo* to give a brown residue. The residue was dissolved in MeCN (10 ml) and purified by HPLC with a linear gradient from 30% MeCN to 70% MeCN. The product (**14**) was eluted at 14.2 min and was obtained as a white solid in 77.1% yield (2.56 g, 7.7 mmol).

Alternatively, a mixture of 4-HPA (**12**) (1.5 g, 10 mmol) in acetone (200 ml) was added to benzyl bromide (4.92 ml, 42 mmol) and anhydrous $K_2CO_3$ (6.92 g, 50 mmol). The mixture was stirred at room temperature for 40 h, and filtered through filter paper. The filtrate was concentrated *in vacuo* to give crude 4-dibenzyloxyphenylacetic acid benzyl ester (**14**) as a brown residue. The residue was dissolved in MeCN (10 ml) and purified by HPLC with a linear gradient from 30% MeCN to 70% MeCN. The product (**14**) was eluted at 14.2 min and was obtained as white solid in 35% yield (1.17 g, 3.5 mmol).

The yield under the former conditions was low, so the latter conditions were selected. mp 72—73 °C. FAB-MS *m/z*: 333 $(M+1)^+$. High-resolution EI-MS *m/z*: Calcd for $C_{22}H_{20}O_3$: 332.1413. Found: 332.1405. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.65 (s, 2H), 5.08 (s, 2H), 5.10 (s, 2H), 6.95 (d, $J=8.5$ Hz, 2H), 7.19 (d, $J=8.5$ Hz, 1H), 7.31—7.44 (m, 10H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 65.6, 69.2, 114.7, 126.4, 127.5, 127.70, 127.9, 128.3, 130.3, 136.1, 137.1, 157.3, 171.1.

**4-Benzyloxyphenylacetic Acid (15)** 4-Benzyloxyphenylacetic acid benzyl ester (**14**) (10 mmol, 3.3 g) was dissolved in 0.3 N NaOH in 100 ml of EtOH. The mixture was allowed to stand at 80 °C for 5 h, then concentrated *in vacuo* to give a white residue. The residue was dissolved in water (70 ml) and the aqueous solution was washed with 100 ml of diethyl ether twice. The pH of the washed solution was adjusted to $<2.0$ by the addition of 1.0 N HCl (10 ml), leading to the formation of a white precipitate of crude 4-benzyloxyphenylacetic acid (**15**). The precipitate was collected and washed with water (50 ml). The solution was concentrated *in vacuo* to give a white residue. The residue was dissolved in MeCN (10 ml) and purified by HPLC with a linear gradient from 20% MeCN containing 0.1% TFA to 70% MeCN containing 0.1% TFA. The product (**15**) was eluted at 22.5 min and was obtained as a white solid in 99% yield (2.40 g, 9.9 mmol). mp 122—123 °C. FAB-MS *m/z*: 243 $(M+1)^+$. High-resolution EI-MS *m/z*: Calcd for $C_{15}H_{14}O_3$: 242.0943. Found: 242.0953. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.47 (s, 2H), 5.08 (s, 2H), 6.94 (d, $J=8.4$ Hz, 2H), 7.16 (d, $J=8.4$ Hz, 2H), 7.32—7.44 (m, 5H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 69.1, 114.5, 127.2, 127.5, 127.7, 128.3, 130.3, 137.1, 157.0, 172.8.

**4-Benzyloxyphenylacetic Acid *N*-Hydroxysuccinimidyl Ester (16)** 4-Benzyloxyphenylacetic acid (**15**) (10 mmol, 2.42 g) was dissolved in a solution of DCC (11 mmol, 2.26 g) and HONSu (11 mmol, 1.27 g) in a mixed solvent of acetone (50 ml) and EtOAc (150 ml). The mixture was stirred in an ice bath at 0 °C for 1 h and allowed to warm to room temperature for 2 h, yielding a white precipitate. This was filtered off, and the filtrate was concentrated *in vacuo* to give crude 4-benzyloxyphenylacetic acid *N*-hydroxysuccinimidyl ester (**16**) as a white residue. The residue was dissolved in MeCN (10 ml) and purified by HPLC with a linear gradient from 50% MeCN to 70% MeCN. The product (**16**) was eluted at 12.1 min and was obtained as a white solid in 72.3% yield (2.45 g, 7.2 mmol). mp 113—114 °C. FAB-MS *m/z*: 340 $(M+1)^+$. High-resolution EI-MS *m/z*: Calcd for $C_{19}H_{17}NO_5$: 339.1107. Found: 339.1109. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.80 (s, 2H), 4.01 (s, 2H), 5.10 (s, 2H), 7.00 (d, $J=8.6$ Hz, 2H), 7.26 (d, $J=8.6$ Hz, 2H), 7.25—7.45 (m, 5H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 25.3, 35.7, 69.1, 114.8, 124.3, 127.6, 127.7, 128.3, 130.4, 137.0, 157.6, 167.5, 170.0.

