Syntheses and Biological Activities of Joro Spider Toxin Analogs to Spidamine and Joramine

Tadashige CHIBA,[a] Toshifumi AKIZAWA,[a] Motomi MATSUKAWA,[a] Nobufumi KAWAI,[b] Yoshiaki KONO,[c] and Masanori YOSHIOKA*,[a]

Faculty of Pharmaceutical Sciences, Setsunan University,[a] 45–1, Nagaotoge-cho, Hirakata, Osaka 573–01, Japan, Department of Physiology, Jichi Ika University,[b] 3311–11, Yakushiji, Minamikawachi-cho, Kawachi-gun, Tochigi 329–04, Japan and National Institute of Health,[c] 1–23–1, Toyama, Shinjuku-ku, Tokyo 162, Japan.
Received April 30, 1996; accepted September 6, 1996

In order to study the structure-activity relationships of spidamine and joramine found in the venom of Joro spider, *Nephila clavata*, we attempted to synthesize various analogs. Six analogs were convergently synthesized according to our previous method for the synthesis of spidamine, *N*-(3-aminopropyl-β-alanyl)-*N'*-(2,4-dihydroxyphenylacetyl-L-asparaginyl)-1,5-pentanediamine and joramine, *N*-(3-aminopropyl-β-alanyl)-*N'*-(4-hydroxyphenylacetyl-L-asparaginyl)-1,5-pentanediamine. The biological activities of the analogs and four intermediates were compared with those of synthetic spidamine and joramine in three bioassay systems, lobster neuromuscular synapse, cockroaches and mosquito larvae. The glutamate receptors in these systems were inhibited by some analogs, and the D-asparagine- or indoleacetyl-containing analogs were found to be strong inhibitors. These compounds have potential application as insecticides.

Key words spidamine; joramine; synthetic analog; spider toxin; excitatory post synaptic potential; insect

In the previous paper, we reported complete characterization of two neurotoxins, spidamine and joramine, in the venom of Joro spider (*Nephila clavata*).[1] Although their contents in the venom are relatively low, their structure–activity relationships (SAR), are of interest.

In a study on SAR of polyamine spider toxins containing 2,4-dihydroxyphenylacetic acid (2,4-DHPA), Teshima *et al.*[2] reported that the α-amino group of arginine (Arg) in a synthetic analog of NSTX-3 from New Guinean Joro spider was necessary for strong binding to glutamate receptors, leading to an irreversible block of the excitatory postsynaptic potentials (EPSP) in the lobster neuromuscular synapse. Kawai and Nakajima[3] also reported SAR of a series of synthetic analogs of JSTX-3. While 2,4-DHPA alone suppressed EPSP, this inhibition was completely removed upon washing the preparation with normal saline. 2,4-DHPA-L-asparaginyl-spermine inhibited EPSP with incomplete recovery after washing. In contrast, JSTX-3 inhibited EPSP irreversibly. Estimation of the relative potency of these analogs indicated that 2,4-DHPA-L-asparaginyl-spermine was one order and 2,4-DHPA-L-asparaginyl-cadaverine (2,4-DHPA–L-Asn–Cad) was two orders of magnitude less potent than JSTX-3.

So far, however, the relationship between the blocking mode of the glutamate receptors and the structures of the polyamine toxins is unknown. In the present study, we found different blocking modes of the glutamate receptors between spidamine and joramine. Although their blocking actions on the glutamate receptors were similar in potency, that of spidamine was almost irreversible, whereas that of joramine was reversible.[4,5]

Recently, Nakanishi *et al.*[6–12] reported that Philanthotoxin-433 (PhTX-433) is both a potent noncompetitive inhibitor of the quisqualate sub-type of glutamate receptors (qGlu-R), as assayed by the twitch contraction of the locust leg muscle, and an allosteric inhibitor of acetylcholine receptors of vertebrates. When the hydrophobicity of regions of PhTX-433 was increased, the inhibitiory activity was increased. These groups may act to anchor the polyamine toxin in a hydrophobic pocket of the receptor channel to support the binding of the polyamine moiety to the channel wall.

We attempted to synthesize various analogs convergently for SAR studies. The activities of the analogs were examined by inhibition assay of EPSP, as well as two biological tests selected from the bioassays described by Kono *et al.*[13]

Syntheses of Polyamine Toxin Analogs

Preparation of Synthons I and II We designed six kinds of polyamine toxin analogs (**11**—**16**) which we synthesized from three appropriately modified synthons, as shown in Fig. 1.[1,14–23]

As synthon I, 7-azido-*N*-Cbz-4-azaheptanoic acid *N*-hydroxysuccinimidyl ester (**4**) was synthesized from *n*-propanolamine (**1**). Michael addition of **1** to methyl acrylate, followed by protection of the amino group with benzyloxycarbonyl (Cbz) gave methyl 7-hydroxyl-*N*-Cbz-4-azaheptanoate (**2**). Conversion of the hydroxyl group of **2** into an azido group gave methyl 7-azido-*N*-Cbz-4-azaheptanoate (**3**) in two steps. Alkaline hydrolysis of **3** furnished the corresponding acid, which was quantitatively transformed into the active ester (**4**) by treatment with *N*-hydroxysuccinimide (HONSu) and *N,N'*-dicyclohexylcarbodiimide (DCC). In this way, synthon I was synthesized in the high yield of 80%.

To obtain synthon II, *tert*-butyloxycarbonyl-L-asparagine *p*-nitrophenyl ester (Boc-L-Asn–ONp) and Boc–D-Asn–ONp, (**5a, b**) were condensed in various combinations with Cad or putrescine (Put) hydrochloride to produce **6a**—**c** in approximately 30% yield (Chart 1).

Preparation of Synthon III and Intermediates Hydroxyphenylacetic acid *N*-succinimidyl esters **8a**—**e** were converted to active esters with HONSu and DCC without further benzylation of the hydroxyl groups. Meanwhile,

* To whom correspondence should be addressed.

