Study on the Structure Activity Relationships of NPTX-594, a Spider Toxin Belonging to the Type-B Acylpolyamine Structure#

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In order to elucidate the structure activity relationships of the spider toxin termed NPTX-594, eleven toxin analogs were designed and synthesized, and their paralytic activities against cricket were tested. As a result of the present study, it was clarified that the Lys residue binding to the 1-amino group of 4,8-diaza-1,12-dodecanediamine (Dada) in the molecule of NPTX-594 is not an essential requisite for toxicity, and can be replaced with neutral or basic amino acids without any considerable loss of the activity. However, the replacement of the Lys residue with acidic amino acid residues, such as Asp or Glu, resulted in an extreme loss of the biological activity.

Spider venom contains many structural types of acylpolyamine toxins[1–15] that are known to be potent and specific blockers against the glutamate receptor.[16] We recently synthesized a novel spider toxin termed NPTX-594 (**1**) (Fig. 1) isolated from *Nephila madagascariencis*, a Madagascar Joro spider, or from *Nephila clavipes*, a Brazilian Joro spider.[17] NPTX-594 is comprised of four residues, i.e., 2,4-dihydroxyphenylacetic acid (Dhpa), Asn, 4,8-diaza-1,12-dodecanediamine (Dada), and Lys.[18] Acylpolyamine spider toxins found in nature are classified into six structural types.[15] NPTX-594 containing Dada as the polyamine component belongs to the structural type-B. In order to elucidate the structure–activity relationships of this novel toxin, we focused on the role of the Lys residue, which binds to the 1-amino group of Dada, to exhibit biological activity. For this purpose, eleven NPTX-594 analogs, such as **2**–**12** (Fig. 2), were designed and synthesized, i.e., the Lys residue was deleted or replaced with several basic, neutral, or acidic amino acid residues.

Results and Discussion

In acylpolyamine spider toxins, the putreanine [Pua = *N*-(4-aminobutyl)-β-alanine or 8-amino-4-azaoctanoic acid] or the Arg residue instead of the Lys residue generally binds to one side of the amino groups in polyamine unit.[19] In the present study we first designed four analogs with the Pua, two Lys equivalents such as *N*-(3-aminopropyl)-β-alanine (Apa = 7-amino-4-azaheptanoic acid) and *N*-(4-aminobutyl)glycine (Abg = 7-amino-3-azaheptanoic acid), and Arg residues at the place of the Lys residue. For this purpose, three unusual basic amino acids, i.e., Pua, Apa, and Abg, were prepared, as shown in Schemes 1–3.

Schemes 1 and 2 show the routes to prepare Pua and Apa based on the Michael reaction between methyl acrylate and amino alcohols **13** and **19** to construct the backbone structures, **14** and **20**, of the corresponding diamino acids.[19,20] The hydoxy groups of both compounds were converted into the amino group via the azide compounds, **15** and **21**, respectively. These diamino acids were finally prepared as the active esters, **18** and **24**, for the coupling with **33** that is the segment corresponding to des-Lys-(NPTX-594). The Abg derivative was prepared by employing the conventional reductive *N*-alkylation method between monoprotected diamine **27**[21,22] and glyoxylic acid, as shown in Scheme 3; this amino acid was also converted to the active ester **29**.

The synthetic routes of NPTX-594 analogs **2**–**12** are shown in Scheme 4. The Boc group in the molecule of **30**,[17] which is a fully protected Dada residue, as the key component for the synthesis of all analogs, was removed by a conventional method. The Asn and Dhpa[20,23,24] residues were then successively introduced to the freed amino terminal side of the Dada residue by means of the active ester method, and the compound **32** was obtained as the common intermediate for the synthesis of all analogs.

