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# An efficient and versatile synthesis of all structural types of acylpolyamine spider toxins

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Dedicated to Professor T. Okuno on the occasion of his 65th birthday

**Abstract**—An efficient and versatile synthesis of acylpolyamine spider toxins of all structural types classified by extensive MS analysis has been achieved. By using 2-nitrobenzenesulfonamide as an effective activating and/or protecting group (the Nosyl strategy), the naturally occurring toxins **1**–**8** corresponding to Types A–F were concisely synthesized in high overall yield.


## 1. Introduction

Acylpolyamine toxins, found in spider and wasp venoms,[1,2] have attracted much interest in the field of neurobiology because of its unique activity as open channel blocker of glutamate receptors.[3,4] In order to investigate biological properties of these toxins in detail, synthesis of a number of congeners and derivatives is necessary because only limited quantity is available from natural sources. Therefore, the acylpolyamine toxins have been an interesting target for organic synthesis.[5–8]

Recent developments of mass spectrometric techniques accelerated and facilitated structural elucidation of acylpolyamine spider toxins even at low picomolar levels.[9,10] Itagaki et al. revealed that, using highly sensitive analytical method with LC-MS and MS/MS, the *Nephila* and *Nephilengys* spider venom glands contain a complex mixture of closely related toxins, a majority of which have not been previously detected by the classical analytical method.[11–13] Furthermore, these new results led to a classification of the toxin structures. These spider toxins consist of three structural elements: a lipophilic head; a polyamine backbone; and a polyamine chain terminal, which are linearly connected in this order. There are a variety of each element and the combination of each element results in a complex mixture of the venom gland constituents as classified into the generalized structures Types A–F based on the distinct polyamine backbone structure (Fig. 1).[11–13] Figure 2 shows the representative toxins for each type. However, the relationships between these structural types and its biological activities are still not documented well,[14] and in the case of novel toxins, the structures should be confirmed by synthesis.[12,15,16] This situation prompted us to establish an efficient and versatile synthetic method for acylpolyamine toxins. We report herein the successful results along this line, which enabled us to synthesize eight naturally occurring acylpolyamine spider toxins, JSTX-3 (**1**), NPTX-8 (**2**), NPTX-1 (**3**), NPTX-473 (**4**), NPTX-501 (**5**), NSTX-3 (**6**), joramine (**7**), and Arg-636 (**8**) (Fig. 2), covering all structural types.[17]

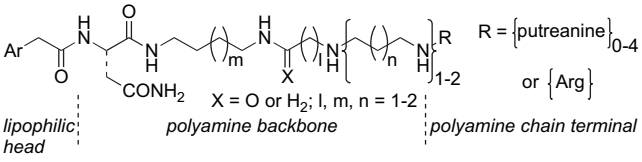


**Figure 1**. Generalized structure of acylpolyamine spider toxins.

## 2. Results and discussion

### 2.1. Basic methodology

Our synthetic strategy is based on the structural classification as mentioned above and the use of 2-nitrobenzenesulfonamide

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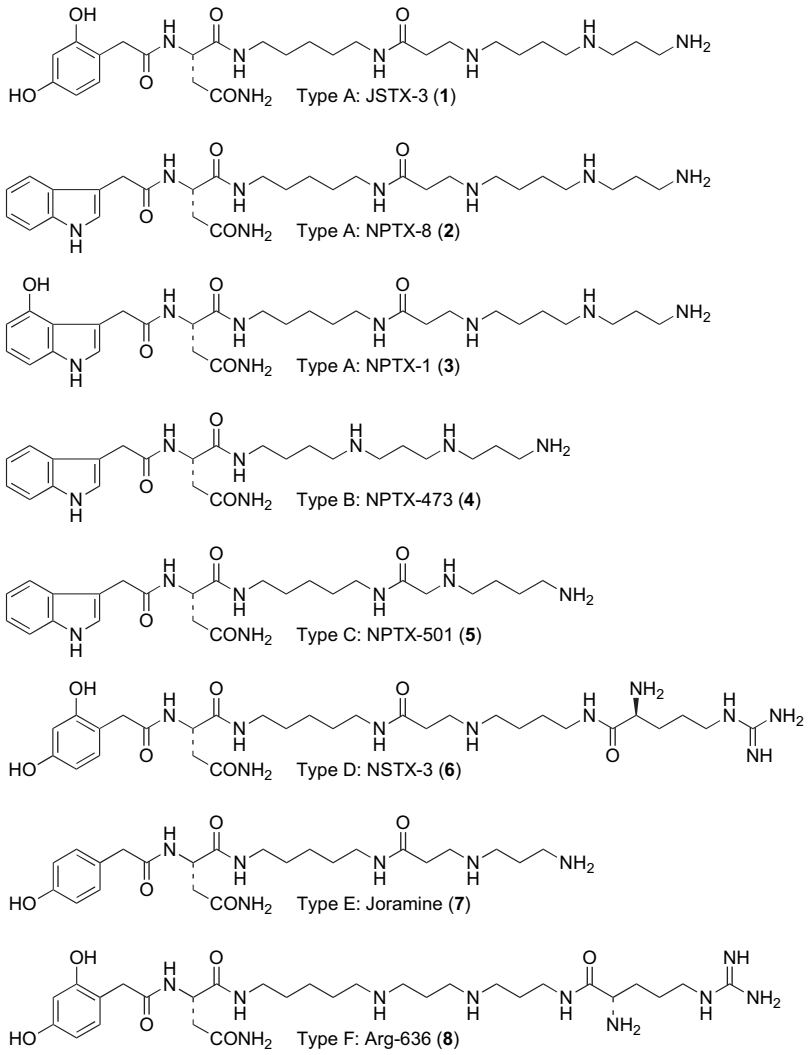
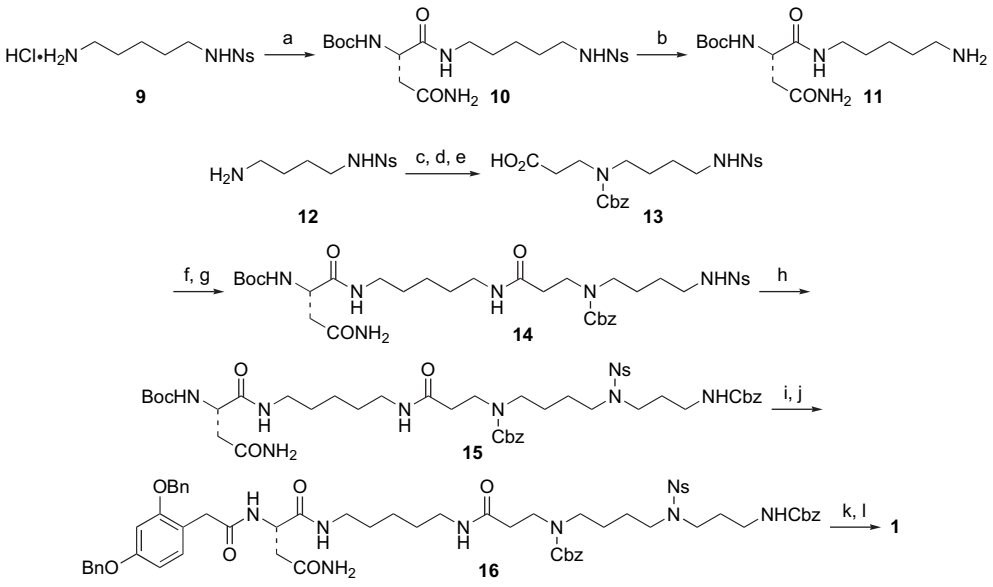



**Figure 2**. Structures of acylpolyamine spider toxins.

(Ns or Nosyl) as an activating and/or protecting group (the Ns strategy).[18–20] The three structural elements can be considered as building blocks, that is, construction of the polyamine backbone, followed by successive connection of the lipophilic head and polyamine chain terminal would afford a variety of natural toxins as well as their analogs. Accordingly, effective preparation of each polyamine backbone should pave the way for convenient and versatile synthesis of these toxins because this element has the richest variation among those three elements (Fig. 2). In order to efficiently construct the polyamine backbones, selective protection and/or activation of amino groups are needed. Fukuyama et al. reported that using the Ns group as both protecting and activating group is exceptionally versatile for the preparation of a variety of secondary amines (Ns strategies),[18–20] and demonstrated its utility for polyamine synthesis.[21–23] Therefore, their protocol is applied to the construction of the polyamine backbones.

## 2.2. Synthesis of Type A toxins

JSTX-3 (**1**), isolated from the venom of *Nephila clavata* in 1986, is one of the first found acylpolyamine spider toxins.[24] This polyamine backbone is classified into Type A, containing cadaverine as a diamine constituent. Thus, the synthesis of **1** started from mono-Ns-cadaverine hydrochloride **9**,[21,22,25] which was readily coupled with commercially available *N*-$^{t}$Boc-L-asparagine-*p*-nitrophenyl ester for 0.5 h to give the asparagyl cadaverine unit **10** (Scheme 1). Initial attempts to remove the Ns group in **10** by the original procedures of the Ns strategy led to poor results; for example, treatment of **10** with thioacetic acid gave the corresponding primary amine **11** only in 20% yield. These results prompted us to improve the procedures for deprotection of 2-nitrobenzenesulfonamide to corresponding primary amine, and as a consequence, we were able to establish the modified procedures using 2-mercaptoethanol/DBU or thiophenol/$Cs_2CO_3$ in DMF.[26] With these modified procedures, the primary amine **11** was obtained in high yield (>85%). On the other hand, the carboxylic acid **13** was prepared from mono-Ns-putrescine **12**[20,21] in excellent yield through sequential reactions by the Michael addition to methyl acrylate,[27] protection by CbzCl, and hydrolysis of the methyl ester. Coupling of the amine **11** with the succinimide ester prepared from **13** by DCC afforded the polyamine backbone precursor **14** in 90% yield. When acid anhydride method with pivaloyl chloride or the coupling agent EDC for this coupling reaction was used, the yields were less than 50%.



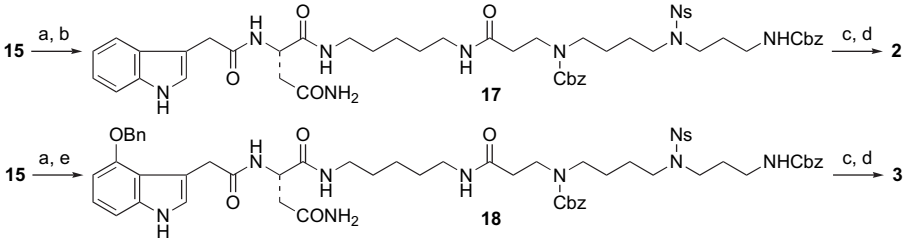

**Scheme 1**. Synthesis of JSTX-3 (**1**). Reagents and conditions: (a) $N^{\alpha}$-Boc-L-Asn-ONp, $Et_3N$/DMF, 0 °C to rt, 0.5 h, 97%; (b) 2-mercaptoethanol, DBU/DMF, rt, 2 h, 85%; (c) methyl acrylate/EtOH, rt, 5 h; (d) CbzCl, $Et_3N/CH_2Cl_2$, 0 °C to rt, 2 h, 86% (two steps); (e) NaOH/$H_2O$–MeOH, 0 °C to rt, 1 h, 96%; (f) HOSu, DCC/$CH_2Cl_2$, 0 °C, 5 h; (g) **11**, DMF, rt, 0.5 h, 90% (two steps); (h) *N*-Cbz-3-bromopropylamine, $Cs_2CO_3$/DMF, 50 °C, 1 h, 92%; (i) TFA/$CHCl_3$, 0 °C to rt, 1 h; (j) 2,4-dibenzyloxyphenylacetic acid-OSu, $Et_3N$/DMF, rt, 2 h, 87% (two steps); (k) 2-mercaptoethanol, DBU/DMF, rt, 0.5 h; (l) $H_2$-$Pd(OH)_2$/AcOH, rt, 3 h, 66% (two steps).

Following the Ns strategy, the polyamine chain was elongated by alkylation of **14** with *N*-Cbz-3-bromopropylamine in the presence of $Cs_2CO_3$ to furnish the fully protected polyamine backbone **15** in 92% yield. After removal of the Boc group in **15** by TFA, the resultant amine was condensed with dibenzyl-2,4-dihydroxyphenylacetic acid *N*-hydroxysuccinimide ester[28] to give the fully protected JSTX-3 **16** in 87% yield. Successive removal of the Ns and Cbz protective groups by 2-mercaptoethanol and hydrogenation, respectively, afforded **1** in high yield. Thus, JSTX-3 (**1**) was synthesized from mono-Ns-cadaverine **9** in a 39% overall yield via nine steps. The synthetic compound was identical with authentic specimen in HPLC co-elution and comparison of the ESI-MS/MS spectra, and other spectroscopic data (IR, $^1H$ and $^{13}C$ NMR, FABHRMS) were consistent with those previously reported.[28]

The synthesis of NPTX-8 (**2**) and -1 (**3**), originally isolated from the Japanese spider *N. clavata*,[29–31] and later found in the Madagascarian spider *Nephilengys borbonica*[11] and Brazilian garden spider *Nephilengys cruentata*[32] as well, demonstrated the convenience of this synthetic method. Both these toxins are also classified into Type A, containing indoleacetate chromophore as the lipophilic head. Therefore, coupling of the polyamine backbone **15** with appropriate indoleacetate moieties instead of 2,4-dihydroxyphenyl moiety would readily lead to these toxins. It was indeed accomplished as shown in Scheme 2. The succinimide ester of indoleacetic acid or 4-benzyloxyindoleacetic acid[33] was coupled with the amine derived from **15** by TFA gave the fully protected toxins **17** or **18**, respectively, from which the natural toxins **2** and **3** were obtained by the same deprotection procedures as above. The synthetic compounds were fully characterized by the spectroscopic data (IR, $^1H$ and $^{13}C$ NMR, FABHRMS, ESI-MS/MS), and identified with the natural toxins.[34,35] Thus, NPTX-8 and -1 were synthesized from mono-Ns-cadaverine **9** in 35 and 36% overall yields, respectively. In this way, a variety of Type A toxins and its analogs can be synthesized.