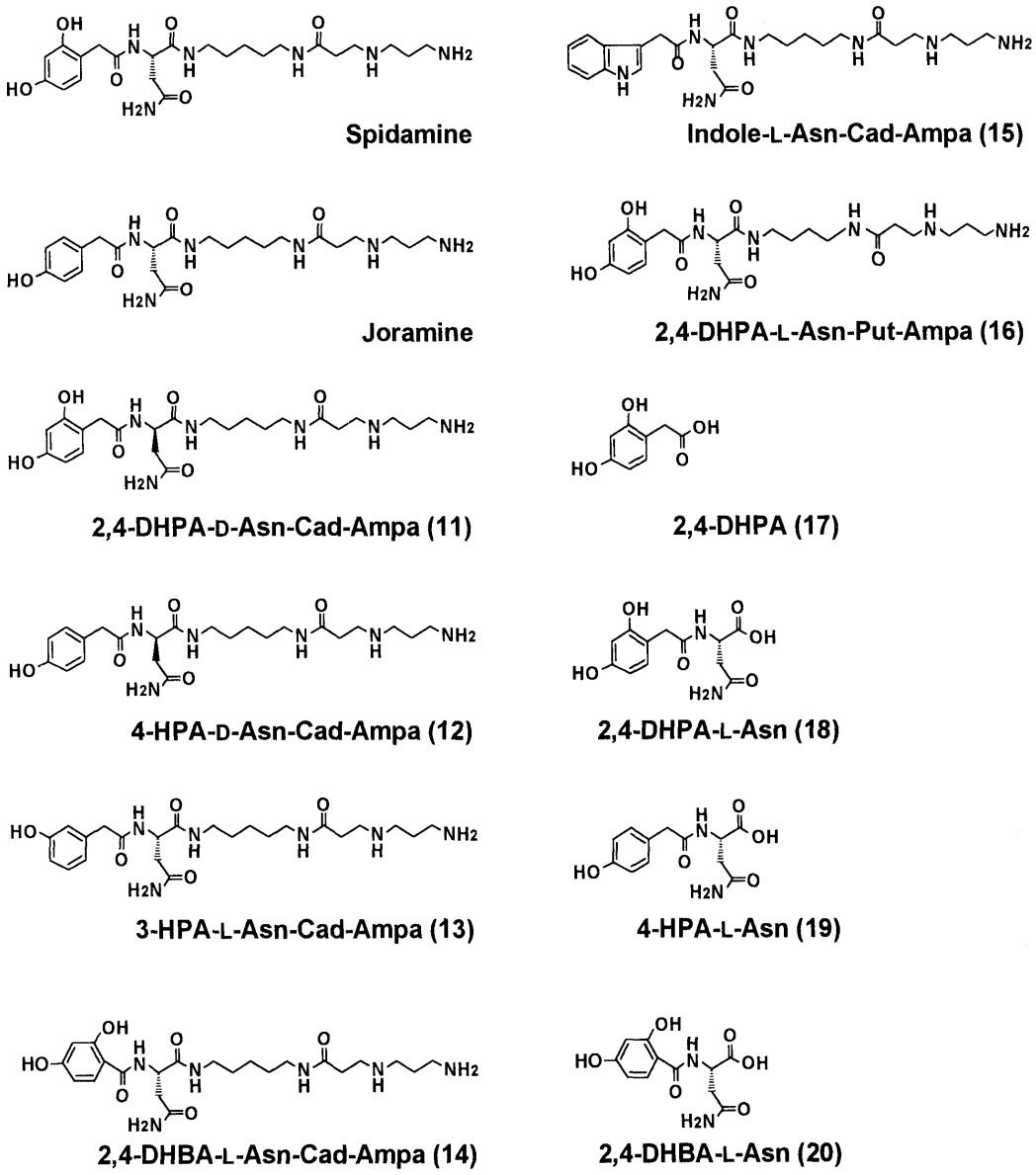

Fig. 1. Structures of Analogs of Spidamine and Joramine (**11**—**16**) and Four Intermediates (**17**—**20**)

either **8b**, **8c**, or **8d** was coupled with L-Asn. In order to confirm the structures of the synthons, the corresponding amides were reduced with nascent hydrogen to give the intermediates 4-hydroxyphenylacetyl-L-asparagine (4-HPA–L-Asn) (**19**), 2,4-DHPA–L-Asn (**18**) and 2,4-dihydroxybenzoyl-L-asparagine (2,4-DHBA–L-Asn) (**20**), respectively. 2,4-Dibenzyloxyphenylacetic acid (2,4-DBPA) was reduced with nascent hydrogen to give 2,4-DHPA (**17**). Each product was purified by HPLC as described in the previous paper.[1] Detailed structural analysis of the intermediates was considered unnecessary because the structures of the whole analogs were subsequently established.

Coupling of Synthon I with II and Synthons I-II with III The active ester **4** was coupled with **6a**—**c**. Removal of the Boc group from each product with trifluoroacetic acid (TFA) afforded the corresponding amines **9a**—**c**, as shown in Chart 2. The hydroxyphenylacetic acid *N*-succinimidyl esters **8a**—**e** were coupled with **9a**—**c**. The protective groups and azido group of **10a**—**f** were reduced with nascent hydrogen to give the targeted analogs **11** through **16**.

The crude products, obtained as TFA salts, were purified by reversed-phase HPLC to give the six purified analogs. The structures of the synthetic analogs were confirmed by analyses of their ^{1}H- and ^{13}C-NMR spectra.

Bioassays of Polyamine Toxin Analogs

Inhibition of EPSP The blocking activity of the synthetic polyamine toxin analogs on the glutamate receptor was tested by using the lobster neuromuscular synapse, as described in the previous paper.[1] Percent inhibition of the EPSP was measured at 5 or 10 min after applying the analogs to the neuromuscular synapse.

As a control experiment, Fig. 2 shows the effects of the synthesized spidamine and joramine on EPSP. Following application of joramine, EPSP was gradually inhibited, while the resting potential and the conductance of the postsynaptic membrane remained unaffected. The amplitude of EPSP was reduced to approximately 40% of the control at 5 min. When the synapse preparation was washed with normal saline, EPSP recovered slowly.

Complete recovery was obtained after washing for 25 min. Spidamine caused a more potent inhibition of EPSP than joramine. After the application of spidamine, EPSP was rapidly inhibited, and inhibition was complete within 5 min. The recovery of EPSP from the effects of spidamine was very slow. At 25 min after spidamine application, the amplitude of the EPSP was only a few percent of the control. By contrast, JSTX-3 inhibited EPSP irreversibly, no recovery being found even after washing as described in the previous paper.[1] Spidamine was one order of magnitude less potent than JSTX-3. The analogs **11**, **12**, **13**, **14** and **15** inhibited EPSP strongly, as shown in Table 1. Intermediates **17** through **20** were inactive. It was unexpected that compounds containing the D-form derivative of Asn, 2,4-DHPA–D-Asn–Cad–Ampa (**11**) and 4-HPA–D-Asn–Cad–Ampa (**12**), and *N*-(3-aminopropyl-*β*-alanyl-*N'*-(3-indoleacetyl-L-asparaginyl)-1,5-pentanediamine (Indole–L-Asn–Cad–Ampa) (**15**) were active as inhibitors.

Effect of the Synthetic Analogs on Mosquito Larvae[13,24] In Culex mosquito, movement of larvae was depressed soon after the release into the aqueous solution of a synthetic analog. Spidamine, joramine, **12**, **18** and **20** at 0.1 mM caused 10, 33, 17, 17 and 14% mortality. Other synthetic analogs, however, were ineffective, as shown in Table 2.