Des-Lys-(NPTX-594) (**2**) was first obtained from **32** by suc-

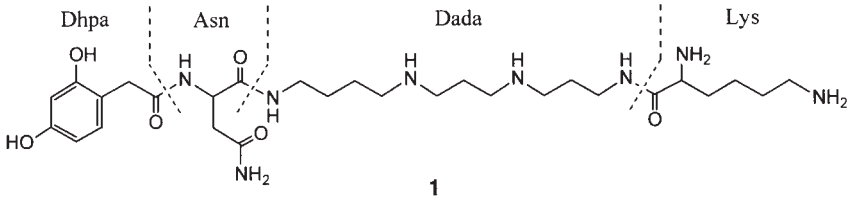

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Fig. 1. The structure of NPTX-594.

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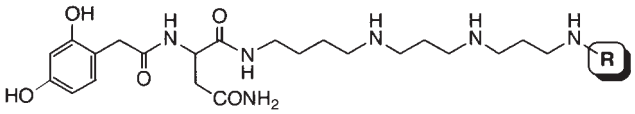

Analogs	R
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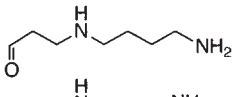
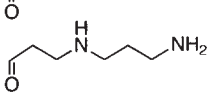
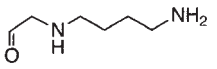
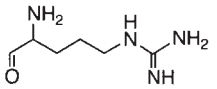
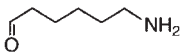
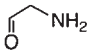
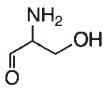
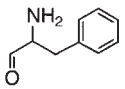
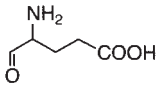
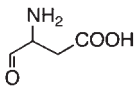
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	

Fig. 2. NPTX-594 analogs designed and synthesized in the present study.

cessive removal of the Troc and the benzyl-type protecting groups. Other analogs **3**–**12** were prepared by the coupling of the corresponding *N*-Z-amino acid succinimidyl esters[25] with **33** obtained from the intermediate **32**. All of the protecting groups in the coupling products **41**–**50** were removed by catalytic hydrogenation in acidic medium, and purified by preparative RPHPLC.

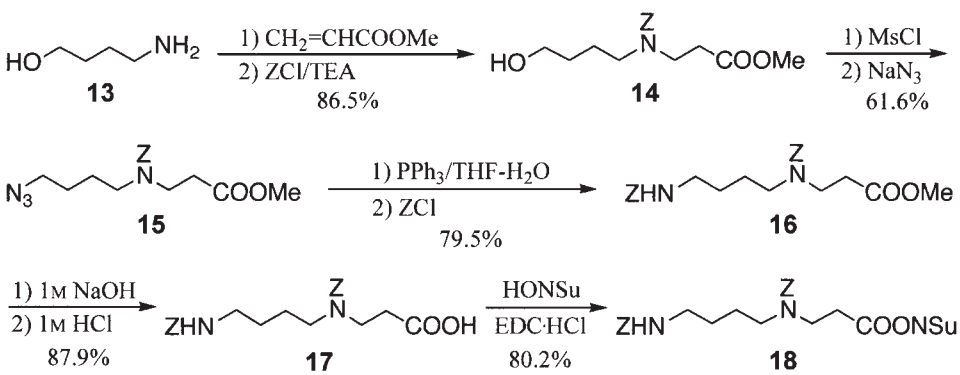
The biological activities of synthetic acylpolyamines **2**–**12** obtained as mentioned above were evaluated by paralysis to crickets (*Grillus bimaculatus*). The ED_{50} values of these analogs are given in Table 1. So far as we examined, the analogs in which the Lys residue is replaced with the basic amino acid residues show comparable biological activities to NPTX-594.[26] On the other hand, replacing the Lys residue with the acidic amino acid residues resulted in an extreme loss of biological activity. As a result of the present study, it is possible to suggest that the Lys residue in NPTX-594 is not an essential requisite for toxicity. In particular, it is noteworthy that the Lys residue is replaceable with neutral amino acid residues.[27] This fact seems to be quite valuable from the standpoint of the design of fluorescence-labeled analogs or analogs for photoaffinity-labeling to elucidate the mode of action of NPTX-594 as a blocker against the glutamate receptor.