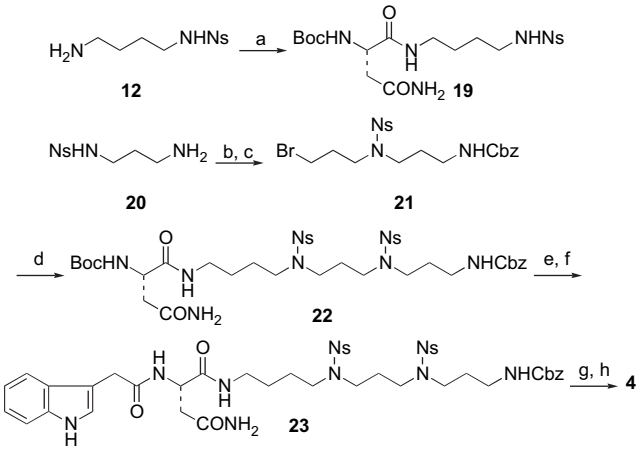
## 2.3. Synthesis of Type B toxin

The polyamine backbone of Type B has no amide bond in its polyamine chain and the putrescine ($C_4$) unit instead of the cadaverine ($C_5$) unit connects to the asparagine moiety. Accordingly, the alkylation procedures of the Ns strategy are




**Scheme 2**. Syntheses of NPTX-8 (**2**) and NPTX-1 (**3**). Reagents and conditions: (a) TFA/$CHCl_3$, 0 °C to rt, 1 h; (b) indoleacetic acid-OSu, $Et_3N$/DMF, rt, 2 h, 84% (two steps); (c) 2-mercaptoethanol, DBU/DMF, rt, 0.5 h; (d) $H_2$-$Pd(OH)_2$/AcOH, rt, 2 h, 61% (two steps); (e) 4-benzyloxyindoleacetic acid-OSu, $Et_3N$/DMF, rt, 2 h, 86% (two steps).

useful for construction of this polyamine chain, and the synthesis should start with mono-Ns-putrescine **12**.[21,22] Scheme 3 shows the synthesis of Type B toxin NSTX-473 (**4**), found in the venom gland of the Madagascarian spider *N. borbonica*.[11] Condensation of the putrescine unit **12** with *N*-$^t$Boc-L-asparagine-*p*-nitrophenyl ester furnished the acylputrescine **19** in 95% yield. On the other hand, mono-Ns-diaminopropane **20**[21,22] was protected by CbzCl, and subsequently alkylated with 1,3-dibromopropane to give the bromide **21** in high yield. Thus, the obtained two segments **19** and **21** were connected by the alkylation procedure of the Ns strategy to afford the Type B polyamine backbone **22** in 94% yield. Removal of the Boc protective group by TFA, followed by coupling with indoleacetic acid succinimide ester furnished the fully protected toxin **23** in 84% yield. Finally, deprotection of the Ns and Cbz groups from **23** in the same manner as for the Type A toxins afforded NPTX-473 (**4**) in a 45% overall yield from **12** via six steps. The synthetic compound was fully characterized by the spectroscopic data (IR, $^1$H and $^{13}$C NMR, FABHRMS, ESI-MS/MS), and consistent with the proposed structure.[11]

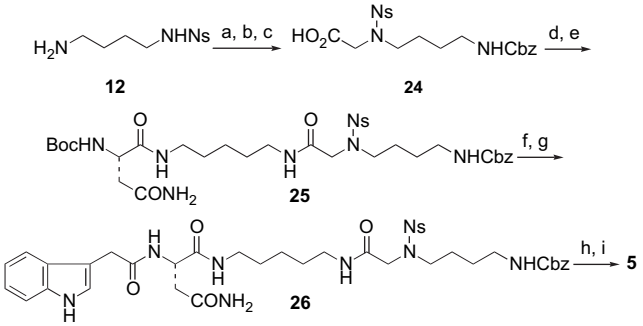



**Scheme 3**. Synthesis of NPTX-473 (**4**). Reagents and conditions: (a) $N^{\alpha}$-Boc-L-Asn-ONp, DMF, 0 °C to rt, 2 h, 95%; (b) CbzCl, $Et_3N/CH_2Cl_2$, 0 °C to rt, 2 h, 88%; (c) 1,3-dibromopropane, $Cs_2CO_3$/DMF, 50 °C, 0.5 h, 91%; (d) **19**, TBAI, $Cs_2CO_3$/DMF, 70 °C, 1 h, 94%; (e) TFA/$CHCl_3$, 0 °C to rt, 1 h; (f) indoleacetic acid-OSu, $Et_3N$/DMF, rt, 2 h, 84% (two steps); (g) 2-mercaptoethanol, DBU/DMF, rt, 0.5 h; (h) $H_2$-Pd(OH)$_2$/AcOH, rt, 2 h, 60% (two steps).

It is noteworthy that the structure of this polyamine chain is the same as that of PhTX-433, a glutamate receptor antagonist from the venom of the solitary wasp *Philanthus triangulum*.[36,37] Therefore, the method established here is applicable to the synthesis of PhTX-433 and its derivatives.[38]

## 2.4. Synthesis of Type C toxin

The Type C structure is close to but simpler than that of Type A; that is, the β-alanine moiety is replaced by the glycine moiety and the $C_3$ unit of the right terminal is missing. Scheme 4 shows the synthesis of NPTX-501 (**5**), a Type C toxin found in Madagascarian spiders *Nephilengys madagascariensis* and *N. borbonica*.[10,11] The primary amine of mono-Ns-putrescine **12** was protected by CbzCl, then alkylated with methyl bromoacetate by the Ns strategy at 50 °C for 0.5 h and the resultant methyl ester was hydrolyzed to yield the carboxylic acid **24** in high yield. Coupling the asparagine unit **11** with the succinimide ester prepared from **24** furnished the Type C acylpolyamine backbone **25** in excellent yield. Removal of the Boc group in **25** by TFA, followed by coupling of the resultant amine with indoleacetic acid succinimide ester afforded the fully protected toxin **26**. Deprotection of the Ns and Cbz by the same procedures as above produced NPTX-501 (**5**) in a 33% overall yield from **12** via nine steps. The synthetic compound was fully characterized by the spectroscopic data (IR, $^1$H and $^{13}$C NMR, FABHRMS, ESI-MS/MS), and consistent with the proposed structure.[10,11]



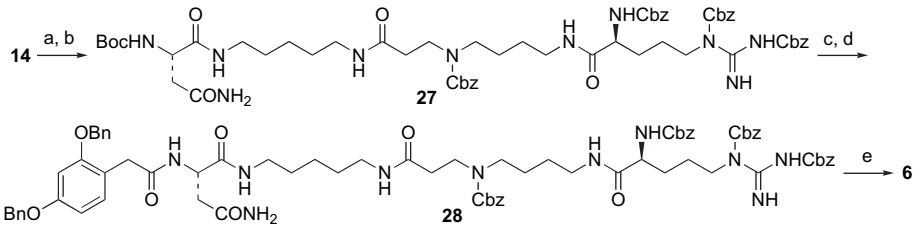

**Scheme 4**. Synthesis of NPTX-501 (**5**). Reagents and conditions: (a) CbzCl, $Et_3N/CH_2Cl_2$, 0 °C to rt, 1 h, 95%; (b) methyl bromoacetate, $Cs_2CO_3$/DMF, 50 °C, 0.5 h, 86%; (c) NaOH/$H_2O$–MeOH, 0 °C to rt, 2 h, 86%; (d) HOSu, DCC/$CH_2Cl_2$, 0 °C, 4 h; (e) **11**, DMF, rt, 0.5 h; (f) TFA/$CHCl_3$, 0 °C to rt, 1 h; (g) indoleacetic acid-OSu, $Et_3N$/DMF, rt, 2 h, 73% (four steps); (h) 2-mercaptoethanol, DBU/DMF, rt, 0.5 h; (i) $H_2$-Pd(OH)$_2$/AcOH, rt, 2 h, 65% (two steps).

## 2.5. Synthesis of Type D toxin

The polyamine backbone of Type D is quite similar to that of Type A and missing only the $C_3$ unit at the right terminal. Therefore, the toxins of these two types can be synthesized from the common polyamine precursor **14**. Thus, the Type D toxin NSTX-3 (**6**), isolated from the Papua New Guinean spider *Nephila maculata*,[24] was synthesized in a manner similar to that of Type A toxins (Schemes 1 and 2) as shown in Scheme 5. Removal of the Ns protective group of **14**,[26] followed by condensation with tri-Cbz-arginine succinimide ester furnished **27** in 76% yield. Deprotection of the Boc protective group of **28** by TFA, and subsequent condensation with 2,4-dihydroxyphenylacetic acid *N*-hydroxysuccinimide ester afforded the fully protected toxin **14** in 87% yield. Finally, hydrogenation of **28** afforded NSTX-3 (**6**) in a 36% overall yield from **9** via nine steps. The synthetic compound was fully characterized by the spectroscopic data (IR, $^1$H and $^{13}$C NMR, FABHRMS, ESI-MS/MS), and identified with the natural[24] and synthetic toxins.[39–41]

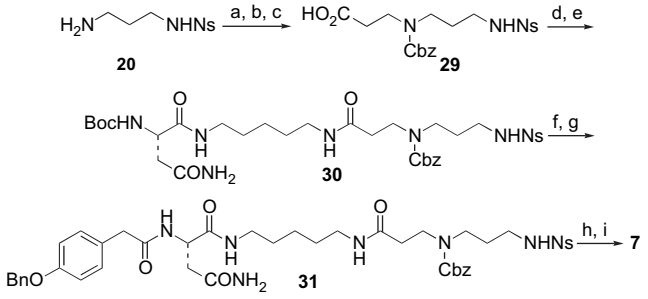
## 2.6. Synthesis of Type E toxin

The Type E polyamine backbone is only slightly different from Type D, that is, the $C_3$ unit is on the right terminal instead of the $C_4$ unit. Accordingly, starting with mono-Ns-1,3-diaminopropane **20** and using the same procedures as that for the Type D would give Type E toxin. Scheme 6 shows the synthesis of joramine (**7**), a minor component of the venom of *N. clavata*.[42] The carboxylic acid **29** was obtained from a series of reactions with Michael addition,



**Scheme 5**. Synthesis of NSTX-3 (**6**). Reagents and conditions: (a) 2-mercaptoethanol, DBU/DMF, rt, 0.5 h; (b) tri-Cbz-arginine-OSu, DMF, rt, 2 h; 76% (two steps); (c) TFA/$CHCl_3$, 0 °C to rt, 1 h; (d) 2,4-dibenzyloxyphenylacetic acid-OSu, $Et_3N$/DMF, rt, 2 h, 87% (two steps); (e) $H_2$-$Pd(OH)_2$/AcOH, rt, 4 h, 74%.

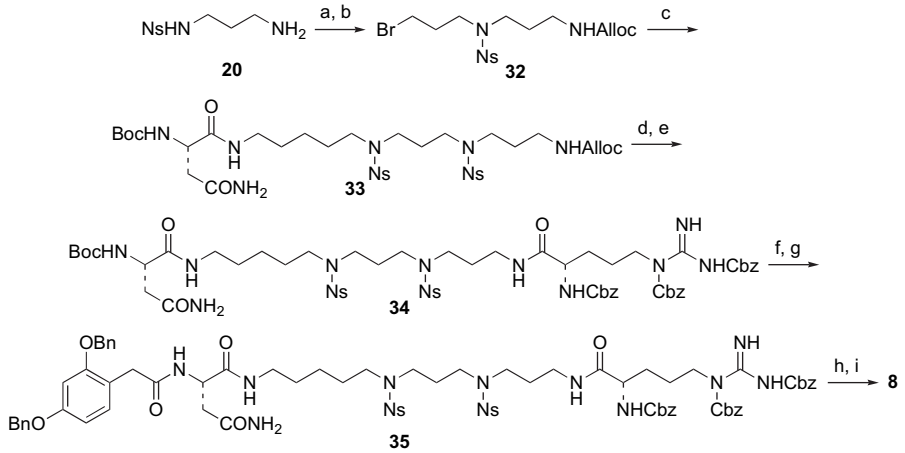
Cbz protection, and ester hydrolysis. The acid **29** was coupled with the amine unit **11** to afford the polyamine backbone **30** in 82% yield. After removal of the Boc group by TFA, the resultant amine was coupled with 4-benzyloxyphenylacetic acid succinimide ester to furnish the fully protected toxin **31**, which was converted to joramine (**7**) by the usual manner. Thus, the Type E toxin joramine (**7**) was synthesized in a 36% overall yield from **20** via nine steps. The synthetic compound was fully characterized by the spectroscopic data (IR, $^1H$ and $^{13}C$ NMR, FABHRMS, ESI-MS/MS), and identified with the natural toxin.[42]



**Scheme 6**. Synthesis of joramine (**7**). Reagents and conditions: (a) methyl acrylate/EtOH, rt, 5 h; (b) CbzCl, $Et_3N$/$CH_2Cl_2$, 0 °C to rt, 2 h, 86% (two steps); (c) NaOH/$H_2O$–MeOH, 0 °C to rt, 1 h, 85%; (d) HOSu, DCC/$CH_2Cl_2$, 0 °C, 5 h; (e) **11**, DMF, rt, 0.5 h, 82% (two steps); (f) TFA/$CHCl_3$, 0 °C to rt, 1 h; (g) 4-benzyloxyphenylacetic acid-OSu, $Et_3N$/DMF, rt, 2 h, 88% (two steps); (h) 2-mercaptoethanol, DBU/DMF, rt, 0.5 h; (i) $H_2$-$Pd(OH)_2$/AcOH, rt, 3 h, 69% (two steps).