**2,4-Dibenzyloxyacetophenone (18)** 2,4-Dihydroxyacetophenone (**17**) (10 mmol, 1.52 g) was treated with sodium hydride (approximately 60% oil suspension; 20 mmol, 800 mg) in 50 ml of MeOH. The mixture was allowed to stand at 70 °C for 1 h, then concentrated *in vacuo* to give a white residue. The residue was dissolved in DMF (50 ml) and the solution was added to benzyl bromide (2.3 ml, 20 mmol). The mixture was stirred at 70 °C for 3 d to give a white precipitate. This was filtered off, and the filtrate was washed with 100 ml of petroleum ether three times. The washed solution was concentrated *in vacuo* to give crude 2,4-dibenzyloxyacetophenone (**18**) as a brown residue. The residue was dissolved in MeCN (10 ml) and purified by HPLC with a linear gradient from 30% MeCN to 95% MeCN. The product (**18**) was eluted at 14.2 min and was

obtained as a white solid in 17% yield (0.56 g, 1.7 mmol).

Alternatively, a solution of 2,4-dihydroxyacetophenone (**17**) (1.52 g, 10 mmol) in acetone (200 ml) was added to benzyl bromide (4.92 ml, 42 mmol) and anhydrous $K_2CO_3$ (6.92 g, 50 mmol). The mixture was stirred at room temperature for 40 h, and filtered through the filter paper. The filtrate was concentrated *in vacuo* to give crude 2,4-dibenzyloxyacetophenone (**18**) as a brown residue. The residue was dissolved in MeCN (10 ml) and purified by HPLC with a linear gradient from 30% MeCN to 95% MeCN. The product (**18**) was eluted at 14.2 min and was obtained as white solid in 81.7% yield (2.71 g, 8.2 mmol).

The yield using the former conditions was low, so the latter conditions were selected. mp 74.5—75 °C. FAB-MS *m/z*: 333 $(M+1)^+$, 331 $(M-1)^-$. High-resolution EI-MS *m/z*: Calcd for $C_{22}H_{20}O_3$: 332.1413. Found: 332.1410; $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.45 (s, 3H), 5.19 (s, 2H), 5.24 (s, 2H), 6.70 (dd, $J$=8.6, 2.3 Hz, 1H), 6.87 (d, $J$=2.3 Hz, 1H), 7.34—7.51 (m, 10H), 7.67 (d, $J$=8.6 Hz). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 31.6, 69.6, 70.2, 100.6, 106.9, 121.0, 127.7, 127.8, 127.9, 128.40, 136.3, 136.4, 196.2.

**2,4-Dibenzyloxyphenylacetic Acid Methyl Ester (19)** 2,4-Dibenzyloxyacetophenone (**18**) (1 mmol, 0.332 g) was dissolved in a solution of TTN (2 mmol, 0.882 g) in a mixed solvent of 70% $HClO_4$ (5.4 ml) and MeOH (20 ml). The mixture was stirred at room temperature for 80 min to give a white precipitate, which was removed by filtration and washed with MeOH (10 ml). The filtrate and washing diluted with water (20 ml) and the solution was extracted with $CH_2Cl_2$ (60 ml). The organic extract was washed with $NaHCO_3$, dried over anhydrous $Na_2SO_4$, and concentrated *in vacuo* to give crude 2,4-dibenzyloxyphenylacetic acid methyl ester (**19**) as a brown oily residue. The residue was dissolved in MeCN (1 ml) and purified by HPLC with a linear gradient from 30% MeCN to 95% MeCN. The product (**19**) was eluted at 25.7 min and was obtained as a white solid in 95.6% yield (0.318 g, 0.96 mmol). mp 61—62 °C. FAB-MS *m/z*: 363 $(M+1)^+$. High-resolution EI-MS *m/z*: Calcd for $C_{23}H_{22}O_4$: 362.1519. Found: 362.1514; $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.28 (s, 3H), 3.55 (s, 2H), 5.08 (s, 4H), 6.56 (dd, $J$=8.2, 2.3 Hz, 1H), 6.73 (d, $J$=2.3 Hz, 1H), 7.10 (d, $J$=8.2 Hz, 1H), 7.31—7.44 (m, 10H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 34.8, 51.2, 69.3, 69.4, 100.5, 105.8, 115.8, 127.0, 127.6, 127.7, 128.3, 131.3, 137.0, 137.1, 157.1, 158.8, 171.7.