Table 1. Biological Activities (Cricket Bioassay) of NPTX-594 and Its Analogs

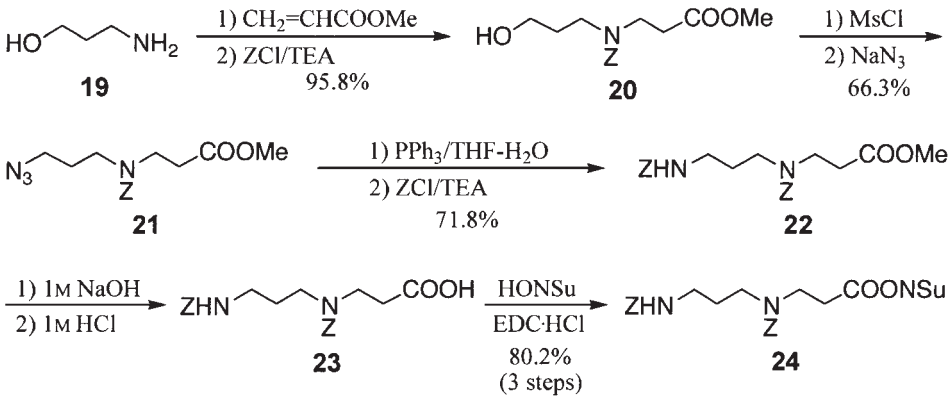
Compounds		ED_{50}/nmol g^{-1} a)
NPTX-594	(**1**)	0.64 ± 0.50
Des-Lys-(NPTX-594)	(**2**)	3.73 ± 3.32
Pua-[Des-Lys-(NPTX-594)]	(**3**)	1.74 ± 1.56
Apa-[Des-Lys-(NPTX-594)]	(**4**)	2.47 ± 2.47
Abg-[Des-Lys-(NPTX-594)]	(**5**)	0.20 ± 0.22
Arg-[Des-Lys-(NPTX-594)]	(**6**)	2.02 ± 1.65
Acp-[Des-Lys-(NPTX-594)]	(**7**)	5.24 ± 3.52
Gly-[Des-Lys-(NPTX-594)]	(**8**)	4.87 ± 9.51
Ser-[Des-Lys-(NPTX-594)]	(**9**)	3.72 ± 1.34
Phe-[Des-Lys-(NPTX-594)]	(**10**)	4.53 ± 2.28
Glu-[Des-Lys-(NPTX-594)]	(**11**)	67.4 ± 47.8
Asp-[Des-Lys-(NPTX-594)]	(**12**)	110 ± 100

a) Crickets (*Grillus bimaculatus*) were injected intrathoracically between the second and third pair of legs, with 5 μL of five different doses of each toxin previously dissolved in Milli-Q water (18.2 MΩ cm). The ED_{50} value for the toxins is given in nanomole (nmol) of toxin per g of cricket, which represents the effective dose to paralyze 50% of treated crickets at 5 min after injection. The inability of crickets to upturn when they were placed on their back was employed as the criterion for paralysis. The ED_{50} value was obtained by probit analysis of data from three groups of 5 crickets.

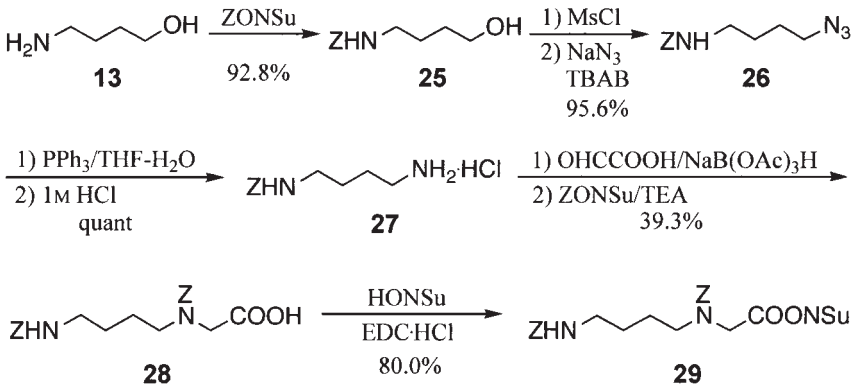
During the course of the present study, we realized that analogs **2**, **3**, and **6** might be the same compounds as NPTX-466, NPTX-608, and NPTX-622 found in the venom of several spiders, respectively. A detailed comparison of these synthetic analogs with natural compounds by mass spectrometry is current-