## 2.7. Synthesis of Type F toxin

The Type F polyamine structure is quite similar to that of Type B; the only difference is the cadaverine ($C_5$) unit instead of the putrescine ($C_4$) unit connected to asparagine. Accordingly, this polyamine backbone can be constructed by basically the same way as used for Type B. Argiotoxin-636 (Argiopine, **8**), isolated from the Argiope spiders[43,44] in 1986 as well as JSTX-3,[45] can be classified into Type F and its synthesis is shown in Scheme 7. In this case, however, in order to attach arginine moiety at the polyamine terminal, selective protection of the terminal primary amine of the polyamine backbone is necessary. Thus, allyloxycarbonyl (Alloc) group was used for the third protective group for construction of polyamine chain. Mono-Ns-diaminopropane **20** was converted to the corresponding allyl carbamate, which was further alkylated by 1,3-dibromopropane in the presence of $Cs_2CO_3$ at 50 °C. By the same way as used for Type B toxin, the resultant bromide **32** was coupled with the asparagine/cadaverne conjugate **10** to afford the Type F polyamine backbone **33** in excellent yield. The Alloc moiety in **33** was selectively removed using tetrakis-(triphenylphosphine)palladium(0) ($Pd(PPh_3)_4$) as a catalyst and pyrrolidine as a nucleophile at room temperature in high yield.[46] Instead of $Pd(PPh_3)_4$, tris(dibenzylideneacetone)-dipalladium(0) was also effective for this deprotection. Thus, the obtained primary amine was coupled with the arginine derivative by its *N*-hydroxysuccinimide ester to give **34**. Boc removal and subsequent acylation of **34**, followed by deprotection in the usual manner afforded



**Scheme 7**. Synthesis of argiotoxin-636 (**8**). Reagents and conditions: (a) AllocCl, $Et_3N$/$CH_2Cl_2$, 0 °C, 0.5 h, 93%; (b) 1,3-dibromopropane, $Cs_2CO_3$/DMF, 50 °C, 0.5 h, 90%; (c) **10**, TBAI, $Cs_2CO_3$/DMF, 70 °C, 1 h; (d) $Pd(PPh_3)_4$, $PPh_3$, pyrrolidine/$CH_2Cl_2$, rt, 0.5 h; (e) tri-Cbz-arginine-OSu, DMF, rt, 1 h, 66% (three steps); (f) TFA/$CHCl_3$, 0 °C to rt, 1 h; (g) 2,4-dibenzyloxyphenylacetic acid-OSu, $Et_3N$/DMF, rt, 2 h, 80% (two steps); (h) 2-mercaptoethanol, DBU/DMF, rt, 0.5 h; (i) $H_2$-$Pd(OH)_2$/AcOH, rt, 2 h, 51% (two steps).

argiotoxin-636 (**8**) in a 26% overall yield from mono-Ns cadaverine **9** via eight steps. The synthetic compound was fully characterized by the spectroscopic data (IR, $^1$H and $^{13}$C NMR, FABHRMS, ESI-MS/MS), and identified with the natural[43,44] and synthetic toxins.[47–50]

## 3. Conclusion

We have established an efficient and versatile synthesis of acylpolyamine spider toxins based on the structural classification of the *Nephila* and *Nephilengys* spider toxins using the 2-nitrobenzenesulfonamide group (the Ns strategy). The naturally occurring toxins **1**–**8** corresponding to each structural type have been efficiently synthesized by this method in a high overall yield with few steps. This method is so versatile that it would enable us to synthesize a variety of analogues as well as naturally occurring toxins. Therefore, it would be highly useful for structure–activity relationship and mode of action studies of the acylpolyamine toxins in more detail. Studies along this line are currently underway.[51,52]

## 4. Experimental

### 4.1. General

Optical rotation was measured on a Jasco DIP-370 polarimeter. IR spectra were recorded on a Perkin–Elmer infrared spectrometer model 1750. NMR spectra were measured on a Varian DPX-300 spectrometer. Low and high resolution mass spectra were recorded on a JEOL JMS D-300 mass spectrometer. ESI-MS/MS spectra were measured on a Micromass Q-TOF Ultima API fitted with an electrospray ion source in positive ionization mode. Preparative HPLC was performed on a Shimadzu LC-10 instrument equipped with a CAPCELL PAK C-18 (5 μm, 10×250 mm) with a flow rate of 5 mL min$^{-1}$ and detection at 210 nm. Column chromatography was carried out on silica gel 60 (70–230 mesh), and preparative TLC was run on silica gel 60F$_{254}$. All dried solvents were purchased from Aldrich Chemical Co. The authentic specimen of JSTX-3 was purchased from Wako Chemical Co.

**4.1.1. *N*-[*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-L-asparaginyl]-*N*′-(2-nitrobenzenesulfonyl)-1,5-diaminopentane (10).** To a stirred solution of *N*-(5-amino-pentyl)-2,4-dinitro-benzenesulfonamide HCl salt[22] (**9**) (500 mg, 1.54 mmol) in DMF (5 mL) were added *N*$^{\alpha}$-Boc-L-asparagine-*p*-nitrophenyl ester (544 mg, 1.54 mmol) and Et$_3$N (156 mg, 1.54 mmol) at 0 °C. After being stirred for 0.5 h at room temperature, the reaction mixture was diluted with EtOAc (200 mL) and washed with saturated aqueous NaHCO$_3$ solution (5×50 mL) and brine (3×50 mL). Aqueous layers were extracted with EtOAc (3×30 mL) and combined organic layers were dried over Na$_2$SO$_4$. Filtration and concentration followed by chromatography on silica gel (1–4% MeOH/CH$_2$Cl$_2$) gave the title compound **10** (745 mg, 97%) as a white powder. $[\alpha]_D^{24}$ −3.85 (*c* 2.83, MeOH). IR (Nujol) 1683, 1657, 1542, 1461, 1162, 856 cm$^{-1}$. $^1$H NMR (300 MHz, CD$_3$OD): $\delta$ 1.26 (m, 2H), 1.36 (s, 9H), 1.41 (m, 4H), 2.52 (dd, 1H, *J*=6.3, 15.3 Hz), 2.58 (dd, 1H, *J*=6.0, 15.3 Hz), 2.96 (t, 2H, *J*=6.9 Hz), 3.07 (t, 1H, *J*=6.6 Hz), 4.29 (dd, 1H, *J*=6.0, 6.3 Hz), 7.75 (m, 3H), 8.00 (m, 1H). $^{13}$C NMR (75 MHz, CD$_3$OD): $\delta$ 24.7, 28.7, 29.7, 30.4, 38.3, 40.2, 44.2, 53.0, 80.9, 125.8, 131.5, 133.5, 134.9, 149.6, 157.5, 173.8, 175.1. HRMS calcd for $C_{14}H_{27}N_4O_4$ (M$^+$−Ns) 315.2032, found 315.2036.

**4.1.2. *N*-[*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-L-asparaginyl]-1,5-diaminopentane (11).** To a stirred solution of sulfonamide **10** (500 mg, 997 μmol) in DMF (2 mL) were added 2-mercaptoethanol (234 mg, 3.00 mmol) and DBU (456 mg, 3.00 mmol) slowly. After being stirred for 0.5 h at room temperature, the reaction mixture was directly subjected to silica gel chromatography (1–8% MeOH/CH$_2$Cl$_2$, then, 7.5% MeOH/CHCl$_3$ containing 2.5% *i*-PrNH$_2$) to afford the title compound **11** (269 mg, 85%) as a white powder. Spectral data ($^1$H and $^{13}$C NMR, IR, MS) obtained were completely in agreement with those reported previously.[53]

**4.1.3. Methyl 8-(2-nitrobenzenesulfonylamino)-4-benzyloxycarbonyl-4-azaoctanoate.** A solution of methyl acrylate (158 mg, 1.84 mmol) in EtOH (10 mL) was added at room temperature over 5 h to a stirring solution of *N*-(5-aminobutyl)-2-nitrobenzenesulfonamide[22] (**12**) (500 mg, 1.83 mmol) in EtOH (20 mL). The solvent was evaporated in vacuo, and the residue was subjected to silica gel chromatography (5–20% MeOH/CH$_2$Cl$_2$) to give the secondary amine as a colorless oil, which was used in the next step without further purification.

To a cold (0 °C) and stirred solution of the secondary amine in CH$_2$Cl$_2$ (20 mL) were added CbzCl (30% in toluene, 1.04 g, 1.83 mmol) and Et$_3$N (185 mg, 1.83 mmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was diluted with EtOAc (200 mL) and washed with saturated NH$_4$Cl solution (3×50 mL) and brine (3×50 mL). Aqueous layers were extracted with EtOAc (3×40 mL) and combined organic layers were dried over Na$_2$SO$_4$. Filtration and concentration followed by chromatography on silica gel (30–50% EtOAc/hexane) gave methyl ester (775 mg, 86%, two steps) as a colorless oil. IR (neat) 1735, 1696, 1542, 1422, 1365, 1166, 853 cm$^{-1}$. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 1.54 (m, 4H), 2.57 (m, 2H), 3.09 (m, 2H), 3.25 (t, 2H, *J*=6.3 Hz), 3.49 (t, 2H, *J*=7.2 Hz), 3.65 (s, 3H), 5.11 (s, 2H), 5.48 (br s, 1H), 7.34 (m, 5H), 7.73 (m, 2H), 7.84 (m, 1H), 8.11 (m, 1H). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 25.0, 26.7, 33.7, 43.0, 43.3, 47.2, 51.8, 67.2, 125.4, 127.9, 128.1, 128.5, 131.0, 132.7, 133.5, 133.7, 136.5, 148.0, 155.9, 172.0. HRMS calcd for $C_{21}H_{24}N_3O_7S$ (M$^+$−OMe) 462.1335, found 462.1353.

**4.1.4. 8-(2-Nitrobenzenesulfonylamino)-4-benzyloxycarbonyl-4-azaoctanoic acid (13).** To a cold (0 °C) and stirred solution of the above methyl ester (700 mg, 1.42 mmol) in MeOH (5 mL) was added 3.0 M aqueous NaOH solution (1.42 mL, 4.26 mmol) slowly. After being stirred for 1 h at room temperature, the reaction mixture was adjusted to pH 2.0 by concentrated HCl. The resultant mixture was diluted with EtOAc (100 mL) and washed with H$_2$O (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over Na$_2$SO$_4$. Filtration and concentration followed by chromatography on silica gel (50% EtOAc/hexane, then, EtOAc) gave the title compound **13** (652 mg, 96%) as a colorless oil.

IR (film) 1686, 1541, 1424, 1365, 1165, 854 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.54 (m, 4H), 2.61 (m, 2H), 3.10 (m, 2H), 3.26 (t, 2H, $J$=6.0 Hz), 3.50 (t, 2H, $J$=7.2 Hz), 5.11 (s, 2H), 5.54 (br s, 1H), 7.36 (m, 5H), 7.71 (m, 2H), 7.82 (m, 1H), 8.10 (m, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 25.0, 26.7, 33.0, 42.9, 43.3, 47.5, 67.3, 125.3, 127.9, 128.1, 128.6, 131.0, 132.8, 133.6, 133.7, 136.4, 148.0, 156.1, 176.3. HRMS calcd for $C_{13}H_{18}N_3O_6S$ ($M^+$−Cbz) 344.0917, found 344.0917.

**4.1.5. *N*-[$N^{\alpha}$-(*tert*-Butoxycarbonyl)-L-asparaginyl]-$N'$-[8-(2-nitrobenzenesulfonylamino)-4-benzyloxycarbonyl-4-azaoctanoyl]-1,5-diaminopentane (14).** To a cold (0 °C) and stirred suspension of carboxylic acid **13** (363 mg, 757 μmol) and HOSu (175 mg, 1.52 mmol) in $CH_2Cl_2$ (5 mL) was added DCC (235 mg, 1.14 mmol) slowly. After being stirred for 5 h at 0 °C, the reaction mixture was filtrated through a pad of silica gel with EtOAc. The filtrate was concentrated in vacuo to give the crude succinimide ester as a colorless oil.

To a stirred solution of obtained ester in DMF (6 mL) was added amine **11** (241 mg, 762 μmol) slowly. After being stirred for 0.5 h at room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with saturated aqueous $NaHCO_3$ solution (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (2–8% EtOH/$CH_2Cl_2$) gave the title compound **14** (532 mg, 90%, two steps) as a colorless solid. $[\alpha]_D^{24}$ −2.61 (*c* 2.30, MeOH). IR (Nujol) 1674, 1542, 1449, 1369, 1165, 854 $cm^{-1}$. $^1H$ NMR (300 MHz, $CD_3OD$): $\delta$ 1.21 (m, 2H), 1.32 (s, 9H), 1.36 (m, 8H), 2.30 (t, 2H, $J$=6.6 Hz), 2.47 (dd, 1H, $J$=6.6, 12.3 Hz), 2.53 (dd, 1H, $J$=5.7, 12.3 Hz), 2.92 (m, 2H), 3.02 (t, 2H, $J$=7.2 Hz), 3.06 (t, 2H, $J$=6.9 Hz), 3.13 (t, 2H, $J$=6.9 Hz), 3.37 (m, 2H), 4.26 (dd, 1H, $J$=5.7, 6.6 Hz), 4.98 (s, 2H), 7.23 (m, 5H), 7.68 (m, 3H), 7.93 (m, 1H). $^{13}C$ NMR (75 MHz, $CD_3OD$): $\delta$ 25.1, 26.6, 28.0, 28.7, 29.8, 29.9, 36.5, 38.4, 40.3, 40.5, 44.0, 45.6, 53.1, 68.3, 80.9, 125.8, 128.9, 129.1, 129.6, 131.5, 133.5, 134.9, 135.0, 138.1, 149.6, 157.5, 157.8, 173.6, 173.8, 175.1. FABHRMS calcd for $C_{35}H_{51}N_7O_{11}SNa$ $(M+Na)^+$ 800.3265, found 800.3276.

**4.1.6. *N*-[$N^{\alpha}$-(*tert*-Butoxycarbonyl)-L-asparaginyl]-$N'$-[12-benzyloxycarbonylamino-9-(2-nitrobenzenesulfonyl)-4-benzyloxycarbonyl-4,9-diazaundecanoyl]-1,5-diaminopentane (15).** To a solution of sulfonamide **14** (200 mg, 257 μmol) and *N*-Cbz-3-bromopropylamine[53] (106 mg, 390 μmol) in DMF (2 mL) was added $Cs_2CO_3$ (169 mg, 519 μmol). After being stirred for 1 h at 50 °C, the reaction mixture was diluted with EtOAc (100 mL) and washed with $H_2O$ (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (2–8% MeOH/$CH_2Cl_2$) gave the title compound **15** (230 mg, 92%) as a colorless oil. $[\alpha]_D^{22}$ −2.28 (*c* 2.46, MeOH). IR (Nujol) 1688, 1667, 1636, 1546, 1462, 1163, 852 $cm^{-1}$. $^1H$ NMR (300 MHz, $CD_3OD$): $\delta$ 1.30 (m, 2H), 1.43 (s, 9H), 1.48 (m, 8H), 1.69 (m, 2H), 2.41 (t, 2H, $J$=6.0 Hz), 2.56 (dd, 1H, $J$=6.0, 16.5 Hz), 2.62 (dd, 1H, $J$=5.7, 16.5 Hz), 3.10 (m, 4H), 3.16 (t, 2H, $J$=6.3 Hz), 3.27 (m, 6H), 3.48 (m, 2H), 4.36 (dd, 1H, $J$=5.7, 6.0 Hz), 5.05 (s, 2H), 5.09 (s, 2H), 7.34 (m, 10H), 7.73 (m, 3H), 7.96 (m, 1H). $^{13}C$ NMR (75 MHz, $CD_3OD$): $\delta$ 25.1, 26.4, 28.7, 29.8, 29.9, 30.0, 36.6, 38.4, 39.1, 40.3, 45.6, 46.4, 53.1, 67.4, 68.3, 80.9, 125.4, 128.8, 129.0, 129.2, 129.5, 129.6, 131.4, 133.1, 134.0, 135.2, 138.1, 138.4, 149.6, 157.8, 158.8, 173.6, 173.8, 175.1. FABHRMS calcd for $C_{46}H_{64}N_8O_{13}SNa$ $(M+Na)^+$ 991.4211, found 991.4217.