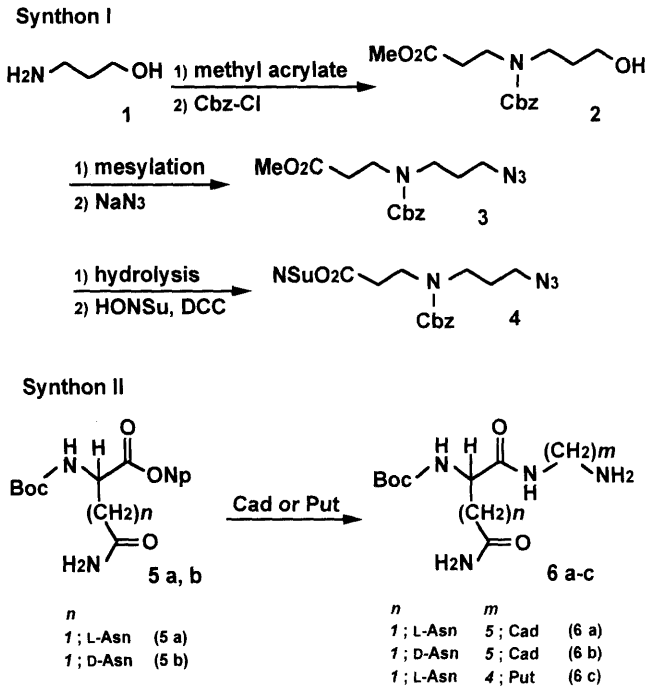

Chart 1. Preparation of Synthons I and II

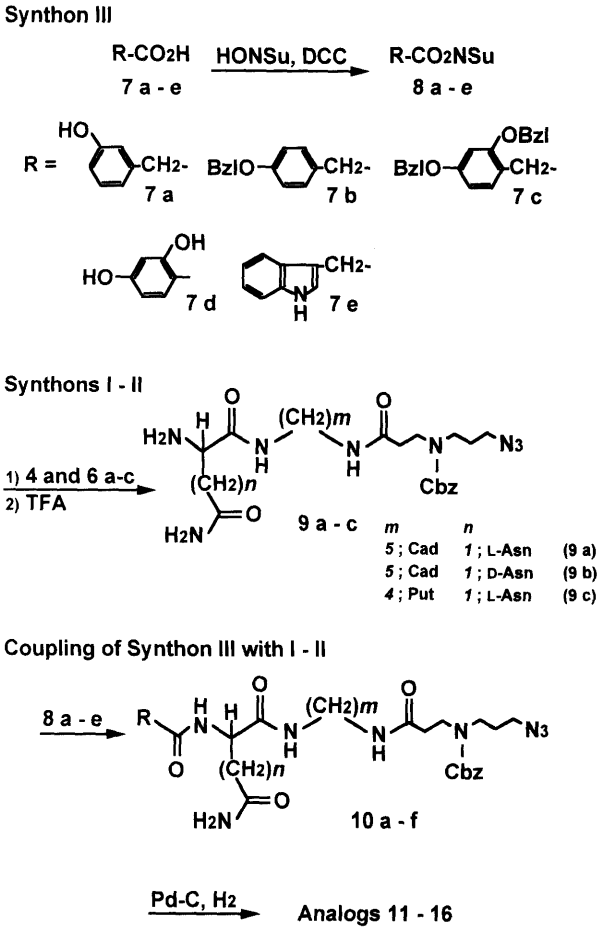

Chart 2. Coupling of Synthons

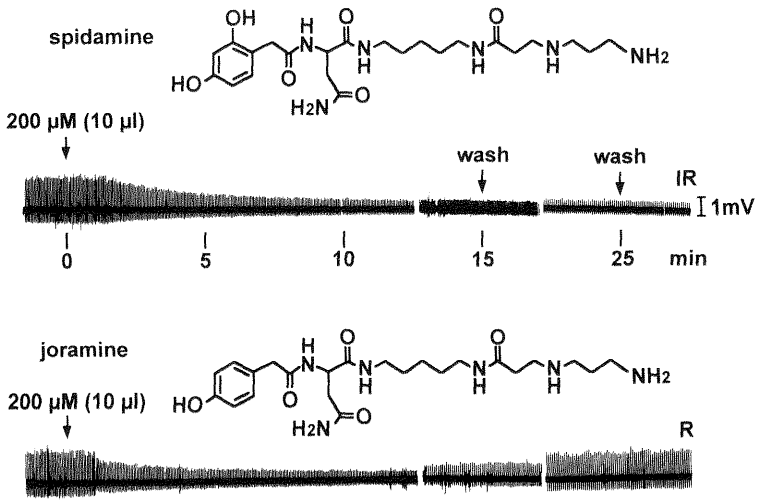

Fig. 2. Comparison of Inhibition by Spidamine and Joramine of EPSP in the Lobster Neuromuscular Synapse
IR, irreversible; R, reversible.

Table 1. Inhibition by Synthetic Analogs of EPSP in the Lobster Neuromuscular Synapse

Compound	Inhibition (%)		
	500 μM	200 μM	50 μM
Spidamine		65 (IR)[a]	41 (R)
Joramine		60 (R)[a]	25 (R)
11			64 (R)
12			43 (R)
13			68 (R)
14			18 (R)
15	100 (IR)		35 (R)
17	No effect		
18	No effect		
19	No effect		
20	No effect		

IR, irreversible; R, reversible. Percent inhibition of the EPSP was measured at 5 min (*a*) 10 min) after applying the compounds to the lobster neuromuscular synapse.

Table 2. Effect of Synthetic Compounds on Mosquito Larvae

Compound	Mortality (%)		
	10^{-3} M	10^{-4} M	10^{-5} M
Spidamine		10	0
Joramine		33	0
11		0	0
12		17	0
14	0	0	
15	0	0	
17	0	0	
18		17	0
19	0	0	
20		14	0
Control	0	0	

Table 3. Effects of Synthetic Compounds on German Cockroaches

Compound	Knock-down rate (%)				
	15 min	30 min	60 min	120 min	1 d
Spidamine	100	100	100	20	0
Joramine	80	20	0	0	0
11	0	0	0	0	0
12	0	0	0	0	0
14	0	0	0	0	0
15	100	100	100	0	0
17	0	0	0	0	0
18	0	0	0	0	0
19	0	0	0	0	0
20	0	0	0	0	0
Control	0	0	0	0	0

Effect of Synthetic Analogs on German Cockroach[13] When 1 mM synthetic analog was injected into adult German cockroaches under CO_2 anesthesia, apparent symptoms of intoxication appeared in all the individuals (five adults in every dosage) as follows. As soon as the cockroaches recovered from CO_2 anesthesia, paralysis appeared in all the hind legs and the animals remained motionless with the legs relaxed for 30—60 min. This quiescence was characteristic. They resumed a normal posture 60 min after the injection and their normal movements by 120 min. On injection of joramine the same symptom appeared, but completely disappeared within 60 min, in contrast to the injection of spidamine and **15**, as shown in Table 3.