Scheme 1. Preparation of the active ester derivative of Pua.



Scheme 2. Preparation of the active ester derivative of Apa.



Scheme 3. Preparation of the active ester derivative of Abg.

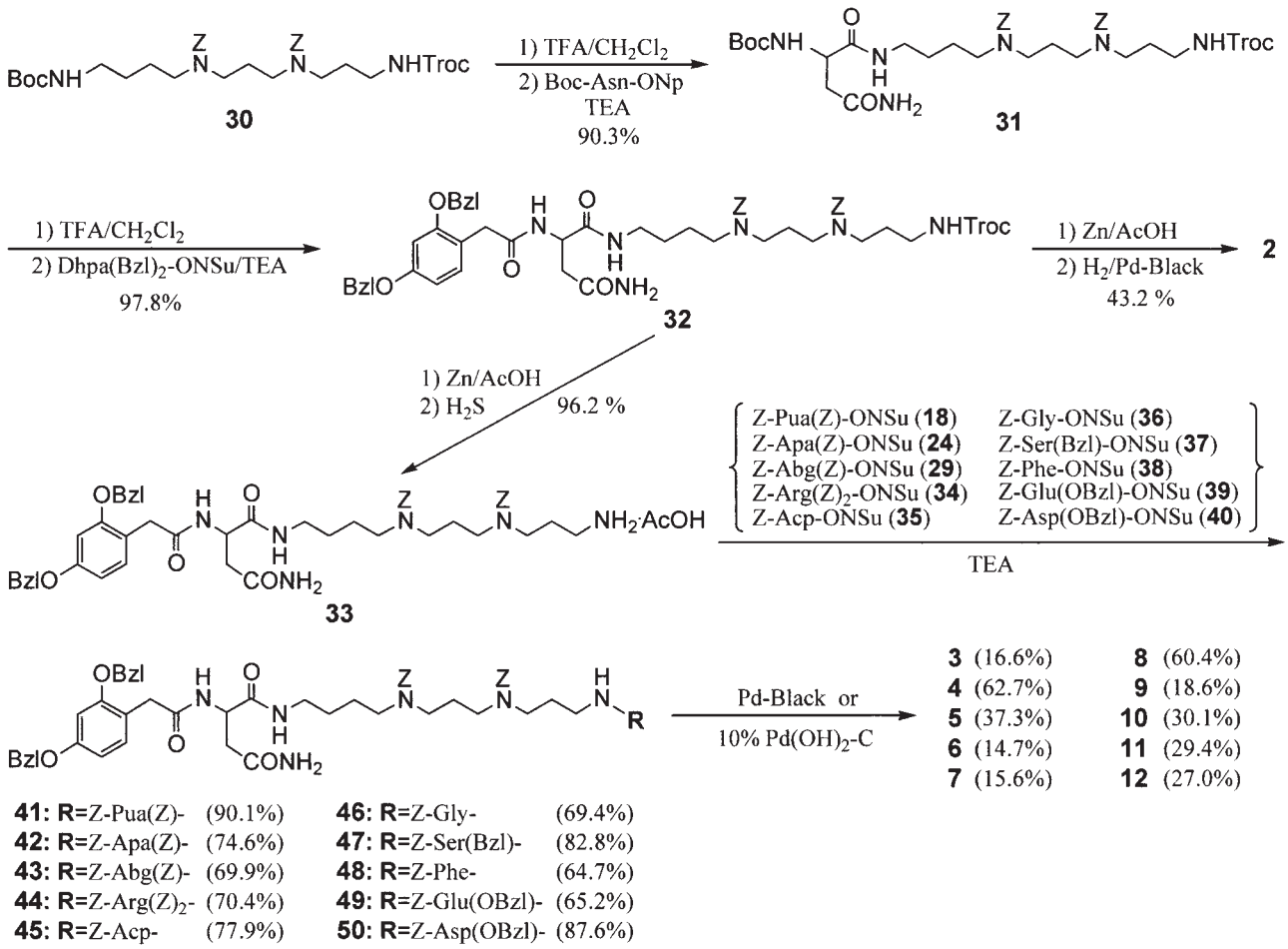
ly being undertaken, and the results will be reported soon elsewhere.