**4.1.7. *N*-[$N^{\alpha}$-(2,4-Dibenzyloxyphenylacetyl)-L-asparaginyl]-$N'$-[12-benzyloxycarbonylamino-9-(2-nitrobenzenesulfonyl)-4-benzyloxycarbonyl-4,9-diazaundecanoyl]-1,5-diaminopentane (16).** To a cold (0 °C) and stirred suspension of polyamine **15** (165 mg, 170 μmol) in $CHCl_3$ (1 mL) was added TFA (1 mL) slowly. After being stirred for 1 h at room temperature, the reaction mixture was concentrated in vacuo to give the crude amine TFA salt as a colorless oil.

To a stirred solution of the amine TFA salt in DMF (1 mL) were added $Et_3N$ (18 mg, 178 μmol) and 2,4-dibenzyloxyphenylacetic acid succinimidyl ester[28] (116 mg, 260 μmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was directly subjected to silica gel chromatography (1–10% EtOH/$CH_2Cl_2$) to afford the title compound **16** (177 mg, 87%, two steps) as a white powder. $[\alpha]_D^{22}$ −0.75 (*c* 2.01, DMF). IR (Nujol) 1695, 1664, 1642, 1544, 1463, 1173, 852 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.16 (quin, 2H, $J$=6.9 Hz), 1.29 (m, 4H), 1.38 (m, 4H), 1.59 (m, 2H), 2.28 (t, 2H, $J$=6.6 Hz), 2.35 (dd, 1H, $J$=7.5, 15.3 Hz), 2.46 (dd, 1H, $J$=6.3, 15.3 Hz), 2.95 (m, 6H), 3.21 (m, 8H), 3.41 (s, 2H), 4.50 (dd, 1H, $J$=6.6, 7.5 Hz), 4.99 (s, 2H), 5.04 (s, 4H), 5.08 (s, 2H), 6.52 (d, 1H, $J$=8.4 Hz), 6.68 (s, 1H), 6.85 (s, 1H), 7.07 (d, 1H, $J$=8.1 Hz), 7.32 (m, 22H), 7.60 (t, 1H, $J$=5.4 Hz), 7.82 (m, 3H), 7.95 (m, 1H). $^{13}C$ NMR (75 MHz, $CD_3OD$): $\delta$ 23.8, 25.3, 28.6, 28.8, 28.9, 34.4, 35.1, 36.4, 37.5, 38.0, 38.5, 38.7, 44.0, 45.5, 46.5, 47.4, 50.0, 65.4, 66.2, 69.4, 69.5, 100.7, 105.9, 117.3, 124.4, 127.3, 127.7, 127.9, 128.0, 128.5, 128.6, 129.8, 131.1, 132.0, 132.6, 134.6, 137.3, 137.4, 147.8, 155.3, 156.3, 157.1, 158.5, 170.1, 170.5, 170.9, 171.8. FABHRMS calcd for $C_{63}H_{74}N_8O_{14}SNa$ $(M+Na)^+$ 1221.4943, found 1221.4943.

**4.1.8. JSTX-3 (1).** To a stirred solution of polyamine **16** (40 mg, 33.4 μmol) in DMF (1 mL) were added 2-mercaptoethanol (8.0 mg, 102 μmol) and DBU (15 mg, 98.5 μmol). After being stirred for 0.5 h at room temperature, the reaction mixture was directly purified on silica gel column (2–12% EtOH/$CH_2Cl_2$, then, 7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) and then, further purification by preparative TLC (7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) gave the secondary amine as a white powder.

A solution of the secondary amine in AcOH (1 mL) was hydrogenated over 20% $Pd(OH)_2$ on carbon (20 mg) for 3 h. The mixture was filtrated through Celite and the filtrate was evaporated to dryness. The resultant residue was dissolved in water and was purified by preparative RP-HPLC with a linear gradient from 5% $H_2O$/MeCN containing 0.1% TFA to 50% $H_2O$/MeCN containing 0.1% TFA in 20 min. JSTX-3 (**1**) was eluted at 9.54 min and was obtained

as a colorless TFA salt (20 mg, 66%, two steps). $[\alpha]_D^{22}$ −4.74 (*c* 0.97, $H_2O$). IR (Nujol) 3518, 2924, 1695, 1547 $cm^{-1}$. $^1H$ NMR (300 MHz, $D_2O$): $\delta$ 1.04 (quin, 2H, *J*=7.8 Hz), 1.29 (m, 4H), 1.63 (m, 4H), 1.94 (quin, 2H, *J*=7.5 Hz), 2.50 (t, 2H, *J*=6.9 Hz), 2.60 (dd, 1H, *J*=7.5, 15.3 Hz), 2.64 (dd, 1H, *J*=5.7, 15.3 Hz), 2.96 (m, 12H), 3.14 (t, 2H, *J*=6.9 Hz), 3.34 (d, 1H, *J*=15.3 Hz), 3.44 (d, 1H, *J*=15.3 Hz), 4.46 (dd, 1H, *J*=5.7, 7.5 Hz), 6.31 (m, 2H), 6.95 (d, 1H, *J*=9.0 Hz). $^{13}C$ NMR (75 MHz, $D_2O$): $\delta$ 22.5, 22.6, 23.1, 23.7, 27.6, 27.7, 30.8, 35.9, 36.4, 36.8, 39.1, 39.2, 43.4, 44.4, 46.7, 46.9, 50.8, 102.8, 107.4, 113.7, 132.4, 155.1, 156.1, 171.5, 172.3, 174.6, 174.9. FABHRMS calcd for $C_{27}H_{48}N_7O_6$ $(M+H)^+$ 566.3670, found 566.3660. ESI-MS/MS: Supplementary data 1 and 2.

**4.1.9. *N*-[$N^{\alpha}$-(Indoleacetyl)-L-asparaginyl]-*N*′-[12-benzyloxycarbonylamino-9-(2-nitrobenzenesulfonyl)-4-benzyloxycarbonyl-4,9-diazaundecanoyl]-1,5-diaminopentane (17).** To a cold (0 °C) and stirred suspension of polyamine **15** (115 mg, 119 μmol) in $CHCl_3$ (1 mL) was added TFA (1 mL) slowly. After being stirred for 1 h at room temperature, the reaction mixture was concentrated in vacuo to give the crude amine TFA salt as a colorless solid.

To a stirred solution of obtained amine TFA salt in DMF (1 mL) were added TEA (13 mg, 129 μmol) and indoleacetic acid succinimide ester[45] (49 mg, 180 μmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with saturated $NaHCO_3$ solution (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (2–10% EtOH/$CH_2Cl_2$) gave the title compound **17** (102 mg, 84%, two steps) as a white powder. $[\alpha]_D^{24}$ +2.55 (*c* 0.51, MeOH). IR (Nujol) 1668, 1543, 1461, 1250, 1161, 852 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.17 (m, 2H), 1.28 (m, 4H), 1.38 (m, 4H), 1.60 (m, 2H), 2.29 (t, 2H, *J*=6.6 Hz), 2.36 (dd, 1H, *J*=6.9, 15.3 Hz), 2.48 (dd, 1H, *J*=6.0, 15.3 Hz), 2.95 (m, 6H), 3.22 (m, 8H), 3.54 (s, 2H), 4.50 (dd, 1H, *J*=6.0, 6.9 Hz), 4.99 (s, 2H), 5.03 (s, 2H), 6.84 (s, 1H), 6.94 (t, 1H, *J*=7.8 Hz), 7.04 (t, 1H, *J*=7.8 Hz), 7.18 (s, 1H), 7.32 (m, 12H), 7.52 (d, 1H, *J*=7.5 Hz), 7.58 (d, 1H, *J*=5.1 Hz), 7.82 (m, 3H), 7.95 (m, 2H), 8.09 (d, 1H, *J*=8.1 Hz), 10.85 (s, 1H). $^{13}C$ NMR (75 MHz, $CD_3OD$): $\delta$ 23.8, 25.3, 28.7, 28.8, 28.9, 32.7, 35.1, 37.5, 38.0, 38.6, 38.7, 43.2, 44.1, 45.5, 46.5, 47.4, 50.0, 65.4, 66.2, 108.9, 111.5, 118.5, 118.8, 121.1, 124.0, 124.4, 127.4, 127.9, 128.0, 128.5, 128.6, 129.8, 132.0, 132.6, 134.6, 136.3, 137.2, 137.4, 147.7, 155.4, 156.3, 170.2, 170.8, 170.9, 171.7. FABHRMS calcd for $C_{51}H_{63}N_9O_{12}SNa$ $(M+Na)^+$ 1048.4214, found 1048.4216.

**4.1.10. NPTX-8 (2).** To a stirred solution of polyamine **17** (40 mg, 39.0 μmol) in DMF (1 mL) were added 2-mercaptoethanol (10 mg, 128 μmol) and DBU (20 mg, 131 μmol). After being stirred for 0.5 h at room temperature, the reaction mixture was directly purified on silica gel column (2–12% EtOH/$CH_2Cl_2$, then, 7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) and then, further purification by preparative TLC (7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) gave the secondary amine as a white powder.

A solution of the secondary amine in AcOH (1 mL) was hydrogenated over 20% $Pd(OH)_2$ on carbon (20 mg) for 2 h. The mixture was filtrated through Celite and the filtrate was evaporated to dryness. The resultant residue was dissolved in water and was purified by preparative RP-HPLC with a linear gradient from 5% $H_2O$/MeCN containing 0.1% TFA to 50% $H_2O$/MeCN containing 0.1% TFA in 20 min. NPTX-8 (**2**) was eluted at 12.26 min and was obtained as a colorless TFA salt (22 mg, 61%, two steps). $[\alpha]_D^{22}$ −2.86 (*c* 0.77, $H_2O$). IR (Nujol) 3481, 2924, 1697, 1539 $cm^{-1}$. $^1H$ NMR (300 MHz, $D_2O$): $\delta$ 1.00 (quin, 2H, *J*=7.2 Hz), 1.23 (quin, 4H, *J*=7.2 Hz), 1.60 (m, 4H), 1.94 (quin, 2H, *J*=8.4 Hz), 2.46 (t, 2H, *J*=6.6 Hz), 2.52 (dd, 1H, *J*=7.5, 15.3 Hz), 2.61 (dd, 1H, *J*=5.7, 15.3 Hz), 2.94 (m, 12H), 3.09 (t, 2H, *J*=6.6 Hz), 3.67 (s, 2H), 4.49 (dd, 1H, *J*=5.7, 7.5 Hz), 7.04 (t, 1H, *J*=7.8 Hz), 7.13 (t, 1H, *J*=7.8 Hz), 7.20 (s, 1H), 7.39 (d, 1H, *J*=7.8 Hz), 7.46 (d, 1H, *J*=7.8 Hz). $^{13}C$ NMR (75 MHz, $D_2O$): $\delta$ 22.5, 22.6, 23.0, 23.6, 27.5, 27.6, 30.7, 32.2, 36.1, 36.4, 39.0, 39.1, 43.4, 44.4, 46.6, 46.9, 50.8, 107.4, 111.9, 118.2, 119.5, 122.1, 124.9, 126.5, 136.2, 171.4, 172.1, 174.4, 175.0. FABHRMS calcd for $C_{29}H_{49}N_8O_4$ $(M+H)^+$ 573.3895, found 573.3853. ESI-MS/MS: Supplementary data 3.

**4.1.11. *N*-[$N^{\alpha}$-(4-Benzyloxyindoleacetyl)-L-asparaginyl]-*N*′-[12-benzyloxycarbonylamino-9-(2-nitrobenzenesulfonyl)-4-benzyloxycarbonyl-4,9-diazaundecanoyl]-1,5-diaminopentane (18).** To a cold (0 °C) and stirred suspension of polyamine **15** (200 mg, 206 μmol) in $CHCl_3$ (1 mL) was added TFA (1 mL) slowly. After being stirred for 1 h at room temperature, the reaction mixture was concentrated in vacuo to give the crude amine TFA salt as a colorless solid.

To a stirred solution of obtained amine TFA salt in DMF (1 mL) were added TEA (21 mg, 208 μmol) and 4-benzyloxyindoleacetic acid succinimide ester[33] (117 mg, 309 μmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with saturated $NaHCO_3$ solution (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (2–10% EtOH/$CH_2Cl_2$) gave the title compound **18** (201 mg, 86%, two steps) as a white powder. $[\alpha]_D^{22}$ +8.07 (*c* 1.50, DMF). IR (Nujol) 1698, 1665, 1639, 1542, 1376, 1248 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.16 (m, 2H), 1.28 (m, 4H), 1.39 (m, 4H), 1.61 (m, 2H), 2.29 (t, 2H, *J*=6.6 Hz), 2.38 (m, 2H), 2.95 (m, 6H), 3.22 (m, 8H), 3.74 (s, 2H), 4.51 (m, 1H), 4.99 (s, 2H), 5.03 (s, 2H), 5.18 (s, 2H), 6.49 (d, 1H, *J*=4.5 Hz), 6.83 (br s, 1H), 6.92 (m, 2H), 7.05 (br s, 1H), 7.32 (m, 15H), 7.47 (m, 2H), 7.56 (m, 1H), 7.80 (m, 4H), 7.94 (m, 2H), 10.88 (s, 1H). $^{13}C$ NMR (75 MHz, $CD_3OD$): $\delta$ 23.8, 24.8, 25.4, 28.7, 28.8, 28.9, 34.5, 35.1, 37.5, 38.0, 38.6, 38.7, 44.1, 45.5, 46.5, 47.4, 49.9, 65.4, 66.3, 69.2, 100.7, 105.3, 108.5, 117.4, 122.0, 122.9, 124.5, 127.4, 127.7, 127.9, 128.0, 128.5, 128.6, 129.8, 132.0, 132.6, 134.6, 137.2, 137.4, 137.9, 138.1, 147.8, 153.0, 155.3, 156.3, 170.2, 170.9, 171.3, 171.9. FABHRMS calcd for $C_{58}H_{70}N_9O_{13}S$ $(M+H)^+$ 1132.4814, found 1132.4833.

**4.1.12. NPTX-1 (3).** To a stirred solution of polyamine **18** (40 mg, 35.3 μmol) in DMF (1 mL) were added 2-mercaptoethanol (9 mg, 115 μmol) and DBU (18 mg, 118 μmol). After being stirred for 0.5 h at room temperature, the reaction mixture was directly purified on silica gel column (2–12% EtOH/$CH_2Cl_2$, then, 7.5% MeOH/$CHCl_3$ containing 2.5% *i*-Pr$NH_2$) and then, further purification by preparative TLC (7.5% MeOH/$CHCl_3$ containing 2.5% *i*-Pr$NH_2$) gave the secondary amine as a white powder.