**2,4-Dibenzyloxyphenylacetic Acid (20)** 2,4-Dibenzyloxyphenylacetic acid methyl ester (**19**) (10 mmol, 3.6 g) was dissolved in 4% LiOH solution (30 ml), MeOH (60 ml), water (20 ml), and DMF (40 ml). The mixture was allowed to stand at room temperature overnight, then concentrated *in vacuo*, and 1.0 N HCl (40 ml) was added to give crude 2,4-dibenzyloxyphenylacetic acid (**20**) as a white precipitate. The precipitate was collected and washed with water (100 ml), to afford a white solid in 99% (3.4 g, 9.9 mmol) yield without further purification. mp 134—135 °C. FAB-MS *m/z*: 349 $(M+1)^+$, 347 $(M-1)^-$. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.43 (s, 2H), 5.06 (s, 2H), 5.08 (s, 2H), 6.54 (dd, $J$=2.4, 8.2 Hz, 1H), 6.69 (d, $J$=2.4 Hz, 1H), 7.08 (d, $J$=8.2 Hz, 1H), 7.28—7.44 (m, 10H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 35.6, 69.2, 69.4, 100.4, 105.7, 117.4, 127.0—128.3, 131.2, 137.1, 137.2, 157.0, 158.3, 173.0.

**2,4-Dibenzyloxyphenylacetic Acid *N*-Hydroxysuccinimidyl Ester (21)** 2,4-Dibenzyloxyphenylacetic acid (**20**) (0.086 mmol, 30 mg) was dissolved in a solution of DCC (0.5 mmol, 103 mg) and HONSu (0.5 mmol, 57.5 mg) in a mixed solvent of acetone (10 ml) and EtOAc (30 ml). The mixture was stirred in an ice bath at 0 °C for 3 h, and was then held at room temperature overnight to give a white precipitate. This was filtered off, and the filtrate was concentrated *in vacuo* to give crude 2,4-dibenzyloxyphenylacetic acid ONSu (**21**) as a white residue. The residue was dissolved in MeCN (1 ml) and purified by HPLC with a linear gradient from 30% MeCN containing 0.1% TFA to 95% MeCN containing 0.1% TFA. The product (**21**) was eluted at 23.4 min and was obtained as a white solid in 80% yield (30.6 mg, 0.069 mmol). mp 138.5—139.5 °C. FAB-MS *m/z*: 446 $(M+1)^+$. High-resolution EI-MS *m/z*: Calcd for $C_{26}H_{23}NO_6$: 445.1526. Found: 445.1522. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.80 (s, 4H), 3.93 (s, 2H), 5.08 (s, 2H), 5.14 (s, 2H), 6.60 (dd, $J$=2.3, 8.3 Hz, 1H), 6.74 (d, $J$=2.3 Hz, 1H), 7.23 (d, $J$=8.3 Hz, 1H), 7.29—7.45 (m, 10H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 25.4, 31.5, 69.4, 100.6, 106.0, 113.3, 127.0, 127.5, 127.6, 127.7, 128.3, 131.4, 136.9, 157.1, 159.2, 167.2, 170.0.