Experimental

All of the melting points are uncorrected, and were measured by a Yanaco MP-J3 (Yanaco Co., Ltd., Kyoto, Japan). Silica-gel column chromatography was carried out with Merck silica gel 60 (Art. 7734, 70–230 mesh) or with Merck silica gel 60 (Art. 9385, 230–400 mesh) at medium pressure (1–5 kg cm^{-2}). ^{1}H NMR spectra were recorded on a Mercury 300 (300 MHz, Varian Co., Ltd., Germany) or a DMX-500 (500 MHz, Bruker Co., Ltd., Germany). MALDI TOF-MS was carried out with a Kratos Kompact MALDI 4 (Shimadzu Co., Ltd., Kyoto, Japan). HRFAB-MS was carried out with a HX110 (JEOL Co., Ltd., Tokyo, Japan). RPHPLC was carried out with a Shimadzu SCL-10A *VP* (Shimadzu Co., Ltd., Kyoto, Japan), and performed on a Cosmosil 5C_{18}-AR (4.6 × 150 mm, Nacalai Tesque Co., Ltd., Kyoto, Japan) for analysis and a YMC-Pack ODS-AM (20 × 250 mm, YMC Co Ltd, Kyoto, Japan) for preparative purification. Boc-Asn-ONp was purchased from Watanabe Chemical Industries Co., Ltd., (Hiroshima Japan). Solid H_2S was purchased from Wako Pure Chemical Industries Co., Ltd., (Osaka, Japan). Celite 545@ was purchased from Nacalai Tesque Co., Ltd., (Kyoto, Japan).

Methyl 8-Azido-4-benzyloxycarbonyl-4-azaoctanoate (15). After methyl acrylate (1.15 g, 13.4 mmol) was added to 4-amino-1-butanol (**13**) (1.00 g, 11.2 mmol), the mixture was stirred for 1 h at 0 °C and additionally for 29 h at r. t. To a solution of the thus-obtained methyl 8-hydroxy-4-azaoctanoate and TEA (1.58 g, 15.7 mmol) in THF (10 mL) was added dropwise ZCl (2.29 g, 13.4 mmol) in THF (10 mL) at r. t. over a 30-minute period. The mixture was stirred for 2.5 h at r. t., and the insoluble material was filtered off. The filtrate was concentrated in vacuo, and the residue was dissolved in AcOEt (50 mL). The solution was washed with H_2O (10 mL), 10% citric acid (10 mL × 3), and saturated aqueous $NaHCO_3$ (10 mL × 3). The organic layer was dried over anhydrous $MgSO_4$, and then concentrated in vacuo to give methyl 4-benzyloxycarbonyl-8-hydroxy-4-azaoctanoate (**14**) (3.00 g, 86.5%) as colorless oil; the product was used for the next reaction without further purification.