A solution of the secondary amine in AcOH (1 mL) was hydrogenated over 20% $Pd(OH)_2$ on carbon (20 mg) for 2 h. The mixture was filtrated through Celite and the filtrate was evaporated to dryness. The resultant residue was dissolved in water and was purified by preparative RP-HPLC with a linear gradient from 5% $H_2O$/MeCN containing 0.1% TFA to 50% $H_2O$/MeCN containing 0.1% TFA in 20 min. NPTX-1 (**3**) was eluted at 11.31 min and was obtained as a colorless TFA salt (20 mg, 61%, two steps). $[\alpha]_D^{22}$ +1.05 (*c* 1.14, $H_2O$). IR (Nujol) 3513, 2924, 1670 $cm^{-1}$. $^1H$ NMR (300 MHz, $D_2O$): $\delta$ 0.90 (quin, 2H, *J*=7.2 Hz), 1.16 (m, 4H), 1.61 (m, 4H), 1.93 (quin, 2H, *J*=7.5 Hz), 2.46 (t, 2H, *J*=6.6 Hz), 2.54 (dd, 1H, *J*=6.0, 16.5 Hz), 2.60 (dd, 1H, *J*=6.0, 16.5 Hz), 2.97 (m, 12H), 3.11 (t, 2H, *J*=6.6 Hz), 3.68 (d, 1H, *J*=15.9 Hz), 3.76 (d, 1H, *J*=15.9 Hz), 4.46 (t, 1H, *J*=6.0 Hz), 6.41 (t, 1H, *J*=4.2 Hz), 6.93 (d, 2H, *J*=4.2 Hz), 7.06 (s, 1H). $^{13}C$ NMR (75 MHz, $D_2O$): $\delta$ 22.5, 22.6, 22.9, 23.6, 27.5, 27.6, 30.7, 33.8, 35.8, 36.4, 39.0, 39.1, 43.4, 44.4, 46.6, 46.9, 50.7, 103.5, 104.4, 106.6, 116.1, 122.9, 124.1, 138.6, 149.6, 171.4, 172.1, 174.5, 175.9. FABHRMS calcd for $C_{29}H_{49}N_8O_5$ $(M+H)^+$ 589.3810, found 589.3849. ESI-MS/MS: Supplementary data 4.

**4.1.13. *N*-[*N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-L-asparaginyl]-*N*′-(2-nitrobenzenesulfonyl)-1,4-diaminobutane (19).** To a stirred solution of *N*-(2-nitrobenzenesulfonyl)-1,4-diaminobutane (500 mg, 1.83 mmol) in DMF (5 mL) was added Boc-L-Asn-ONp (647 mg, 1.83 mmol) at 0 °C. After being stirred for 2 h at room temperature, the reaction mixture was diluted with EtOAc (200 mL) and washed with saturated aqueous $NaHCO_3$ solution (5×50 mL) and brine (3×50 mL). Aqueous layers were extracted with EtOAc (3×40 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (1–12% MeOH/$CH_2Cl_2$) gave the title compound **19** (845 mg, 95%) as a white powder. $[\alpha]_D^{22}$ −2.13 (*c* 2.44, DMF). IR (Nujol) 1681, 1659, 1543, 1461, 1161 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.35 (m, 13H), 2.34 (m, 2H), 2.86 (m, 2H), 2.96 (quin, 2H, *J*=6.0 Hz), 4.13 (m, 1H), 6.79 (d, 1H, *J*=8.1 Hz), 6.86 (br s, 1H), 7.24 (br s, 1H), 7.69 (br s, 1H), 7.83 (m, 2H), 7.92 (m, 2H), 8.04 (m, 1H). $^{13}C$ NMR (75 MHz, $CD_3OD$): $\delta$ 26.3, 26.6, 28.4, 37.7, 38.2, 42.6, 51.7, 78.4, 124.6, 129.6, 132.8, 133.0, 134.2, 148.0, 155.3, 171.5, 175.8. HRMS calcd for $C_{14}H_{20}N_5O_6S$ ($M^+$−Boc) 386.1134, found 386.1113.

**4.1.14. *N*-(2-Nitrobenzenesulfonyl)-*N*′-(benzyloxycarbonyl)-1,3-diaminopropane.** To a cold (0 °C) and stirred suspension of *N*-(2-nitrobenzenesulfonyl)-1,3-diaminopropane[22] (**20**) (400 mg, 1.54 mmol) in $CH_2Cl_2$ (10 mL) were added CbzCl (30% in toluene, 876 mg, 1.54 mmol) and TEA (156 mg, 1.54 mmol) slowly. After being stirred for 1 h at room temperature, the reaction mixture was diluted with EtOAc (200 mL) and washed with saturated $NH_4Cl$ solution (3×50 mL) and brine (3×50 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (30–40% EtOAc/hexane) gave the title compound (530 mg, 88%) as a colorless oil. IR (film) 1702, 1540, 1442, 1364, 1259, 1166, 854 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.71 (quin, 2H, *J*=5.7 Hz), 3.15 (q, 2H, *J*=5.7 Hz), 3.28 (q, 2H, *J*=5.7 Hz), 5.06 (br s, 1H), 5.08 (s, 2H), 5.86 (br s, 1H), 7.33 (m, 5H), 7.71 (m, 2H), 7.83 (m, 1H), 8.10 (m, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 30.2, 37.6, 40.7, 66.8, 125.3, 128.0, 128.1, 128.5, 130.8, 132.7, 133.5, 133.8, 136.4, 148.0, 156.8. HRMS calcd for $C_{11}H_{15}N_2O_2$ ($M^+$−Ns) 207.1133, found 207.1118.

**4.1.15. 7-Benzyloxycarbonylamino-4-(2-nitrobenzenesulfonyl)-4-azaheptan-1-yl bromide (21).** To a solution of the above sulfonamide (420 mg, 1.07 mmol) and 1,3-dibromopropane (648 mg, 3.21 mmol) in DMF (5 mL) was added $Cs_2CO_3$ (525 mg, 1.61 mmol). After being stirred for 0.5 h at 50 °C, the reaction mixture was diluted with EtOAc (100 mL) and washed with $H_2O$ (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (20–50% EtOAc/hexane) gave the title compound **21** (501 mg, 91%) as a colorless oil. IR (film) 1718, 1543, 1455, 1372, 1247, 1161, 852 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.79 (quin, 2H, *J*=6.6 Hz), 2.08 (quin, 2H, *J*=7.2 Hz), 3.23 (m, 2H), 3.38 (m, 6H), 5.09 (s, 2H), 5.14 (br s, 1H), 7.35 (m, 5H), 7.65 (m, 3H), 7.99 (m, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 28.2, 29.8, 31.2, 37.7, 45.4, 46.1, 66.6, 124.3, 128.0, 128.1, 128.5, 130.9, 131.8, 132.8, 133.8, 136.5, 148.0, 156.4. HRMS calcd for $C_{14}H_{20}N_2O_2Br$ ($M^+$−Ns) 327.0708, found 327.0710.

**4.1.16. *N*-(*N*$^{\alpha}$-*tert*-Butoxycarbonyl-L-asparaginyl)-12-benzyloxycarbonylamino-5,9-[di-(2-nitrobenzenesulfonyl)]-5,9-diaza-1-aminoundecane (22).** To a solution of sulfonamide **19** (244 mg, 500 μmol) and bromide **21** (387 mg, 752 μmol) in DMF (3 mL) were added $Cs_2CO_3$ (489 mg, 1.50 mmol) and TBAI (19.0 mg, 51.4 μmol). After being stirred for 1 h at 70 °C, the reaction mixture was diluted with EtOAc (200 mL) and washed with $H_2O$ (3×40 mL) and brine (3×40 mL). Aqueous layers were extracted with EtOAc (3×40 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (2–4% MeOH/$CH_2Cl_2$) gave the title compound **22** (433 mg, 94% from **19**) as a colorless solid. $[\alpha]_D^{24}$ −2.43 (*c* 1.48, MeOH). IR (Nujol) 1712, 1670, 1544, 1459, 1372, 1161, 852 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.44 (s, 9H), 1.53 (m, 4H), 1.78 (m, 4H), 2.51 (dd, 1H, *J*=6.0, 15.6 Hz), 2.88 (dd, 1H, *J*=4.8, 15.6 Hz), 3.25 (m, 12H), 4.41 (m, 1H), 5.08 (s, 2H), 5.50 (br s, 1H), 5.68 (br s, 1H), 6.08 (br s, 2H), 6.85 (br s, 1H), 7.35 (m, 5H), 7.61 (m, 2H), 7.68 (m, 4H), 7.96 (m, 2H). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 25.1, 26.7, 27.6, 28.3, 28.4, 38.0, 38.4, 45.1, 45.4, 45.5, 47.6, 51.2, 66.6, 80.3, 124.2, 124.3, 128.1, 128.5, 130.6, 131.8, 131.9, 132.7, 132.9, 133.6, 133.7, 136.6, 148.0, 155.7, 156.6,

171.4, 173.4. FABHRMS calcd for $C_{39}H_{52}N_8O_{14}S_2Na$ $(M+Na)^+$ 943.2942, found 943.2951.

**4.1.17. *N*-[$N^{\alpha}$-(Indoleacetyl)-L-asparaginyl]-12-benzyloxycarbonylamino-5,9-[di-(2-nitrobenzenesulfonyl)]-5,9-diaza-1-aminoundecane (23).** To a cold (0 °C) and stirred suspension of polyamine **22** (156 mg, 170 μmol) in $CHCl_3$ (1 mL) was added TFA (1 mL) slowly. After being stirred for 1 h at room temperature, the reaction mixture was concentrated in vacuo to give the crude amine TFA salt as a colorless solid.

To a stirred solution of obtained amine TFA salt in DMF (1 mL) were added TEA (18 mg, 178 μmol) and indoleacetic acid succinimide ester[45] (70 mg, 257 μmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with saturated $NaHCO_3$ solution (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (2–8% EtOH/$CH_2Cl_2$) gave the title compound **23** (139 mg, 84%, two steps) as a white powder. $[\alpha]_D^{22}$ +5.06 (*c* 1.76, acetone). IR (Nujol) 1666, 1542, 1461, 1376, 1256, 1160, 852 $cm^{-1}$. $^1H$ NMR (300 MHz, $CD_3COCD_3$): $\delta$ 1.21 (quin, 2H, *J*=6.3 Hz), 1.39 (quin, 2H, *J*=7.2 Hz), 1.75 (quin, 2H, *J*=6.9 Hz), 1.78 (quin, 2H, *J*=6.6 Hz), 2.52 (dd, 1H, *J*=6.0, 15.6 Hz), 2.72 (dd, 1H, *J*=5.1, 15.6 Hz), 3.02 (m, 2H), 3.13 (m, 2H), 3.20 (m, 2H), 3.23 (m, 2H), 3.30 (m, 2H), 3.37 (m, 2H), 3.69 (s, 2H), 4.65 (m, 1H), 5.06 (s, 2H), 6.31 (br s, 1H), 6.43 (br s, 1H), 6.94 (br s, 1H), 7.01 (t, 1H, *J*=6.9 Hz), 7.09 (t, 1H, *J*=6.9 Hz), 7.33 (m, 7H), 7.53 (br s, 1H), 7.59 (d, 1H, *J*=6.9 Hz), 7.83 (m, 6H), 8.00 (m, 3H), 10.13 (br s, 1H). $^{13}C$ NMR (75 MHz, $CD_3COCD_3$): $\delta$ 25.1, 26.6, 27.2, 33.3, 36.7, 38.1, 38.2, 38.4, 44.8, 45.3, 45.7, 47.4, 50.2, 65.9, 109.2, 111.7, 118.8, 119.2, 121.8, 124.1, 124.3, 124.4, 124.5, 127.8, 128.0, 128.1, 128.6, 130.4, 130.5, 132.4, 132.5, 132.9, 133.1, 134.3, 134.5, 137.0, 137.8, 148.4, 156.7, 171.9, 172.3, 173.8. FABHRMS calcd for $C_{44}H_{51}N_9O_{13}S_2Na$ $(M+Na)^+$ 1000.2946, found 1000.2936.

**4.1.18. NPTX-473 (4).** To a stirred solution of polyamine **23** (40 mg, 40.9 μmol) in DMF (1 mL) were added 2-mercaptoethanol (20 mg, 256 μmol) and DBU (40 mg, 263 μmol). After being stirred for 0.5 h at room temperature, the reaction mixture was directly purified on silica gel column (2–12% EtOH/$CH_2Cl_2$, then, 7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) and then, further purification by preparative TLC (7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) gave the secondary amine as a white powder.

A solution of the secondary amine in AcOH (1 mL) was hydrogenated over 20% $Pd(OH)_2$ on carbon (20 mg) for 2 h. The mixture was filtrated through Celite and the filtrate was evaporated to dryness. The resultant residue was dissolved in water and was purified by preparative RP-HPLC with a linear gradient from 5% $H_2O$/MeCN containing 0.1% TFA to 50% $H_2O$/MeCN containing 0.1% TFA in 20 min. NPTX-473 (**4**) was eluted at 12.02 min and was obtained as a colorless TFA salt (20 mg, 60%, two steps). $[\alpha]_D^{22}$ −3.81 (*c* 0.42, $H_2O$). IR (Nujol) 3300, 2924, 1670, 1549 $cm^{-1}$. $^1H$ NMR (300 MHz, $D_2O$): $\delta$ 1.32 (m, 4H), 1.87 (quin, 2H, *J*=8.1 Hz), 1.93 (quin, 2H, *J*=6.6 Hz), 2.56 (dd, 1H, *J*=8.1, 15.3 Hz), 2.64 (dd, 1H, *J*=7.2, 15.3 Hz), 2.78 (m, 4H), 2.98 (m, 8H), 3.69 (s, 2H), 4.47 (dd, 1H, *J*=7.2, 8.1 Hz), 7.06 (t, 1H, *J*=8.1 Hz), 7.16 (t, 1H, *J*=8.1 Hz), 7.22 (s, 1H), 7.41 (d, 1H, *J*=8.1 Hz), 7.48 (d, 1H, *J*=8.1 Hz). $^{13}C$ NMR (75 MHz, $D_2O$): $\delta$ 22.6, 22.7, 23.9, 25.5, 32.4, 36.2, 36.7, 38.6, 44.1, 44.7, 45.5, 47.2, 51.3, 107.8, 112.2, 118.6, 119.8, 122.4, 125.2, 126.8, 136.5, 172.8, 174.6, 175.4. FABHRMS calcd for $C_{24}H_{39}N_7O_3$ $(M+H)^+$ 474.3193, found 474.3171. ESI-MS/MS: Supplementary data 5.