Discussion

Six analogs and four intermediates were obtained as TFA salts by the convergent synthesis as described in the previous paper.[1] However, hydroxyphenylacetic acid *N*-succinimidyl esters **8a, d** were used without benzylation of phenolic groups. This direct reaction of **8a, d** reduced the number of steps, but the amounts of by-products were increased and the yields were low. The by-products had to be extracted and removed by HPLC. The products **13** and **20** were not completely analyzed, although their structures could be reasonably ascribed by analogy with those of the other products.

In the SAR of the synthetic analogs concerning EPSP, as shown in Table 1, **11**, **12**, **13**, **14** and **15** were all active, but the intermediates **17** through **20** were inactive as expected. In the previous paper,[1] the 2,4-DHPA head was shown to be essential to the irreversible inhibition. The polyamine frame was involved in the binding with the receptor. It was difficult to decide the mode of inhibition for 50 μM solution of analogs **11**, **12**, **13**, **14** and **15**. We did not have a sufficient amount of analog **13** for the assays using the cockroach and mosquito larvae. Nevertheless, analogs **11** and **12** substituted with D-Asn were active. This phenomenon has been noted in PhTX analogs.[11,12]

To examine insecticidal activity, we used mosquito larvae. Spidamine, joramine and intermediates **17** and **20** were as active as JSTX-3. Clavamine was inactive for EPSP, but was active in this assay.[22] Spidamine and joramine were active in both assays, though both are among the lowest-molecular-weight toxins containing 2,4-DHPA. Their insecticidal activity was low, but they were active against EPSP and the larvae. Thus, the physiological activity of the primitive toxins might have diversified in the higher polyamine toxins.[3] Analogs **13** and **14** were active, which means that the complete 2,4-DHPA residue is not essential for the inhibition. Analog **15** containing an indoleacetyl residue showed irreversible inhibition, like the indoleacetyl analogs of NPTX.[14-16,18,19] These phenomena may help us to elucidate the mechanism of the insecticidal action.

As shown in Table 3, motor neurons innervated with qGlu-R were inhibited by spidamine, joramine and analog **15**, but not by the other analogs. These motor neurons are different from those of larvae. Thus, individual polyamine toxins in the venom may target specific receptors of prey. It is also interesting that the knock-down character of all three compounds was different from that in the case of kinate and JSTX-3. The legs paralyzed by spidamine, joramine and analog **15** were atonic, as with clavamine, while those in the case of kinate and JSTX-3 were stiff.

The synthetic analogs not only represent potential insecticides, but also may be useful as tools for various studies in the fields of biochemistry, chelate chemistry and pharmacology.

Experimental

Bioassay. Mortality of Synthetic Analogs on Mosquito Larvae[13] Five to ten first-instar larvae of the mosquito, *Culex pipiens molestus*, were released into a well of a 96-wellmicrotiter plate which contained 300 μl of an aqueous solution of a synthetic analog. Dead larvae were counted 3 h after the release. Low concentrations were tested 4 times, but high concentrations were tested only once.

Effects of Synthetic Analogs on German Cockroach[13] German cockroaches, *Blattella germanica*, were used for assaying the biological activity of analogs. Two microliters of a 1 mM solution of a synthetic analog was injected into the thoracic body cavity of the cockroach under CO_2 anesthesia using a microsyringe. After the injection, the cockroaches were confined individually in polyethylene cups, so that poisoning symptoms could be observed. Five independent determinations were carried out in each assay. All experiments were performed at room temperature.

Purification by HPLC Purification was carried out on a Capcell Pak C18 (10 × 250 mm, Shiseido Corporation, Ltd., Tokyo) column at 40 °C with a flow rate of 3.0 ml/min. The eluent composition was as described in each case. Final fractions were lyophilized.

Measurements of Chemical Characteristics Melting points were measured with a Yanagimoto MP-S3 apparatus. Optical rotation was measured with a JASCO DIP-140 digital polarimeter. FAB-MS (glycerin matrix) and EI-MS (high-resolution; HR) were measured with a JEOL JMX-DX300 mass spectrometer. ^{1}H- and ^{13}C-NMR (100 MHz for carbon NMR resonance) were measured with a JEOL JNM-GSX 400 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 ppm downfield from TMS or 3-(trimethylsilyl)propionic acid sodium salt-d_4 as appropriate for each synthetic compound. Coupling constants (J values) are given in Hz.

Synthesis. Methyl 7-Hydroxy-*N*-Cbz-4-azaheptanoate (2) Methyl acrylate (19.4 ml, 0.2 mol) was added to *n*-propanolamine (**1**, 15.0 ml, 0.2 mol). The mixture was placed in an ice bath at 0 °C for 1 h and then was allowed to warm to room temperature. Methyl 7-hydroxy-4-azaheptanoate was obtained as a clear oil in 97.9% yield (31.8 g, 1.96 mol). The product was used without further purification. The crude product (0.1 mol, 16.1 g) was dissolved in a solution of benzyloxycarbonyl chloride (0.1 mol, 14.2 ml), and $NaHCO_3$ (0.5 mol, 41.5 g) in a mixed solvent of DMSO (100 ml) and ethyl acetate (EtOAc) (150 ml). The mixture was stirred at room temperature overnight, then concentrated *in vacuo* to give an oily residue. The residue was dissolved in EtOAc (500 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 **2** as a yellow oily residue. The residue was dissolved in MeCN (20 ml) and purified by HPLC with a linear gradient from water containing 0.1% TFA to 70% MeCN containing 0.1% TFA. The product **2** was eluted at 23.9 min and was obtained as a clear oil in 85% yield (25.0 g, 85.0 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) δ: 1.66 (qu, 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) δ: 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-Azido-*N*-Cbz-4-azaheptanoate (3) Methyl 7-hydroxy-*N*-Cbz-4-azaheptanoate (**2**, 23.6 g, 80 mmol) was dissolved in a solution of methanesulfonyl chloride (12.8 ml, 160 mmol) and pyridine (12.8 ml, 160 mmol) in 100 ml of CH_2Cl_2. The mixture was stirred in an ice bath at 0 °C for 4 h to give a white precipitate. This was removed by filtration, and the filtrate was concentrated *in vacuo* to give a yellow oily residue. The residue was dissolved in EtOAc (800 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 as a yellow oily residue. The product was used without further purification. The crude product (18.5 g, approximately 50 mmol) was dissolved in a solution of sodium azide (6.5 g, 100 mmol) in 100 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 (1000 ml), and the organic solution was washed with 500 ml of water three times. The washed solution was dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to give crude **3** as a yellow 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 **3** was eluted at 31.1 min and was obtained as a clear oil in 55% yield (8.8 g, 27.5 mmol). FAB-MS m/z: 321 $(M+1)^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.74 (qu, 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) δ: 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 *N*-Hydroxysuccinimidyl Ester (4) Compound **3** (8.0 g, 25 mmol) was dissolved in 50 ml of a solution of 1.0 N NaOH in MeOH. The mixture was allowed to stand at 60 °C for 1.5 h, then concentrated *in vacuo* to give crude 7-azido-*N*-Cbz-4-azaheptanoic acid as an oily residue, which was adjusted to pH 7.0 by the addition of 25 ml of 1.0 N HCl. The residue was dissolved in MeCN (20 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 was eluted at 16.4 min and was obtained as a clear oil in 99% yield (7.9 g, 24.8 mmol). The product (6.0 g, 20 mmol) was dissolved in a slution of DCC (4.6 g, 22 mmol) and HONSu (2.6 g, 22 mmol) in a mixed solvent of acetone (15 ml) and EtOAc (25 ml). The mixture was stirred in an ice bath at 0 °C for 12 h to give a white precipitate, and then allowed to warm to room temperature. The precipitate was removed by filtration, and the filtrate was concentrated *in vacuo* to give crude **4** as a yellow oily residue. The residue was dissolved in MeCN (20 ml) and purified by HPLC with a linear gradient from 30% MeCN to 95% MeCN. The product **4** was eluted at 17.9 min and was obtained as a clear oil in 80% yield (6.4 g, 16 mmol). FAB-MS m/z: 404 $(M+1)^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.74 (qu, 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) δ: 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 (6a) Boc-L-Asn-ONp (**5a**, 7.0 g, 20 mmol) was dissolved in a solution of Cad-2HCl (10.6 g, 60 mmol) and TEA (8.4 ml, 60 mmol) in 200 ml of DMSO. The mixture was stirred at room temperature overnight, then concentrated *in vacuo* to give crude **6a** as a yellow powder. 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 **6a** was eluted at 10.5 min and was obtained as a white solid in 35% yield (2.2 g, 7.0 mmol). mp: 141—143 °C. FAB-MS m/z: 317 $(M+1)^+$. 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) δ: 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) δ: 22.9, 26.5, 28.1, 28.3, 37.5, 38.2, 38.6, 51.5, 78.1, 155.0, 171.2, 171.6.