***N*-(4-Benzyloxyphenylacetyl-L-asparaginyl)-*N'*-(7-azido-*N*-Cbz-4-azaheptanoyl)-1,5-pentanediamine (22)** 4-Benzyloxyphenylacetic acid ONSu (**16**) (0.1 mmol, 33.9 mg) was dissolved in a solution of *N*-(Asn)-*N'*-(7-azido-*N*-Cbz-4-azaheptanoyl)-Cad (**11**) (0.1 mmol, 50.4 mg) in DMF (5 ml) containing TEA (0.1 mmol, 14 $\mu$l). The mixture was stirred at room temperature for 12 h to give a white precipitate. This was removed by filtration, and the filtrate was concentrated *in vacuo* to give crude *N*-(4-benzyloxyphenylacetyl-Asn)-*N'*-(7-azido-*N*-Cbz-4-azaheptanoyl)-Cad (**22**) as a white residue. The residue was dissolved in MeCN (1 ml) and purified by HPLC with a linear gradient from 30% MeCN containing 0.1% TFA to 50% MeCN containing 0.1% TFA. The product (**22**) was eluted at 32.9 min and was obtained as a white solid in 90% yield (65.6 mg, 0.09 mmol). mp 145—146 °C. FAB-MS *m/z*: 729 $(M+1)^+$. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 1.20 (m, 2H), 1.34 (m, 4H), 1.93 (q, $J$=6.7 Hz, 2H), 2.32 (t, $J$=7.2 Hz, 2H), 2.38 (dd, $J$=7.3, 16.8 Hz, 1H), 2.47 (dd, $J$=6.1, 16.8 Hz, 1H), 3.00 (m, 4H), 3.31 (m, 2H), 3.38 (s, 2H), 3.42 (m, 2H), 3.58 (t, $J$=6.1 Hz, 2H), 4.49 (dd, $J$=7.5, 14.2 Hz, 1H), 5.06 (s, 2H), 5.07 (s, 2H), 6.78 (s, 1H), 6.91 (d, $J$=8.9 Hz, 2H), 7.16 (d, $J$=8.9 Hz, 2H), 7.21 (s, 1H), 7.29—7.44 (m, 10H), 7.58 (t, $J$=5.6 Hz, 1H), 7.79 (t, $J$=5.3 Hz, 1H), 8.06 (d, $J$=7.9 Hz, 1H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 23.6, 28.5, 28.6, 31.1, 34.9, 37.4, 38.4, 41.2, 42.8, 44.6, 49.8, 66.1, 69.2, 114.5, 127.3, 127.4, 127.6, 128.3, 128.4, 129.8, 130.0, 136.9, 137.2, 155.1, 156.9, 169.9, 170.2, 170.6, 171.4.

**Joramine (23)** *N*-(4-Benzyloxyphenylacetyl-Asn)-*N'*-(7-azido-*N*-Cbz-4-azaheptanoyl)-Cad (**22**) (0.1 mmol, 72.8 mg) was added to a suspension of 10% Pd–C (50 mg) and ammonium formate (0.5 mmol, 32 mg) in 3 ml of DMF. The mixture was allowed to stand at room temperature for 30 min, then washed with water (10 ml) and filtered. The filtrate was concentrated by lyophilization to give crude joramine (**23**) as an oily residue. The residue was dissolved in water (1 ml) and purified by HPLC with a linear gradient from water containing 0.1% TFA to 50% MeCN containing 0.1% TFA. The product (**23**) was eluted at 13.6 min and was obtained as a clear oil in 98% yield (46.9 mg, 0.98 mmol). A portion (50 nmol) of the product was hydrolyzed at 115 °C for 24 h and subjected to analyzed on a Dabsyl analyzer.[4] Equivalent quantities of Asp and Cad were obtained. UV spectrum $\lambda_{max}$ 276.4 nm, $\varepsilon$=854; $[\alpha]_D$ +4.90° ($c$=0.42 in $H_2O$, at 25 °C). FAB-MS *m/z*: 479 $(M+1)^+$. High-resolution FAB-MS *m/z*: Calcd for $C_{23}H_{39}N_6O_5$: 479.2985. Found: 479.2973. IR (diamond): 3280, 2940, 1670, 1520 cm$^{-1}$. $^1$H-NMR (600 MHz, DMSO-$d_6$) $\delta$: 1.91 (q, $J$=7.6 Hz, 2H), 1.20 (q, $J$=7.7 Hz, 2H), 1.43 (m, 4H), 2.37 (dd, $J$=7.6, 13.2 Hz, 1H), 2.46 (dd, $J$=6.1, 14.1 Hz, 1H), 2.51 (t, $J$=7.0 Hz, 2H), 2.88 (t, $J$=7.6 Hz, 2H), 3.00 (t, $J$=7.6 Hz, 2H) 3.11 (t, $J$=7.0 Hz, 2H), 3.14 (m, 4H), 3.33 (s, 2H), 4.48 (dd, $J$=7.6, 11.8 Hz, 1H), 6.67 (d, $J$=8.6 Hz, 2H), 6.83 (s, 1H), 7.03 (d, $J$=8.6 Hz, 2H), 7.29 (s, 1H), 7.64 (t, $J$=5.6 Hz, 1H), 7.99 (br, 2H), 8.09 (t, $J$=5.6 Hz, 1H), 8.10 (d, $J$=7.9 Hz, 1H), 8.67 (br, 1H). $^{13}$C-NMR (150 MHz, DMSO-$d_6$) $\delta$: 23.5, 23.6, 28.5, 30.7, 36.1, 37.3, 38.4, 41.2, 43.0, 43.9, 49.8, 114.9, 126.2, 129.9, 155.8, 168.8, 170.6, 171.4.