**4.1.19. *N*-(2-Nitrobenzenesulfonyl)-*N*′-(benzyloxycarbonyl)-1,4-diaminobutane.** To a cold (0 °C) and stirred suspension of *N*-(2-nitrobenzenesulfonyl)-1,4-diaminobutane[22] (**12**) (400 mg, 1.46 mmol) in $CH_2Cl_2$ (10 mL) were added CbzCl (30% in toluene, 830 mg, 1.46 mmol) and TEA (148 mg, 1.46 mmol) slowly. After being stirred for 1 h at room temperature, the reaction mixture was diluted with EtOAc (200 mL) and washed with saturated $NH_4Cl$ solution (3×50 mL) and brine (3×50 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (30–50% EtOAc/hexane) gave the title compound (564 mg, 95%) as a colorless oil. IR (film) 1703, 1540, 1442, 1364, 1252, 1166, 854 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.55 (m, 4H), 3.10 (m, 2H), 3.16 (m, 2H), 4.89 (br s, 1H), 5.08 (s, 2H), 5.46 (br s, 1H), 7.35 (m, 5H), 7.72 (m, 2H), 7.84 (m, 1H), 8.12 (m, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 26.8, 26.9, 40.3, 43.3, 66.6, 125.3, 128.0, 128.5, 131.0, 132.8, 133.6, 136.5, 148.0, 156.5. HRMS calcd for $C_{12}H_{17}N_2O_2$ ($M^+$−Ns) 221.1290, found 221.1299.

**4.1.20. Methyl 7-benzyloxycarbonylamino-3-(2-nitrobenzenesulfonyl)-3-azaheptanoate.** To a solution of the above sulfonamide (514 mg, 1.26 mmol) and methyl bromoacetate (289 mg, 1.89 mmol) in DMF (4 mL) was added $Cs_2CO_3$ (616 mg, 1.89 mmol). After being stirred for 0.5 h at 50 °C, the reaction mixture was diluted with EtOAc (150 mL) and washed with $H_2O$ (3×30 mL) and brine (3×30 mL). Aqueous layers were extracted with EtOAc (3×30 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (30–50% EtOAc/hexane) gave the ester (520 mg, 86%) as a colorless oil. IR (film) 1752, 1714, 1546, 1439, 1372, 1254, 1218, 1164, 853 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.54 (m, 4H), 3.17 (m, 2H), 3.41 (t, 2H, *J*=7.2 Hz), 3.65 (s, 3H), 4.16 (s, 2H), 5.00 (br s, 1H), 5.08 (s, 2H), 7.35 (m, 5H), 7.60 (m, 1H), 7.68 (m, 2H), 8.06 (m, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 24.8, 26.8, 40.3, 47.6, 48.1, 52.3, 66.6, 124.1, 128.0, 128.1, 128.5, 130.8, 131.7, 133.2, 133.6, 136.6, 147.9, 156.5, 162.8. HRMS calcd for $C_{13}H_{18}N_3O_6S$ ($M^+$−Cbz) 344.0917, found 344.0918.

**4.1.21. Methyl 7-benzyloxycarbonylamino-3-(2-nitrobenzenesulfonyl)-3-azaheptanoic acid (24).** To a cold (0 °C) and stirred solution of the above ester (500 mg, 1.04 mmol) in MeOH (5 mL) was added 3.0 M aqueous NaOH solution (1.04 mL, 3.12 mmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was adjusted to pH 2.0 by concentrated HCl. The resultant

mixture was diluted with EtOAc (100 mL) and washed with $H_2O$ (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (2–40% MeOH/$CH_2Cl_2$) gave the title compound **24** (414 mg, 86%) as a colorless oil. IR (film) 1729, 1714, 1548, 1455, 1371, 1259, 1162, 853 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.34 (m, 2H), 1.41 (m, 2H), 3.02 (m, 2H), 3.31 (m, 2H), 4.04 (s, 2H), 5.02 (s, 2H), 5.07 (br s, 1H), 7.29 (m, 5H), 7.54 (m, 3H), 8.00 (m, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 24.5, 26.6, 40.3, 48.1, 49.0, 66.6, 124.1, 127.9, 128.0, 128.4, 130.7, 132.0, 133.0, 133.5, 136.5, 147.7, 156.6, 174.4. HRMS calcd for $C_{14}H_{19}N_2O_4$ ($M^+$−Ns) 279.1345, found 279.1347.

**4.1.22. *N*-[$N^{\alpha}$-(Indoleacetyl)-L-asparaginyl]-$N'$-[7-benzyloxycarbonylamino-3-(2-nitrobenzenesulfonyl)-3-azaheptanoyl]-1,5-diaminopentane (26).** To a cold (0 °C) and stirred suspension of carboxylic acid **24** (186 mg, 400 μmol) and HOSu (92 mg, 799 μmol) in $CH_2Cl_2$ (5 mL) was added DCC (124 mg, 601 μmol) slowly. After being stirred for 4 h at 0 °C, the reaction mixture was filtrated through a pad of silica gel with EtOAc. The filtrate was concentrated in vacuo to give the crude succinimidyl ester as a colorless oil.

To a stirred solution of obtained ester in DMF (3 mL) was added amine **11** (127 mg, 401 μmol) slowly. After being stirred for 0.5 h at room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with saturated aqueous $NaHCO_3$ solution (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (2–8% MeOH/$CH_2Cl_2$) gave the polyamine **25** (252 mg), which was used in the next step without further purification. $^1H$ NMR (300 MHz, $CD_3OD$): $\delta$ 1.29 (m, 2H), 1.43 (s, 9H), 1.45 (m, 8H), 2.57 (dd, 1H, $J$=6.3, 13.2 Hz), 2.66 (dd, 1H, $J$=6.3, 13.2 Hz), 3.06 (t, 2H, $J$=6.6 Hz), 3.12 (t, 2H, $J$=6.0 Hz), 3.15 (t, 2H, $J$=6.6 Hz), 3.39 (t, 2H, $J$=6.6 Hz), 4.02 (s, 2H), 4.36 (t, 1H, $J$=6.3 Hz), 5.04 (s, 2H), 7.32 (m, 5H), 7.75 (m, 3H), 8.10 (m, 1H). $^{13}C$ NMR (75 MHz, $CD_3OD$): $\delta$ 25.0, 26.0, 27.9, 28.7, 29.9, 38.4, 40.3, 41.2, 50.4, 53.1, 67.3, 80.9, 125.4, 128.8, 129.0, 129.5, 131.9, 133.1, 133.9, 135.3, 138.5, 149.5, 157.5, 158.9, 170.2, 173.8, 175.1.

To a cold (0 °C) and stirred suspension of polyamine **25** (120 mg, 157 μmol) in $CHCl_3$ (1 mL) was added TFA (1 mL) slowly. After being stirred for 1 h at room temperature, the reaction mixture was concentrated in vacuo to give the crude amine TFA salt as a colorless solid.

To a stirred solution of obtained amine TFA salt in DMF (1 mL) were added TEA (16 mg, 158 μmol) and indoleacetic acid succinimide ester[54] (65 mg, 239 μmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with saturated $NaHCO_3$ solution (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (2–10% EtOH/$CH_2Cl_2$) gave the title compound **26** (113 mg, 73%, four steps) as a white powder. $[\alpha]_D^{22}$ +5.59 (*c* 2.22, DMF). IR (Nujol) 1660, 1538, 1456, 1376, 1257, 1158, 852 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.14 (m, 2H), 1.28 (m, 6H), 1.45 (m, 2H), 2.37 (dd, 1H, $J$=7.8, 15.3 Hz), 2.48 (dd, 1H, $J$=6.0, 15.3 Hz), 2.94 (m, 6H), 3.27 (m, 2H), 3.54 (s, 2H), 3.93 (s, 2H), 4.50 (dd, 1H, $J$=6.0, 7.8 Hz), 4.98 (s, 2H), 6.84 (br s, 1H), 6.94 (t, 1H, $J$=6.9 Hz), 7.05 (t, 1H, $J$=6.9 Hz), 7.18 (br s, 1H), 7.21 (t, 1H, $J$=5.4 Hz), 7.33 (m, 7H), 7.51 (d, 1H, $J$=8.1 Hz), 7.59 (t, 1H, $J$=5.4 Hz), 7.80 (m, 2H), 7.91 (m, 2H), 8.10 (m, 2H), 10.84 (s, 1H). $^{13}C$ NMR (75 MHz, DMSO-$d_6$): $\delta$ 23.7, 24.7, 26.7, 28.8, 32.7, 37.5, 38.7, 48.2, 48.7, 50.1, 65.4, 108.9, 111.5, 118.5, 118.9, 121.2, 124.0, 124.3, 127.4, 127.9, 128.0, 128.6, 130.3, 132.3, 132.4, 134.5, 136.3, 137.5, 147.8, 156.3, 167.1, 170.8, 170.9, 171.7. FABHRMS calcd for $C_{39}H_{48}N_8O_{10}SNa$ $(M+Na)^+$ 843.3112, found 843.3115.

**4.1.23. NPTX-501 (5).** To a stirred solution of polyamine **26** (40 mg, 48.7 μmol) in DMF (1 mL) were added 2-mercaptoethanol (12 mg, 154 μmol) and DBU (24 mg, 158 μmol). After being stirred for 0.5 h at room temperature, the reaction mixture was directly purified on silica gel column (2–12% EtOH/$CH_2Cl_2$, then, 7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) and then, further purification by preparative TLC (7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) gave the secondary amine as a white powder.

A solution of the secondary amine in AcOH (1 mL) was hydrogenated over 20% $Pd(OH)_2$ on carbon (20 mg) for 2 h. The mixture was filtrated through Celite and the filtrate was evaporated to dryness. The resultant residue was dissolved in water and was purified by preparative RP-HPLC with a linear gradient from 5% $H_2O$/MeCN containing 0.1% TFA to 50% $H_2O$/MeCN containing 0.1% TFA in 20 min. NPTX-501 (**5**) was eluted at 13.21 min and was obtained as a colorless TFA salt (23 mg, 65%, two steps). $[\alpha]_D^{22}$ −0.41 (*c* 1.22, $H_2O$). IR (Nujol) 3554, 2924, 1698, 1541 $cm^{-1}$. $^1H$ NMR (300 MHz, $D_2O$): $\delta$ 0.99 (quin, 2H, $J$=7.2 Hz), 1.23 (m, 4H), 1.55 (m, 4H), 2.51 (dd, 1H, $J$=7.8, 15.3 Hz), 2.60 (dd, 1H, $J$=6.0, 15.3 Hz), 2.84 (m, 4H), 2.97 (m, 4H), 3.56 (s, 2H), 3.66 (s, 2H), 4.48 (dd, 1H, $J$=6.0, 7.8 Hz), 7.03 (t, 1H, $J$=8.1 Hz), 7.13 (t, 1H, $J$=8.1 Hz), 7.19 (s, 1H), 7.38 (d, 1H, $J$=8.1 Hz), 7.44 (d, 1H, $J$=8.1 Hz). $^{13}C$ NMR (75 MHz, $D_2O$): $\delta$ 22.4, 22.9, 23.7, 27.6, 32.1, 36.1, 38.6, 39.0, 39.1, 46.8, 47.7, 50.8, 107.4, 111.9, 118.2, 119.5, 122.1, 124.8, 126.5, 136.2, 165.4, 172.1, 174.4, 174.9. FABHRMS calcd for $C_{25}H_{40}N_7O_4$ $(M+H)^+$ 502.3142, found 502.3108. ESI-MS/MS: Supplementary data 6.

**4.1.24. *N*-[$N^{\alpha}$-(*tert*-Butoxycarbonyl)-L-asparaginyl]-$N'$-[8-($N^{\alpha}$,$N^{\delta}$,$N^{\omega}$-tribenzyloxycarbonylarginyl)-amino-4-benzyloxycarbonyl-4-azaoctanoyl]-1,5-diaminopentane (27).** To a stirred solution of polyamine **14** (300 mg, 386 μmol) in DMF (1 mL) were added 2-mercaptoethanol (91 mg, 1.16 mmol) and DBU (176 mg, 1.16 mmol). After being stirred for 0.5 h at room temperature, the reaction mixture was directly subjected to silica gel chromatography (2–12% EtOH/$CH_2Cl_2$, then, 7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) to afford the secondary amine as a white powder.

To a stirred solution of obtained amine in DMF (1 mL) was added tri-Cbz-arginine succinimide ester[48] (389 mg, 577 μmol) slowly. After being stirred for 1 h at room temperature, the reaction mixture was directly subjected to silica gel chromatography (2–8% EtOH/$CH_2Cl_2$) to afford the title compound **27** (337 mg, 76%) as a white powder. $[\alpha]_D^{22}$ +6.36 (*c* 1.32, DMSO). IR (Nujol) 1725, 1688, 1652, 1259, 1103 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.25 (m, 2H), 1.35 (m, 19H), 1.55 (m, 4H), 2.29 (t, 2H, *J*=6.9 Hz), 2.35 (m, 2H), 2.98 (m, 6H), 3.15 (m, 2H), 3.42 (m, 2H), 3.84 (m, 3H), 4.16 (dd, 1H, *J*=5.2, 6.6 Hz), 4.97 (s, 2H), 5.03 (s, 4H), 5.20 (s, 2H), 6.81 (d, 1H, *J*=8.4 Hz), 6.87 (s, 1H), 7.31 (m, 20H), 7.67 (br s, 1H), 7.85 (m, 2H), 9.14 (br s, 2H). $^{13}C$ NMR (75 MHz, DMSO-$d_6$): $\delta$ 23.8, 25.3, 26.5, 28.4, 28.9, 29.5, 34.4, 35.2, 37.6, 38.5, 38.7, 43.3, 44.0, 44.6, 46.6, 46.7, 51.7, 54.8, 65.6, 66.2, 66.3, 68.4, 78.4, 127.5, 127.8, 127.9, 128.1, 128.4, 128.5, 128.6, 128.7, 135.5, 137.2, 137.3, 155.2, 155.3, 155.4, 156.1, 159.9, 163.1, 170.2, 171.4, 171.7, 171.8. FABHRMS calcd for $C_{59}H_{79}N_{10}O_{14}$ $(M+H)^+$ 1151.5778, found 1151.5778.

**4.1.25. *N*-[$N^{\alpha}$-(*tert*-Butoxycarbonyl)-L-asparaginyl]-$N'$-[8-($N^{\alpha}$,$N^{\delta}$,$N^{\omega}$-tribenzyloxycarbonylarginyl)-amino-4-benzyloxycarbonyl-4-azaoctanoyl]-1,5-diaminopentane (28).** To a cold (0 °C) and stirred suspension of polyamine **27** (100 mg, 86.9 μmol) in $CHCl_3$ (1 mL) was added TFA (1 mL) slowly. After being stirred for 1 h at room temperature, the reaction mixture was concentrated in vacuo to give the crude amine TFA salt as a colorless solid.