Boc-D-asparaginyl-1,5-pentanediamine (6b) Boc-D-Asn-ONp (**5b**, 7.0 g, 20 mmol) was treated in the same manner as described for the synthesis of **6a**. The product **6b** was eluted at 10.5 min and was obtained as a white solid in 35% yield (2.2 g, 7.0 mmol). mp: 141—143 °C. FAB-MS m/z: 317 $(M+1)^+$. 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) δ: 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) δ: 22.9, 26.5, 28.1, 28.3, 37.5, 38.2, 38.6, 51.5, 78.1, 155.0, 171.2, 171.6.

Boc-L-asparaginyl-1,4-butanediamine (6c) Boc-L-Asn-ONp (**5d**, 7.0 g, 20 mmol) was dissolved in a solution of 1,4-butanediamine dihydrochloride (Put-2HCl) (9.7 g, 60 mmol) and TEA (8.4 ml, 60 mmol) in 200 ml of DMSO. The mixture was treated in the same manner as described for the synthesis of **6a**. The product **6c** was eluted at 10.1 min and was obtained as a clear oil in 40% yield (2.4 g, 8.0 mmol). FAB-MS m/z: 303 $(M+1)^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.38 (m, 9H), 1.44 (s, 2H), 1.51 (m, 2H), 2.39 (m, 2H), 2.85 (m, 2H), 3.05 (m, 2H), 4.18 (br, 1H), 6.74 (br, 1H), 6.81 (s, 1H), 7.23 (s, 1H), 7.72 (br, 2H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 26.7, 28.0, 28.1, 37.7, 38.5, 38.7, 51.4, 78.0, 154.8, 170.9, 172.0.

3-Hydroxyphenylacetic Acid *N*-Hydroxysuccinimidyl Ester (8a) 3-Hydroxyphenylacetic acid (**7a**, 152 mg, 1 mmol) was dissolved in a solution of 40 ml of DCC (226 mg, 1.1 mmol) and HONSu (127 mg, 1.1 mmol) in acetone (10 ml) and EtOAc (30 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 **8a** as a white residue. The residue was dissolved in MeCN (1 ml) and purified by HPLC with a linear gradient from water to 70% MeCN. The product **8a** was eluted at 13.7 min and was obtained as a clear oil in 50% yield (118 mg, 0.50 mmol). FAB-MS *m/z*: 249 $(M+1)^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.80 (s, 2H), 3.98 (s, 2H), 6.70 (dd, $J=2.1$, 8.1 Hz, 1H), 6.75 (s, 1H), 6.76 (d, $J=6.7$ Hz), 7.14 (t, $J=8.1$ Hz, 1H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 25.4, 36.6, 114.4, 116.2, 119.8, 129.4, 133.3, 157.4, 167.2, 170.0.

2,4-Dihydroxybenzoic Acid *N*-Hydroxysuccinimidyl Ester (8d) 2,4-Dihydroxybenzoic acid (**7d**, 154 mg, 1 mmol) was treated in the same manner as described for the synthesis of **8a**. The product **8d** was eluted at 20.5 min and was obtained as a white solid in 55% yield (13.0 mg, 0.055 mmol). FAB-MS *m/z*: 251 $(M+1)^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.86 (s, 4H), 6.45 (d, $J=8.6$ Hz, 1H), 6.46 (s, 1H), 7.77 (d, $J=8.6$ Hz, 1H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 25.5, 101.3, 103.2, 108.6, 133.0, 161.2, 162.5, 165.1, 170.5.

3-Indoleacetic Acid *N*-Hydroxysuccinimidyl Ester (8e) 3-Indoleacetic acid (**7e**, 175 mg, 1 mmol) was treated in the same manner as described for the synthesis of **8a**. The product **8e** was eluted at 19.2 min and was obtained as a white solid in 85% yield (231 mg, 0.85 mmol). FAB-MS *m/z*: 273 $(M+1)^+$. High-resolution EI-MS *m/z*: Calcd for $C_{14}H_{12}N_2O_4$: 272.0798. Found: 272.0789. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.79 (s, 4H), 4.14 (s, 2H), 7.02 (d, $J=7.5$ Hz, 1H), 7.11 (t, $J=7.3$ Hz, 1H), 7.35 (d, $J=1.8$ Hz, 1H), 7.38 (d, $J=8.4$ Hz, 1H), 7.54 (d, $J=7.7$ Hz, 1H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 25.4, 27.4, 104.8, 111.5, 118.3, 118.7, 121.3, 124.5, 126.8, 136.1, 167.5, 170.