***N*-(2,4-Dibenzyloxyphenylacetyl-L-asparaginyl)-*N'*-(7-azido-*N*-Cbz-4-azaheptanoyl)-1,5-pentanediamine (24)** 2,4-Dibenzyloxyphenylacetic acid ONSu (**21**) (0.1 mmol, 44.5 mg) was dissolved in a solution of *N*-(Asn)-*N'*-(7-azido-*N*-Cbz-4-azaheptanoyl)-Cad (**11**) (0.1 mmol, 50.4 mg) in 50 ml of DMF containing TEA (0.1 mmol, 14 $\mu$l). The mixture was stirred at room temperature for 12 h to give a white precipitate. This was filtered off, and the filtrate was concentrated *in vacuo* to give crude *N*-(2,4-dibenzyloxyphenylacetyl-Asn)-*N'*-(7-azido-*N*-Cbz-4-azaheptanoyl)-Cad (**24**) as a white residue. The residue was dissolved in MeCN (1 ml) and purified by HPLC with a linear gradient from 30% MeCN containing 0.1% TFA to 70% MeCN containing 0.1% TFA. The product (**24**) was eluted at 27.3 min and was obtained as a white solid in 90% yield (75 mg, 0.09 mmol). mp 167—168 °C. FAB-MS *m/z*: 835 $(M+1)^+$. $^1$H-NMR (400 MHz, DMSO-$d_6$) $\delta$: 1.18 (m, 2H), 1.29—1.36 (m, 4H), 1.92 (q, $J$=6.7 Hz, 2H), 2.32 (t, $J$=7.2 Hz, 2H), 2.37 (dd, $J$=6.7, 16.8 Hz, 1H), 2.44 (dd, $J$=6.4, 16.8 Hz, 1H), 2.98 (m, 4H), 3.26—3.32 (m, 4H), 3.42 (s, 2H), 3.58 (t, $J$=6.4 Hz, 2H), 4.51 (dd, $J$=6.4, 14.3 Hz, 1H), 5.06 (s, 2H), 5.07 (s, 2H), 5.09 (s, 2H), 6.54 (dd, $J$=2.3, 8.5 Hz, 1H), 6.69 (d, $J$=2.3 Hz, 1H), 6.77 (s, 1H), 7.08 (d, $J$=8.5 Hz, 1H), 7.22 (s, 1H), 7.28—7.45 (m, 15H), 7.53 (t, $J$=5.7 Hz, 1H), 7.78 (t, $J$=5.3 Hz, 1H), 7.87 (d, $J$=7.9 Hz, 1H). $^{13}$C-NMR (100 MHz, DMSO-$d_6$) $\delta$: 23.5, 28.6, 31.1, 34.9, 36.1, 37.2, 38.3, 38.4, 42.8, 44.6, 49.8, 66.1, 69.3, 100.5, 105.8, 117.1, 127.0, 127.2, 127.5, 127.6, 127.7, 128.3, 130.8, 136.9, 137.1, 155.1, 156.9, 158.3, 169.8, 170.2, 170.5, 171.5.

**Spidamine (25)** *N*-(2,4-Dibenzyloxyphenylacetyl-Asn)-*N'*-(7-azido-*N*-Cbz-4-azaheptanoyl)-Cad (**24**) (0.1 mmol, 83.4 mg) was dissolved in a suspension of 10% Pd–C (50 mg) and ammonium formate (0.5 mmol, 32 mg) in 3 ml of DMF. The mixture was allowed to stand to room temperature for 30 min, then washed with water (10 ml) and filtered. The filtrate was concentrated by lyophilization to give crude spidamine (**25**)