To a stirred solution of obtained amine TFA salt in DMF (1 mL) were added TEA (9 mg, 89.0 μmol) and 2,4-dibenzyloxyphenylacetic acid succinimide ester[28] (58 mg, 130 μmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was directly subjected to silica gel chromatography (2–8% MeOH/$CH_2Cl_2$) to afford the title compound **28** (104 mg, 87%) as a white powder. $[\alpha]_D^{22}$ +2.57 (*c* 1.05, DMF). IR (Nujol) 1691, 1664, 1645, 1614, 1260 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.17 (m, 2H), 1.31 (m, 8H), 1.50 (m, 4H), 2.29 (t, 2H, *J*=6.6 Hz), 2.36 (dd, 1H, *J*=6.3, 15.9 Hz), 2.43 (dd, 1H, *J*=6.0, 15.9 Hz), 2.96 (m, 6H), 3.14 (m, 2H), 3.27 (m, 2H), 3.41 (s, 2H), 3.84 (m, 3H), 4.53 (m, 1H), 4.97 (s, 2H), 5.03 (s, 6H), 5.08 (s, 2H), 5.20 (s, 2H), 6.52 (d, 1H, *J*=9.3 Hz), 6.68 (s, 1H), 6.85 (s, 1H), 7.07 (d, 1H, *J*=8.4 Hz), 7.35 (m, 32H), 7.60 (t, 1H, *J*=7.2 Hz), 7.83 (m, 2H), 7.96 (d, 1H, *J*=8.1 Hz)), 9.14 (br s, 2H). $^{13}C$ NMR (75 MHz, DMSO-$d_6$): $\delta$ 22.8, 23.8, 25.3, 26.5, 28.8, 28.9, 29.5, 36.4, 37.5, 38.5, 38.6, 38.7, 44.6, 46.5, 49.4, 50.0, 54.7, 65.6, 66.2, 66.3, 68.3, 69.4, 69.5, 100.7, 105.9, 117.3, 127.3, 127.5, 127.9, 128.0, 128.6, 128.7, 131.1, 135.5, 137.2, 137.3, 137.4, 155.1, 155.3, 156.1, 157.1, 158.5, 159.9, 163.1, 170.1, 170.5, 170.8, 171.6, 171.7. FABHRMS calcd for $C_{76}H_{88}N_{10}O_{15}Na$ $(M+Na)^+$ 1403.6328, found 1403.6323.

**4.1.26. NSTX-3 (6).** A solution of the polyamine **28** (26 mg, 18.8 μmol) in AcOH (1 mL) was hydrogenated over 20% $Pd(OH)_2$ on carbon (20 mg) for 4 h. The mixture was filtrated through Celite and the filtrate was evaporated to dryness. The resultant residue was dissolved in water and was purified by preparative RP-HPLC with a linear gradient from 5% $H_2O$/MeCN containing 0.1% TFA to 50% $H_2O$/MeCN containing 0.1% TFA in 20 min. NSTX-3 (**6**) was eluted at 9.66 min and was obtained as a colorless TFA salt (14 mg, 74%). $[\alpha]_D^{22}$ +2.95 (*c* 1.73, $H_2O$). IR (Nujol) 3444, 2925, 1696, 1523 $cm^{-1}$. $^1H$ NMR (300 MHz, $D_2O$): $\delta$ 1.03 (quin, 2H, *J*=7.5 Hz), 1.29 (m, 4H), 1.52 (m, 6H), 1.77 (dt, 2H, *J*=9.6, 6.9 Hz), 2.50 (t, 2H, *J*=6.9 Hz), 2.52 (dd, 1H, *J*=7.8, 15.0 Hz), 2.63 (dd, 1H, *J*=5.7, 15.0 Hz), 2.93 (t, 4H, *J*=8.1 Hz), 3.08 (t, 4H, *J*=6.9 Hz), 3.13 (t, 4H, *J*=6.9 Hz), 3.34 (d, 1H, *J*=15.3 Hz), 3.43 (d, 1H, *J*=15.3 Hz), 3.81 (t, 1H, *J*=6.9 Hz), 4.46 (dd, 1H, *J*=5.7, 7.8 Hz), 6.31 (m, 2H), 6.94 (d, 1H, *J*=9.0 Hz). $^{13}C$ NMR (75 MHz, $D_2O$): $\delta$ 22.8, 23.0, 23.5, 25.3, 27.6, 27.7, 27.9, 30.8, 35.9, 36.8, 38.7, 39.1, 39.2, 40.2, 43.3, 46.9, 50.8, 52.9, 102.7, 107.4, 113.6, 132.3, 155.1, 156.1, 156.7, 169.2, 171.5, 172.2, 174.6. FABHRMS calcd for $C_{30}H_{53}N_{10}O_7$ $(M+H)^+$ 665.4107, found 665.4083. ESI-MS/MS: Supplementary data 7.

**4.1.27. Methyl 7-(2-nitrobenzenesulfonyl)-amino-4-benzyloxycarbonyl-4-azahenpanoate.** A solution of methyl acrylate (166 mg, 1.93 mmol) in EtOH (10 mL) was added at room temperature over 5 h to a stirring solution of *N*-(2-nitrobenzenesulfonyl)-1,3-diaminopropane[18] (**20**) (500 mg, 1.93 mmol) in EtOH (20 mL). The solvent was evaporated in vacuo, and the residue was subjected to silica gel chromatography (5–20% MeOH/$CH_2Cl_2$) to give the crude secondary amine as a colorless oil.

To a cold (0 °C) and stirred solution of obtained secondary amine in $CH_2Cl_2$ (20 mL) were added CbzCl (30% in toluene, 1.10 g, 1.93 mmol) and $Et_3N$ (195 mg, 1.93 mmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was diluted with EtOAc (200 mL) and washed with saturated $NH_4Cl$ solution (3×50 mL) and brine (3×50 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (20–50% EtOAc/hexane) gave the methyl ester (796 mg, 86%, two steps) as a colorless oil. IR (film) 1732, 1695, 1539, 1480, 1368, 1125, 854 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.73 (quin, 2H, *J*=6.6 Hz), 2.57 (m, 2H), 3.11 (m, 2H), 3.37 (t, 2H, *J*=6.3 Hz), 3.49 (t, 2H, *J*=7.2 Hz), 3.64 (s, 3H), 5.12 (s, 2H), 5.44 (br s, 1H), 7.33 (m, 5H), 7.72 (m, 2H), 7.83 (m, 1H), 8.09 (m, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 28.5, 33.6, 40.6, 43.1, 44.7, 51.8, 67.4, 125.2, 127.9, 128.1, 128.5, 130.7, 132.6, 133.3, 134.2, 136.3, 148.0, 155.6, 171.9. HRMS calcd for $C_{20}H_{22}N_3O_7S$ ($M^+-OMe$) 448.1179, found 448.1183.

**4.1.28. 7-(2-Nitrobenzenesulfonyl)-amino-4-benzyloxycarbonyl-4-azahenpanoic acid (29).** To a cold (0 °C) and stirred solution of the above ester (600 mg, 1.25 mmol) in MeOH (4 mL) was added 3.0 M aqueous NaOH solution (1.25 mL, 3.75 mmol) slowly. After being stirred for 1 h at room temperature, the reaction mixture was adjusted to pH 2.0 by concentrated HCl. The resultant mixture was diluted with EtOAc (200 mL) and washed with $H_2O$ (3×50 mL) and brine (3×50 mL). Aqueous layers were extracted with EtOAc (3×40 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (1–40% MeOH/$CH_2Cl_2$) gave the title compound **29** (494 mg, 85%) as a colorless oil. IR (film) 1694, 1540, 1484, 1424, 1365, 1166, 854 $cm^{-1}$.

$^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.75 (quin, 2H, $J$=6.6 Hz), 2.59 (m, 2H), 3.09 (m, 2H), 3.37 (m, 2H), 3.50 (t, 2H, $J$=6.6 Hz), 5.12 (s, 2H), 5.47 (br s, 1H), 7.33 (m, 5H), 7.70 (m, 2H), 7.83 (m, 1H), 8.08 (m, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 28.4, 33.4, 40.7, 42.9, 43.7, 67.4, 125.2, 127.8, 128.0, 128.5, 130.7, 132.6, 133.3, 136.2, 147.9, 156.5, 175.9. HRMS calcd for $C_{12}H_{16}N_3O_6S$ ($M^+-Na$) 330.0760, found 330.0757.

**4.1.29. *N*-[$N^{\alpha}$-(*tert*-Butoxycarbonyl)-L-asparaginyl]-*N*′-[7-(2-nitrobenzenesulfonylamino)-4-benzyloxycarbonyl-4-azaheptanoyl]-1,5-diaminopentane (30).** To a cold (0 °C) and stirred suspension of carboxylic acid **29** (377 mg, 810 μmol) and HOSu (186 mg, 1.62 mmol) in $CH_2Cl_2$ (5 mL) was added DCC (252 mg, 1.22 mmol) slowly. After being stirred for 5 h at 0 °C, the reaction mixture was filtrated through a pad of silica gel with EtOAc. The filtrate was concentrated in vacuo to give the crude succinimidyl ester as a colorless oil.

To a stirred solution of obtained ester in DMF (6 mL) was added amine **11** (256 mg, 0.81 mmol) slowly. After being stirred for 0.5 h at room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with saturated aqueous $NaHCO_3$ solution (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (2–10% EtOH/$CH_2Cl_2$) gave the title compound **30** (505 mg, 82%, two steps) as a colorless solid. $[\alpha]_D^{24}$ −2.53 (*c* 2.96, MeOH). IR (Nujol) 1685, 1636, 1540, 1480, 1367, 1164, 854 cm$^{-1}$. $^1$H NMR (300 MHz, $CD_3OD$): $\delta$ 1.26 (m, 2H), 1.39 (s, 9H), 1.43 (m, 4H), 1.69 (quin, 2H, $J$=7.2 Hz), 2.36 (t, 2H, $J$=6.6 Hz), 2.52 (dd, 1H, $J$=5.7, 16.2 Hz), 2.59 (dd, 1H, $J$=5.7, 16.2 Hz), 2.84 (m, 2H), 3.07 (m, 2H), 3.13 (t, 2H, $J$=6.6 Hz), 3.26 (m, 2H), 3.44 (m, 2H), 4.32 (dd, 1H, $J$=5.1, 5.7 Hz), 5.05 (s, 2H), 7.28 (m, 5H), 7.75 (m, 3H), 7.99 (m, 1H). $^{13}$C NMR (75 MHz, $CD_3OD$): $\delta$ 25.1, 28.7, 29.8, 29.9, 35.8, 36.4, 38.4, 40.3, 41.8, 41.9, 45.0, 46.2, 53.1, 63.4, 80.9, 125.9, 128.9, 129.1, 129.6, 131.5, 133.6, 134.8, 135.0, 138.1, 150.0, 157.5, 158.1, 173.5, 173.8, 175.1. FABHRMS calcd for $C_{34}H_{49}N_7O_{11}SNa$ ($M^+$+Na) 786.3129, found 786.3119.

**4.1.30. *N*-[$N^{\alpha}$-(4-Benzyloxyphenylacetyl)-L-asparaginyl]-*N*′-[7-(2-nitrobenzenesulfonylamino)-4-benzyloxycarbonyl-4-azaheptanoyl]-1,5-diaminopentane (31).** To a cold (0 °C) and stirred suspension of polyamine **30** (170 mg, 223 μmol) in $CHCl_3$ (1 mL) was added TFA (1 mL) slowly. After being stirred for 1 h at room temperature, the reaction mixture was concentrated in vacuo to give the crude amine TFA salt as a colorless solid.

To a stirred solution of obtained amine TFA salt in DMF (1 mL) were added $Et_3N$ (23 mg, 228 μmol) and 4-benzyloxyphenylacetic acid succinimide ester[31] (114 mg, 336 μmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with saturated $NaHCO_3$ solution (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (2–10% EtOH/$CH_2Cl_2$) gave the title compound **31** (174 mg, 88%, two steps) as a white powder. $[\alpha]_D^{22}$ −3.88 (*c* 2.55, DMF). IR (Nujol) 1641, 1542, 1457, 1376, 1239, 1164, 853 cm$^{-1}$. $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.14 (m, 2H), 1.32 (m, 4H), 1.62 (m, 2H), 2.27 (t, 2H, $J$=7.2 Hz), 2.34 (dd, 1H, $J$=7.8, 15.6 Hz), 2.43 (dd, 1H, $J$=6.3, 15.6 Hz), 2.87 (m, 2H), 2.96 (m, 4H), 3.18 (m, 2H), 3.36 (m, 4H), 4.47 (dd, 1H, $J$=6.3, 7.8 Hz), 5.02 (s, 2H), 5.05 (s, 2H), 6.85 (br s, 1H), 6.90 (d, 2H, $J$=8.4 Hz), 7.14 (d, 2H, $J$=8.4 Hz), 7.32 (m, 10H), 7.41 (m, 1H), 7.67 (t, 1H, $J$=5.7 Hz), 7.84 (m, 3H), 7.95 (m, 2H), 8.07 (br s, 1H), 8.15 (d, 1H, $J$=8.4 Hz). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 23.8, 28.0, 28.8, 28.9, 34.3, 35.0, 37.6, 38.6, 38.7, 40.7, 41.4, 43.5, 44.8, 50.1, 66.3, 69.4, 114.7, 124.6, 127.5, 127.8, 127.9, 128.0, 128.5, 128.6, 129.6, 130.3, 132.7, 132.8, 134.3, 137.2, 137.4, 148.0, 157.1, 170.1, 170.5, 170.9, 171.6. FABHRMS calcd for $C_{44}H_{54}N_7O_{11}S$ $(M+H)^+$ 888.3602, found 888.3604.

**4.1.31. Joramine (7).** To a stirred solution of polyamine **31** (40 mg, 45.1 μmol) in DMF (1 mL) were added 2-mercaptoethanol (11 mg, 141 μmol) and DBU (22 mg, 145 μmol). After being stirred for 0.5 h at room temperature, the reaction mixture was directly purified on silica gel column (2–12% EtOH/$CH_2Cl_2$, then, 7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) and then, further purification by preparative TLC (7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) gave the primary amine as a white powder.