***N*-(7-Azido-*N*-Cbz-4-azaheptanoyl)-*N'*-(L-asparaginyl)-1,5-pentanediamine (9a)** Boc-L-Asn-Cad (**6a**, 316 mg, 1 mmol) was dissolved in a solution of **4** (403 mg, 1 mmol) 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*-(7-azido-*N*-Cbz-4-azaheptanoyl)-*N'*-(Boc-L-Asn)-Cad 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 was eluted at 14.0 min and was obtained as a white solid in 80% yield (483 mg, 0.80 mmol). The product (60.4 mg, 0.1 mmol) 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 **9a** as a clear oily residue. The product **9a** was obtained as a clear oil in 96% yield (48.4 mg, 0.096 mmol). FAB-MS *m/z*: 505 $(M+1)^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.25 (m, 2H), 1.35—1.45 (m, 4H), 1.73 (qu, $J=7.0$ 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, 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) δ: 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.

***N*-(7-Azido-*N*-Cbz-4-azaheptanoyl)-*N'*-(D-asparaginyl)-1,5-pentanediamine (9b)** Boc-D-Asn-Cad (**6b**, 316 mg, 1 mmol) was treated in the same manner as described for the synthesis of **9a**. The product **9b** was obtained as a clear oil in 96% yield (48.4 mg, 0.096 mmol). FAB-MS *m/z*: 505 $(M+1)^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.25 (m, 2H), 1.35—1.45 (m, 4H), 1.73 (qu, $J=7.0$ 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, 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) δ: 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.

***N*-(7-Azido-*N*-Cbz-4-azaheptanoyl)-*N'*-(L-asparaginyl)-1,4-butanediamine (9c)** Boc-L-Asn-Put (**6c**, 302 mg, 1 mmol) was treated in the same manner as described for the synthesis of **9a**. The product **9c** was obtained as a clear oil in 96% yield (48.4 mg, 0.096 mmol). FAB-MS *m/z*: 505 $(M+1)^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.33—1.43 (m, 4H), 1.75 (qu, $J=6.8$ Hz, 2H), 2.36 (t, $J=6.8$ Hz, 2H), 2.60 (dd, $J=7.8$, 16.5 Hz, 1H), 2.61 (dd, $J=5.4$, 16.5, 1H), 3.02—3.17 (m, 4H), 3.21—3.31 (m, 4H), 3.60 (m, 2H), 4.03 (br, 1H), 5.03 (s, 2H), 7.17 (s, 1H), 7.32—7.42 (m, 5H), 7.61 (s, 1H), 7.85 (t, $J=5.3$ Hz, 1H), 8.00 (br, 2H), 8.22 (t, $J=5.4$ Hz, 1H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 24.0, 27.1, 28.5, 28.2, 34.6, 35.5, 38.1, 38.9, 43.5, 44.6, 48.1, 49.0, 65.8, 127.8, 127.9, 128.3, 137.2, 155.7, 167.1, 169.3, 169.9.

***N*-(7-Azido-*N*-Cbz-4-azaheptanoyl)-*N'*-(2,4-dibenzyloxyphenylacetyl-D-asparaginyl)-1,5-pentanediamine (10a)** 2,4-Dibenzyloxyphenylacetic acid *N*-hydroxysuccinimidyl ester (**8c**, 44.5 mg, 0.1 mmol)[1] was dissolved in a solution of **9b** (50.4 mg, 0.1 mmol) in 5 ml of DMF containing TEA (14 μl, 0.1 mmol). 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 **10a** 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 **10a** 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) δ: 1.18 (m, 2H), 1.29—1.36 (m, 4H), 1.92 (qu, $J=6.7$ 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) δ: 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.

***N*-(3-Aminopropyl-β-alanyl)-*N'*-(2,4-dihydroxyphenylacetyl-D-asparaginyl)-1,5-pentanediamine (11)** *N*-(7-Azido-*N*-Cbz-4-azaheptanoyl)-*N'*-(2,4-dibenzyloxyphenylacetyl-D-asparaginyl)-1,5-pentanediamine (**10a**, 83.4 mg, 0.1 mmol) was dissolved in a suspension of 10% Pd–C (50 mg) and ammonium formate (32 mg, 0.5 mmol) 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 **11** 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 **11** was eluted at 13.4 min and was obtained as a clear oil in 98% yield (48.4 mg, 0.98 mmol). UV spectrum; λ_{max} 279.2 nm, $\varepsilon=1690$. $[\alpha]_D=+3.00°$ ($c=0.21$ 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: 495.2908. IR (diamond): 3280, 2940, 1670, 1540 cm^{-1}. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 1.21 (qu, $J=7.5$ Hz, 2H), 1.37 (m, 4H), 1.91 (qu, $J=7.6$ 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) δ: 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.

***N*-(7-Azido-*N*-Cbz-4-azaheptanoyl)-*N'*-(4-benzyloxyphenylacetyl-D-asparaginyl)-1,5-pentanediamine (10b)** 4-Benzyloxyphenylacetic acid *N*-hydroxysuccinimidyl ester (**8b**, 33.9 mg, 0.1 mmol)[1] was treated in the same manner as described for the synthesis of **10a** except that 70% MeCN was used in place of 50% in HPLC. The product **10b** was eluted at 27.4 min and was obtained as a white solid in 95% yield (69.2 mg, 0.095 mmol). mp 145—146 °C. FAB-MS *m/z*: 729 $(M+1)^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.20 (m, 2H), 1.34 (m, 4H), 1.93 (qu, $J=6.7$ 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) δ: 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.

***N*-(3-Aminopropyl-β-alanyl)-*N'*-(4-hydroxyphenylacetyl-D-asparaginyl)-1,5-pentanediamine (12)** Compound **10b**, (72.8 mg, 0.1 mmol) was treated in the same manner as described for the synthesis of **11**. The product **12** was eluted at 13.6 min and was obtained as a clear oil in 98% yield (46.9 mg, 0.98 mmol). UV spectrum λ_{max} 276.4 nm, $\varepsilon=854$. $[\alpha]_D$ +3.64° ($c=0.57$ 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:

479.2956. IR (diamond): 3280, 2940, 1670, 1520 cm^{-1}. ^{1}H-NMR (600 MHz, DMSO-d_6) δ: 1.91 (qu, J=7.6 Hz, 2H), 1.20 (qu, 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) δ: 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*-(3-Aminopropyl-β-alanyl)-*N'*-(3-hydroxyphenylacetyl-L-asparaginyl)-1,5-pentanediamine (13)** Compound **8a** (23.5 mg, 0.1 mmol) was dissolved in a solution of **9a** (50.4 mg, 0.1 mmol) in 5 ml of DMF containing TEA (14 μl, 0.1 mmol). 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*-(7-azido-*N*-Cbz-4-azaheptanoyl)-*N'*-(3-hydroxyphenylacetyl-L-Asn)-Cad as a white residue. The residue was dissolved in a suspension of 10% Pd–C (50 mg) and ammonium formate (32 mg, 0.5 mmol) 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 **13** 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 70% MeCN containing 0.1% TFA. The product 13 was eluted at 10.1 min and was obtained as a clear oil in 25% yield (12.0 mg, 0.025 mmol). FAB-MS m/z: 479 $(M+1)^+$. ^{1}H-NMR (400 MHz, D_2O) δ: 1.22 (m, 2H), 1.43 (m, 4H), 2.10 (m, 2H), 2.67 (t, J=6.7 Hz, 2H), 2.66—2.81 (m, 2H), 3.09—3.19 (m, 6H), 3.32 (t, J=6.7 Hz, 2H), 3.59 (s, 2H), 4.62 (br, 1H), 6.82 (d, J=1.8 Hz, 1H), 6.84 (dd, J=1.8, 7.8 Hz, 1H), 6.87 (d, J=7.8 Hz, 1H), 7.29 (t, J=7.8 Hz, 1H). ^{13}C-NMR (100 MHz, D_2O) δ: 23.4, 24.0, 28.1, 28.2, 31.3, 36.7, 37.0, 39.6, 39.7, 42.5, 44.1, 44.9, 51.5, 114.7, 116.4, 121.6, 122.7, 130.7, 137.1, 138.5, 171.9, 172.6, 174.8, 175.0.