as an oily residue. The residue was dissolved in water (1 ml) and purified by HPLC with a linear gradient from water containing 0.1% TFA to 50% MeCN containing 0.1% TFA. The product (**25**) was eluted at 13.4 min and was obtained as a clear oil in 98% yield (48.4 mg, 0.98 mmol). A portion (50 nmol) of the product was hydrolyzed at 115 °C for 24 h and analyzed with the Dabsyl analyzer.[4] Equivalent quantities of Asp and Cad were obtained. UV spectrum $\lambda_{max}$ 279.2 nm, $\varepsilon = 1690$; $[\alpha]_D = -4.96°$ ($c = 0.61$ in $H_2O$, at 25 °C). FAB-MS $m/z$: 495 $(M+1)^+$. High-resolution FAB-MS $m/z$: Calcd for $C_{23}H_{39}N_6O_6$: 495.2934. Found: 495.2914. IR (diamond): 3280, 2940, 1670, 1540 $cm^{-1}$. $^1$H-NMR (600 MHz, DMSO-$d_6$) $\delta$: 1.21 (q, $J = 7.5$ Hz, 2H), 1.37 (m, 4H), 1.91 (q, $J = 7.6$ Hz, 2H), 2.41 (dd, $J = 7.6$, 14.1 Hz, 1H), 2.46 (dd, $J = 6.2$, 14.4 Hz, 1H), 2.51 (br, 2H), 2.88 (br, 2H), 3.00 (m, 2H), 3.03 (m, 2H), 3.04 (m, 2H), 3.11 (br, 2H), 3.29 (d, $J = 15.0$ Hz, 1H), 3.32 (d, $J = 15.0$ Hz, 1H), 4.47 (dd, $J = 7.0$, 12.2 Hz, 1H), 6.16 (dd, $J = 2.3$, 8.3 Hz, 1H), 6.29 (d, $J = 2.3$ Hz, 1H), 6.83 (s, 1H), 6.84 (d, $J = 8.3$ Hz, 1H), 7.32 (s, 1H), 7.58 (t, $J = 5.6$ Hz, 1H), 7.95 (br, 2H), 8.00 (d, $J = 8.1$ Hz, 1H), 8.07 (t, $J = 5.5$ Hz, 1H), 8.67 (br, 1H). $^{13}$C-NMR (150 MHz, DMSO-$d_6$) $\delta$: 23.5, 28.5, 30.7, 36.1, 36.7, 37.1, 38.4, 43.0, 43.9, 49.8, 102.6, 106.2, 112.8, 131.0, 155.9, 157.1, 168.8, 170.6, 171.3, 171.6.

## Discussion

We have synthesized spidamine and joramine, which possess a common side chain, by utilizing three synthons, **7**, **9** and **16** or **21**. In the preparation of synthon **7**, we employed a Cbz group, which could be removed conveniently by nascent hydrogen in the final step of the synthesis, as shown in Chart 1. In the case of synthon **9**, the incorporation of Boc-Asn was low, when one equivalent of **8** was added to one equivalent of Cad, but the yield of **9** reached 35% when three or more equivalents of Cad were used. For preparation of the synthons **16** and **21**, we examined two conditions of benzylation; the use of benzyl bromide at 70 °C was more favorable for **16**, while benzyl bromide containing anhydrous $K_2CO_3$ at room temperature was superior for **21** (Charts 3 and 4, respectively).

In these studies, chemical and physical characteristics were checked by HPLC, UV, optical rotation, IR, MS, and NMR measurements. The yields of joramine and spidamine exceeded 10%, which compares to favorbly with that of *ca.* 7% for NSTX-3 or JSTX-3.[11,12] Interestingly, NOEs of the methylene of 2,4-DHPA to the amide between the Asn and 2,4-DHPA residue could be observed in the NMR spectrum of spidamine, but not in that of joramine. The kind of coupling seen in spidamine is probably present in other toxins, such as JSTXs, argiopins,[15] and clavamine,[16] possessing 2,4-DHPA linked to Asn-Cad, because the signals of their methylene protons appear as two double-doublets is the $^1$H-NMR.

JSTXs and argiopins cause an irreversible block of EPSP. Clavamine, possessing a similar side chain connected to a tripeptide, was inactive in the case of EPSP, but was active *in vivo* for various insects. Its insecticidal mechanism is not known. Both synthetic compounds showed the same activities toward EPSP as the natural products which we obtained previously.[4] Further, their affinities to the receptor for EPSP were quantitatively the same, although the reaction of spidamine was irreversible, whereas that of joramine was reversible. The structure–activity relationship of synthetic JSTX-3 analogs discussed in our previous paper[17] imply that the specificity to various receptors may be dependent on the side chain species of polyamine toxins possessing 2,4-DHPA, whereas the irreversible toxic reaction may be due to the 2,4-DHPA head. Thus, we may regard the intact head as a common chemical "blade" which the spider attaches to a variety of "handles" to obtain chemical weapons for attacking variety kinds of prey. If this view is valid, the 2,4-DHPA head may be useful in the design of simple synthetic insecticides. The present synthetic methods should be applicable to various other analogs.

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