A solution of the secondary amine in AcOH (1 mL) was hydrogenated over 20% $Pd(OH)_2$ on carbon (20 mg) for 3 h. The mixture was filtrated through Celite and the filtrate was evaporated to dryness. The resultant residue was dissolved in water and was purified by preparative RP-HPLC with a linear gradient from 5% $H_2O$/MeCN containing 0.1% TFA to 50% $H_2O$/MeCN containing 0.1% TFA in 20 min. Joramine (**7**) was eluted at 10.24 min and was obtained as a colorless TFA salt (22 mg, 69%). $[\alpha]_D^{22}$ −10.29 (*c* 0.35, $H_2O$). IR (Nujol) 3479, 2924, 1696, 1515 cm$^{-1}$. $^1$H NMR (300 MHz, $D_2O$): $\delta$ 1.05 (quin, 2H, $J$=6.9 Hz), 1.28 (quin, 4H, $J$=6.9 Hz), 1.96 (quin, 2H, $J$=8.1 Hz), 2.53 (t, 2H, $J$=6.9 Hz), 2.57 (dd, 1H, $J$=8.1, 15.3 Hz), 2.64 (dd, 1H, $J$=6.0, 15.3 Hz), 3.00 (m, 8H), 3.18 (t, 2H, $J$=6.9 Hz), 3.42 (s, 2H), 4.46 (dd, 1H, $J$=6.0, 8.1 Hz), 6.74 (d, 2H, $J$=8.1 Hz), 7.05 (d, 2H, $J$=8.1 Hz). $^{13}$C NMR (75 MHz, $D_2O$): $\delta$ 22.8, 23.3, 27.4, 27.5, 30.6, 36.0, 36.2, 38.9, 39.0, 41.0, 43.4, 44.2, 50.7, 115.4, 126.4, 130.2, 154.4, 171.2, 171.9, 174.2, 174.6. FABHRMS calcd for $C_{23}H_{38}N_6O_5$ $(M+H)^+$ 479.3008, found 479.2959. ESI-MS/MS: Supplementary data 8.

**4.1.32. 7-Allyloxycarbonylamino-4-(2-nitrobenzenesulfonyl)-4-azaheptan-1-yl bromide (32).** To a solution of *N*-(2-nitrobenzenesulfonyl)-*N*′-(allyloxycarbonyl)-1,3-diaminopropane (600 mg, 1.75 mmol), which was prepared from sulfonamide **20** in 93% yield,[18] and 1,3-dibromopropane (1.06 g, 5.20 mmol) in DMF (10 mL) was added $Cs_2CO_3$ (855 mg, 2.62 mmol). After being stirred for 0.5 h at 50 °C, the reaction mixture was diluted with EtOAc (200 mL) and washed with $H_2O$ (3×40 mL) and brine (3×40 mL). Aqueous layers were extracted with EtOAc (3×50 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (20–40% EtOAc/hexane) gave the title

compound **32** (730 mg, 90%) as a colorless oil. IR (film) 1715, 1546, 1440, 1373, 1247, 1162, 931, 852 $cm^{-1}$. $^1H$ NMR (300 MHz, $CDCl_3$): $\delta$ 1.80 (quin, 2H, $J$=6.3 Hz), 2.10 (quin, 2H, $J$=6.3 Hz), 3.25 (q, 2H, $J$=6.3 Hz), 3.40 (m, 6H), 4.56 (d, 2H, $J$=5.4 Hz), 5.08 (br s, 1H), 5.22 (dd, 1H, $J$=1.8, 10.8 Hz), 5.31 (dd, 1H, $J$=1.8, 15.9 Hz), 5.92 (ddd, 1H, $J$=5.4, 10.8, 15.9 Hz), 7.66 (m, 1H), 7.72 (m, 2H), 8.04 (m, 1H). $^{13}C$ NMR (75 MHz, $CDCl_3$): $\delta$ 28.1, 29.7, 31.1, 37.5, 45.3, 45.6, 65.4, 117.5, 124.2, 130.9, 131.6, 132.7, 133.6, 147.9, 156.2. HRMS calcd for $C_{10}H_{18}N_2O_2Br$ ($M^+-Ns$) 277.0551, found 277.0564.

**4.1.33. *N*-($N^{\alpha}$-*tert*-Butoxycarbonyl-L-asparaginyl)-12-($N^{\alpha}$,$N^{\delta}$,$N^{\omega}$-tribenzyloxycarbonylarginyl)-amino-6,10-[di-(2-nitrobenzenesulfonyl)]-6,10-diaza-1-aminoundecane (34).** To a solution of sulfonamide **10** (200 mg, 399 μmol) and bromide **32** (278 mg, 599 μmol) in DMF (2 mL) were added $Cs_2CO_3$ (391 mg, 1.20 mmol) and TBAI (15.0 mg, 40.6 μmol). After being stirred for 1 h at 70 °C, the reaction mixture was diluted with EtOAc (150 mL) and washed with $H_2O$ (3×30 mL) and brine (3×30 mL). Aqueous layers were extracted with EtOAc (3×30 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (2–4% MeOH/$CH_2Cl_2$) gave the title compound **33** (332 mg), which was used in the next step without further purification. $^1H$ NMR (300 MHz, $CD_3COCD_3$): $\delta$ 1.27 (m, 2H), 1.40 (s, 9H), 1.48 (m, 4H), 1.78 (quin, 2H, $J$=6.9 Hz), 1.86 (quin, 2H, $J$=7.2 Hz), 2.59 (dd, 1H, $J$=6.6, 15.9 Hz), 2.73 (dd, 1H, $J$=4.8, 15.9 Hz), 3.16 (m, 4H), 3.36 (m, 8H), 4.35 (dd, 1H, $J$=4.8, 6.6 Hz), 4.50 (d, 2H, $J$=5.4 Hz), 5.14 (dd, 1H, $J$=1.2, 10.8 Hz), 5.27 (dd, 1H, $J$=1.2, 16.2 Hz), 5.92 (ddd, 1H, $J$=5.4, 10.8, 16.2 Hz), 6.36 (m, 3H), 6.98 (br s, 1H), 7.33 (br s, 1H), 7.87 (m, 6H), 8.06 (m, 2H). $^{13}C$ NMR (75 MHz, $CD_3COCD_3$): $\delta$ 24.6, 28.3, 28.8, 28.9, 30.1, 38.4, 39.3, 39.8, 46.1, 46.3, 46.7, 48.8, 52.7, 65.8, 79.9, 117.5, 125.5, 125.6, 131.5, 133.3, 133.4, 135.1, 135.4, 135.5, 149.5, 156.6, 157.4, 172.3, 173.9.

To a cold (0 °C) and stirred solution of polyamine **33** (135 mg, 153 μmol) in $CH_2Cl_2$ (4 mL) were added $PPh_3$ (8 mg, 30.5 μmol), $Pd(PPh_3)_4$ (9 mg, 7.8 μmol), and pyrrolidine (54 mg, 759 μmol). After being stirred for 0.5 h at room temperature, the reaction mixture was concentrated in vacuo. The residue was subjected to silica gel chromatography (2–10% EtOH/$CH_2Cl_2$, then 7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) to afford the primary amine as a colorless oil.

To a stirred solution of amine obtained in DMF (1 mL) was added tri-Cbz-arginine succinimide ester[48] (154 mg, 229 μmol) slowly. After being stirred for 1 h at room temperature, the reaction mixture was diluted with EtOAc (100 mL) and washed with saturated $NaHCO_3$ solution (3×20 mL) and brine (3×20 mL). Aqueous layers were extracted with EtOAc (3×20 mL) and combined organic layers were dried over $Na_2SO_4$. Filtration and concentration followed by chromatography on silica gel (2–6% MeOH/$CH_2Cl_2$) gave the title compound **34** (146 mg, 66%, three steps from **10**) as a white powder. $[\alpha]_D^{22}$ +3.06 (*c* 1.11, DMSO). IR (Nujol) 1727, 1681, 1646, 1544, 1456, 1376, 1255, 1162, 852 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.09 (m, 2H), 1.34 (m, 13H), 1.55 (m, 8H), 2.32 (dd, 1H, $J$=6.3, 15.0 Hz), 2.39 (dd, 1H, $J$=6.0, 15.0 Hz), 2.69 (m, 4H), 3.17 (m, 8H), 3.84 (m, 3H), 4.16 (dd, 1H, $J$=6.0, 6.3 Hz), 4.97 (s, 2H), 5.02 (s, 2H), 5.20 (s, 2H), 6.81 (d, 1H, $J$=7.5 Hz), 6.86 (br s, 1H), 7.32 (m, 17H), 7.67 (br s, 1H), 7.87 (m, 9H), 9.14 (br s, 2H). $^{13}C$ NMR (75 MHz, DMSO-$d_6$): $\delta$ 23.3, 25.3, 26.9, 27.5, 28.3, 28.4, 28.7, 29.3, 36.2, 37.6, 38.6, 44.6, 44.8, 45.0, 45.6, 47.3, 51.7, 54.8, 65.6, 66.3, 68.4, 78.3, 124.5, 124.6, 127.8, 127.9, 128.1, 128.4, 128.5, 128.7, 129.9, 131.8, 131.9, 132.7, 134.7, 134.8, 135.5, 137.2, 137.3, 147.7, 155.2, 155.3, 156.2, 159.9, 163.1, 171.4, 171.8. FABHRMS calcd for $C_{62}H_{78}N_{12}O_{19}S_2Na$ $(M+Na)^+$ 1381.4845, found 1381.4835.

**4.1.34. *N*-($N^{\alpha}$-2,4-Dibenzyloxyphenylacetyl-L-asparaginyl)-12-($N^{\alpha}$,$N^{\delta}$,$N^{\omega}$-tribenzyloxycarbonylarginyl)-amino-6,10-[di-(2-nitrobenzenesulfonyl)]-6,10-diaza-1-aminoundecane (35).** To a cold (0 °C) and stirred suspension of polyamine **34** (90 mg, 66.2 μmol) in $CHCl_3$ (1 mL) was added TFA (1 mL) slowly. After being stirred for 1 h at room temperature, the reaction mixture was concentrated in vacuo to give the crude amine TFA salt as a colorless solid.

To a stirred solution of obtained amine TFA salt in DMF (1 mL) were added $Et_3N$ (7 mg, 69.2 μmol) and 2,4-dibenzyloxyphenylacetic acid succinimide ester[28] (45 mg, 101 μmol) slowly. After being stirred for 2 h at room temperature, the reaction mixture was directly subjected to silica gel chromatography (2–10% MeOH/$CH_2Cl_2$) to afford the title compound **35** (84 mg, 80%, two steps) as a white powder. $[\alpha]_D^{24}$ −0.43 (*c* 0.70, DMSO). IR (Nujol) 1721, 1658, 1545, 1457, 1377, 1262, 1162, 852 $cm^{-1}$. $^1H$ NMR (300 MHz, DMSO-$d_6$): $\delta$ 1.06 (m, 2H), 1.23 (m, 2H), 1.33 (m, 2H), 1.54 (m, 8H), 2.35 (dd, 1H, $J$=7.2, 15.0 Hz), 2.42 (dd, 1H, $J$=6.3, 15.0 Hz), 2.89 (m, 2H), 2.97 (m, 2H), 3.16 (m, 8H), 3.37 (m, 2H), 3.83 (m, 3H), 4.51 (m, 1H), 4.96 (s, 2H), 5.01 (s, 2H), 5.02 (s, 2H), 5.07 (s, 2H), 5.18 (s, 2H), 6.51 (s, 2H), 6.67 (br s, 1H), 6.85 (br s, 1H), 7.07 (m, 2H), 7.33 (m, 26H), 7.61 (m, 1H), 7.85 (m, 10H), 9.14 (br s, 2H). $^{13}C$ NMR (75 MHz, DMSO-$d_6$): $\delta$ 23.4, 25.3, 26.8, 27.5, 28.2, 28.7, 29.3, 36.2, 36.4, 37.5, 38.7, 44.6, 44.7, 45.0, 45.6, 47.3, 50.0, 54.8, 65.6, 66.3, 68.4, 69.4, 69.5, 127.3, 127.8, 127.9, 128.0, 128.1, 128.5, 128.6, 128.7, 129.9, 131.2, 131.8, 131.9, 132.7, 134.7, 134.8, 135.5, 137.2, 137.3, 137.4, 147.7, 155.2, 156.2, 157.1, 158.5, 160.0, 163.1, 170.5, 170.9, 171.7, 171.8. FABHRMS calcd for $C_{79}H_{88}N_{12}O_{20}S_2Na$ $(M+Na)^+$ 1611.5577, found 1611.5571.

**4.1.35. Argiotoxin-636 (8).** To a stirred solution of polyamine **35** (40 mg, 25.2 μmol) in DMF (1 mL) were added 2-mercaptoethanol (39 mg, 499 μmol) and DBU (77 mg, 506 μmol). After being stirred for 0.5 h at room temperature, the reaction mixture was directly purified on silica gel column (2–12% EtOH/$CH_2Cl_2$, then, 7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) and then, further purification by preparative TLC (7.5% MeOH/$CHCl_3$ containing 2.5% *i*-$PrNH_2$) gave the secondary amine as a white powder.

A solution of the secondary amine in AcOH (1 mL) was hydrogenated over 20% $Pd(OH)_2$ on carbon (20 mg) for 2 h. The mixture was filtrated through Celite and the filtrate was evaporated to dryness. The resultant residue was dissolved in water and was purified by preparative RP-HPLC with a linear gradient from 5% $H_2O$/MeCN containing

0.1% TFA to 50% $H_2O$/MeCN containing 0.1% TFA in 20 min. Arg-636 (**8**) was eluted at 9.06 min and was obtained as a colorless TFA salt (14 mg, 51%, two steps). $[\alpha]_D^{22}$ +4.69 (*c* 0.98, $H_2O$). IR (Nujol) 3394, 2923, 1674 $cm^{-1}$. $^1H$ NMR (300 MHz, $D_2O$): $\delta$ 1.07 (quin, 2H, *J*=7.2 Hz), 1.32 (m, 2H), 1.45 (quin, 2H, *J*=7.2 Hz), 1.50 (quin, 2H, *J*=7.2 Hz), 1.77 (m, 4H), 1.95 (m, 2H), 2.56 (dd, 1H, *J*=6.9, 15.0 Hz), 2.62 (dd, 1H, *J*=6.9, 15.0 Hz), 2.95 (m, 8H), 3.10 (m, 6H), 3.33 (d, 1H, *J*=15.6 Hz), 3.43 (d, 1H, *J*=15.6 Hz), 3.83 (t, 1H, *J*=7.2 Hz), 4.44 (t, 1H, *J*=6.9 Hz), 6.30 (m, 2H), 6.95 (d, 1H, *J*=8.7 Hz). $^{13}C$ NMR (75 MHz, $D_2O$): $\delta$ 22.5, 22.7, 23.5, 24.9, 25.3, 27.5, 27.9, 35.8, 36.4, 36.8, 38.8, 40.2, 44.1, 44.4, 45.2, 47.5, 50.9, 52.9, 102.7, 107.4, 113.7, 132.4, 155.1, 156.1, 156.7, 169.6, 172.4, 174.6, 174.9. FABHRMS calcd for $C_{29}H_{53}N_{10}O_6$ ($M^+$+H) 665.4162, found 665.4127. ESI-MS/MS: Supplementary data 9.

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## Supplementary data

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