***N*-(3-Aminopropyl-β-alanyl)-*N'*-(2,4-dihydroxybenzoyl-L-asparaginyl)-1,5-pentanediamine (14)** Compound **8d** (23.7 mg, 0.1 mmol) was treated in the same manner as described for the synthesis of **13**. The product **14** was eluted at 10.7 min and was obtained as a clear oil in 30% yield (14.4 mg, 0.030 mmol). FAB-MS m/z: 481 $(M+1)^+$. ^{1}H-NMR (400 MHz, D_2O) δ: 1.30 (m, 2H), 1.51 (m, 4H), 2.14 (m, 2H), 2.69 (t, J=6.7 Hz, 2H), 2.87 (d, J=6.7 Hz, 2H), 3.12—3.25 (m, 6H), 3.34 (t, J=6.7 Hz, 2H), 4.85 (t, J=6.7 Hz, 1H), 6.45 (d, J=1.8 Hz, 1H), 6.53 (dd, J=1.8, 8.7 Hz, 1H), 7.69 (d, J=8.7 Hz, 1H). ^{13}C-NMR (150 MHz, D_2O) δ: 26.1, 26.4, 30.6, 30.7, 33.7, 39.4, 39.5, 42.1, 42.2, 46.6, 47.4, 53.9, 106.0, 111.1, 111.8, 133.7, 162.5, 164.1, 172.0, 174.3, 175.3, 177.7.

***N*-(3-Aminopropyl-β-alanyl)-*N'*-(3-indoleacetyl-L-asparaginyl)-1,5-pentanediamine (15)** Compound **8e** (23.8 mg, 0.1 mmol) was treated in the same manner as described for the synthesis of **13**. The product **15** was eluted at 12.3 min and was obtained as a clear oil in 65% yield (32.6 mg, 0.065 mmol). FAB-MS m/z: 502 $(M+1)^+$. ^{1}H-NMR (400 MHz, D_2O) δ: 1.14 (m, 2H), 1.32 (m, 2H), 1.39 (m, 2H), 2.09 (m, 2H), 2.63 (t, J=6.7 Hz, 2H), 2.69 (m, 2H), 2.85—3.15 (m, 8H), 3.28 (t, J=6.7 Hz, 2H), 3.80 (s, 2H), 4.64 (br, 1H), 7.18 (t, J=7.3 Hz, 1H), 7.28 (t, J=7.6 Hz, 1H), 7.34 (s, 1H), 7.53 (d, J=7.9 Hz, 1H), 7.60 (d, J=7.9 Hz, 1H). ^{13}C-NMR (100 MHz, D_2O) δ: 26.2, 26.7, 30.8, 34.0, 35.5, 39.4, 39.7, 42.2, 42.3, 46.8, 47.6, 53.9, 110.6, 115.1, 121.0, 121.4, 122.7, 125.2, 128.0, 129.7, 139.4, 174.5, 175.2, 177.6, 178.0.

***N*-(3-Aminopropyl-β-alanyl)-*N'*-(2,4-dihydroxyphenylacetyl-L-asparaginyl)-1,4-butanediamine (16)** Compound **8c** (44.5 mg, 0.1 mmol) was dissolved in a solution of **9d** (49.0 mg, 0.1 mmol) in 5 ml of DMF containing TEA (14 μl, 0.1 mmol). 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*-(7-azido-*N*-Cbz-4-azaheptanoyl)-*N'*-(2,4-dibenzyloxyphenyl-acetyl-L-Asn)-Put as a white residue. The residue (82.0 mg, 0.1 mmol) was dissolved in a suspension of 10% Pd–C (50 mg) and ammonium formate (32 mg, 0.5 mmol) 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 **16** 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 **16** was eluted at 13.1 min and was obtained as a clear oil in 98% yield (48.4 mg, 0.98 mmol). FAB-MS m/z: 481 $(M+1)^+$. ^{1}H-NMR (400 MHz, D_2O) δ: 1.54 (m, 4H), 2.20 (m, 2H), 2.76 (t, J=6.8 Hz, 2H), 2.79—2.89 (m, 2H), 3.16—3.33 (m, 8H), 3.41 (t, J=6.8 Hz, 2H), 3.62 (d, J=12.2 Hz, 1H), 3.66 (d, J=13.4 Hz, 1H), 4.72 (br, 1H), 6.56 (m, 2H), 7.18 (m, 1H). ^{13}C-NMR (150 MHz, DMSO-d_6) δ: 23.7, 25.6, 25.8, 31.0, 36.2, 36.7, 37.0, 39.1, 39.2, 43.9, 44.7, 51.0, 103.1, 107.7, 113.9, 132.5, 155.3, 156.3, 171.7, 172.4, 174.8, 175.0.

2,4-Dihydroxyphenylacetic Acid (17) 2,4-Dibenzyloxyphenylacetic acid (34.8 mg, 0.1 mmol)[1] was dissolved in a suspension of 10% Pd–C (50 mg) and ammonium formate (32 mg, 0.5 mmol) 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 the crude **17** 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 30% MeCN containing 0.1% TFA. The product **17** was eluted at 11.5 min and was obtained as a clear oil in 99% yield (15.2 mg, 0.99 mmol). FAB-MS m/z: 155 $(M+1)^+$. ^{1}H-NMR (400 MHz, D_2O) δ: 4.66 (s, 2H), 6.45—6.48 (m, 2H), 7.06 (d-like, J=8.9 Hz, 1H). ^{13}C-NMR (100 MHz, D_2O) δ: 38.0, 105.9, 110.5, 116.6, 135.2, 158.1, 159.0, 180.2.

2,4-Dihydroxyphenylacetyl-L-asparagine (18) Compound **8c** (44.5 mg, 0.1 mmol) was dissolved in a solution of L-Asn (39.6 mg, 0.3 mmol) in 5 ml of DMF containing TEA (14 μl, 0.1 mmol). 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 2,4-dibenzyloxyphenylacetyl-L-asparagine, as a white residue. The residue was dissolved in a suspension of 10% Pd–C (50 mg) and ammonium formate (32 mg, 0.5 mmol) 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 **18** 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 70% MeCN containing 0.1% TFA. The product **18** was eluted at 7.9 min and was obtained as a clear oil in 90% yield (25.5 mg, 0.90 mmol). FAB-MS m/z: 283 $(M+1)^+$. ^{1}H-NMR (400 MHz, D_2O) δ: 2.79 (m, 2H), 3.53 (dd, J=15.8, 19.5 Hz, 2H), 4.71 (t, J=6.1 Hz, 1H), 6.45 (s, 1H), 6.46 (dd, J=2.4, 9.2 Hz, 1H), 7.07 (d, J=9.2 Hz, 1H). ^{13}C-NMR (100 MHz, D_2O) δ: 39.3, 39.4, 52.6, 105.9, 110.5, 116.6, 135.1, 158.1, 159.0, 177.7, 177.7.

4-Hydroxyphenylacetyl-L-asparagine (19) Compound **8b** (33.9 mg, 0.1 mmol) was treated in the same manner as described for the synthesis of **18**. The product **19** was eluted at 8.5 min and was obtained as a clear oil in 95% yield (25.3 mg, 0.95 mmol). FAB-MS m/z: 267 $(M+1)^+$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 2.53 (m, 2H), 3.34 (s, 2H), 4.51 (m, 1H), 6.68 (d, J=8.5 Hz, 2H), 7.04 (d, J=8.5 Hz, 2H), 8.06 (d, J=7.9 Hz, 1H). ^{13}C-NMR (100 MHz, DMSO-d_6) δ: 36.7, 41.1, 48.8, 115.0, 126.2, 129.9, 155.8, 170.4, 171.3, 172.7.

2,4-Dihydroxybenzoyl-L-asparagine (20) Compound **8d** (25.1 mg, 0.1 mmol) was treated in the same manner as described for the synthesis of **18**, except that 70% MeCN was used in place of 30% in HPLC. The product **20** was eluted at 11.7 min and was obtained as a clear oil in 96% yield (24.1 mg, 0.96 mmol). FAB-MS m/z: 269 $(M+1)^+$. ^{1}H-NMR (400 MHz, D_2O) δ: 2.91 (m, 2H), 4.87 (dd, J=5.5, 7.0 Hz, 1H), 6.45 (d, J=2.4 Hz, 1H), 6.52 (dd, J=2.4, 8.9 Hz, 1H), 7.69 (d, J=8.9 Hz, 1H).

Acknowledgments The authors are grateful to Dr. K. Nomoto of Suntory Institute for Bioorganic Research, Osaka for the measurements of high-resolution FAB-MS. We would also like to thank Prof. H. Pan-Hou of this faculty for technical advice, Mr. S. Yamaguchi of this faculty for measurements of MS, and Dr. M. Nishi of this faculty for measurements of NMR.

References

1) Chiba T., Akizawa T., Matsukawa M., Nishi M., Kawai N., Yoshioka M., *Chem. Pharm. Bull.*, **44**, 972—979 (1996).
2) Teshima T., Matsumoto T., Wakamiya T., Shiba T., Nakajima T., Kawai N., *Tetrahedron*, **46**, 3813—3818 (1990).
3) Kawai N., Nakajima T., *J. Toxicol.*, **9**, 203—223 (1990).
4) Chiba T., Akizawa T., Matsukawa M., Pan-Hou H., Yoshioka M., *Chem. Pharm. Bull.*, **42**, 1964—1969 (1994).
5) Chiba T., Akizawa T., Matsukawa M., Pan-Hou H., Kawai N., Yoshioka M., *Chem. Pharm. Bull.*, **43**, 2177—2181 (1995).
6) Nakanishi K., Goodnow R., Konno K., Niwa M., Bukownik R., Kallimopoulos T. A., Usherwood P., Eldefrawi A. T., Eldefrawi M. E., *Pure & Appl. Chem.*, **62**, 1223—1230 (1990).
7) Goodnow R., Konno K., Niwa M., Kallimopoulos T., Bukownik R., Lenares D., Nakanishi K., *Tetrahedron*, **46**, 3267—3286 (1990).

8) Choi S. K., Goodnow R. A., Kalivretenos A., Chiles G. W., Fushiya S., Nakanishi K., *Tetrahedron*, **48**, 4793—4822 (1992).
9) Choi S. K., Nakanishi K., Usherwood P. N. R., *Tetrahedron*, **49**, 5777—5790 (1993).
10) Kalivretenos A. G., Nakanishi K., *J. Org. Chem.*, **58**, 6596—6608 (1993).
11) Nakanishi K., Choi S. K., Hwang D., Lerro K., Orlando M., Kalivretenos A. G., Eldefrawi A., Eldefrawi M., Usherwood P. N. R., *Pure & Appl. Chem.*, **66**, 671—678 (1994).
12) Choi S. K., Kalivretenos A. G., Usherwood P. N. R., Nakanishi K., *Chemistry & Biology*, **2**, 23—27 (1995).
13) Kono Y., Narai N., Numata M., Yoshioka M., *Appl. Ent. Zool.*, **22**, 392—394 (1987).
14) Miyashita M., Matsushita M., Sato H., Toki T., Nakajima T., Irie H., *Chem. Lett.*, 929—932 (1993).
15) Miyashita M., Sato H., Yoshikishi A., Toki T., Matsushita M., Irie H., Yanami T., Kikuchi Y., Takasaki C., Nakajima T., *Tetrahedron Lett.*, **33**, 2833—2836 (1992).
16) Miyashita M., Sato H., Matsushita M., Kusumegi Y., Toki T., Yoshikishi A., Yanami T., Kikuchi Y., Takasaki C., Nakajima T., Irie H., *Tetrahedron Lett.*, **33**, 2837—2840 (1992).
17) Bycroft B. W., Chan W. C., Hone N. D., Millington S., Nash I. A., *J. Am. Chem. Soc.*, **116**, 7415—7416 (1994).
18) Matsushita M., Kanemura T., Hatakeyama S., Irie H., Toki T., Miyashita M., *Tetrahedron*, **51**, 10687—10698 (1995).
19) Matsushita M., Kanemura T., Hatakeyama S., Irie H., Toki T., Miyashita M., *Tetrahedron Lett.*, **36**, 5231—5234 (1995).
20) Houssin R., Bernier J. L., Henichart J. P., *Synthesis*, **1988**, 259—261.
21) Mattingly P. G., *Synthesis*, **1990**, 366—368.
22) Atwell G. J., Denny W. A., *Synthesis*, **1984**, 1032—1033.
23) Teshima T., Wakamiya T., Aramaki Y., Nakajima T., Kawai N., Shiba T., *Tetrahedron Lett.*, **28**, 3509—3510 (1987).
24) Yoshioka M., Narai N., Kawai N., Numata M., Nakajima T., *Biog. Amines*, **7**, 375—384 (1990).