To a solution of the thus-obtained **14** (2.00 g, 6.46 mmol) in pyridine (20 mL) was added MsCl (0.747 g, 7.11 mmol) at 0 °C, and the mixture was stirred for 1 h at 0 °C. After the addition of diethyl ether (50 mL), the reaction mixture was washed with saturated aqueous $CuSO_4$ (20 mL × 3), saturated aqueous $NaHCO_3$ (10 mL × 3), and brine (10 mL × 3). The organic layer was dried over anhydrous $MgSO_4$, and then concentrated in vacuo. To a solution of the residue in DMF (40 mL) was added NaN_3 (2.52 g, 38.8 mmol) at 80 °C, and the solution was stirred for 1 h at 80 °C. After the addition of AcOEt (100 mL), the reaction mixture was washed with 10% citric acid (10 mL × 3), saturated aqueous $NaHCO_3$ (10 mL × 3), and brine (10 mL × 3). The organic layer was dried over anhydrous $MgSO_4$, and then concentrated in vacuo. The thus-obtained crude product was purified by silica-gel column chromatography (Art. 9385, 80 g, 2 × 50 cm, benzene:AcOEt = 20:1). The fractions containing the desired product were combined, and concentrated in vacuo to give **15** as colorless oil (1.33 g, 61.6%). ^{1}H NMR ($CDCl_3$) δ 1.59 (4H, m, C^6H_2, C^7H_2), 2.56 and 2.63 (each 1H, m, C^2H_2), 3.50 (4H, m, C^3H_2, C^5H_2), 3.54 (2H, t, C^8H_2), 3.65 (3H, s, CH_3), 5.12 (2H, s, CH_2/Z), 7.28–7.40 (5H, m, Ph/Z). MALDI-TOF MS: found m/z 335.2 $(M + H)^+$ (calcd for



Scheme 4. Synthetic routes to NPTX-594 analogs.

$C_{16}H_{22}N_4O_4$ + H: 335.2), 357.2 $(M + Na)^+$ (calcd for $C_{16}H_{22}N_4O_4$ + Na: 357.2).

Methyl 4,N^8-Bis(benzyloxycarbonyl)-8-amino-4-azaoctanoate (16). To a solution of **15** (1.00 g, 2.99 mmol) in THF (10 mL) and H_2O (1 mL) was added PPh_3 (0.942 g, 3.59 mmol) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and additionally for 20 h at r. t., and concentrated in vacuo. The residue was dissolved in AcOEt (50 mL), and it was extracted with 1 M HCl (10 mL × 3). The acidic aqueous extract was neutralized with Na_2CO_3, and diluted with THF (20 mL). To a mixture basified by the addition of Na_2CO_3 (0.634 g, 5.98 mmol) was added dropwise ZCl (0.765 g, 5.98 mmol). The reaction mixture was stirred for 5 h at r. t., and then extracted with AcOEt (30 mL × 3). The extract was dried over anhydrous $MgSO_4$, and concentated in vacuo. The crude product was purified by silica-gel column chromatography (Art. 7734, 38 g, 1 × 40 cm, $CHCl_3$:MeOH = 20:1). The fractions containing the desired product were combined, and concentrated in vacuo to give **16** as colorless oil (1.05 g, 79.5%). ^{1}H NMR ($CDCl_3$) δ 1.53 (4H, m, C^6H_2, C^7H_2), 2.58 (2H, t, C^2H_2), 3.16–3.28 (4H, m, C^3H_2, C^5H_2), 3.52 (2H, t, C^8H_2), 3.65 (3H, s, CH_3), 5.09 (2H, s, CH_2/Z), 5.12 (2H, s, CH_2/Z), 7.31–7.40 (10H, m, Ph/Z). MALDI TOF-MS: found m/z 443.5 $(M + H)^+$ (calcd for $C_{24}H_{30}N_2O_6$ + H: 443.2), 465.4 $(M + Na)^+$ (calcd for $C_{24}H_{30}N_2O_6$ + Na: 465.2).

4,N^8-Bis(benzyloxycarbonyl)-8-amino-4-azaoctanoic Acid [Z-Pua(Z)-OH] (17). Methyl 4,N^8-bis(benzyloxycarbonyl)-8-amino-4-azaoctanoate (**16**) (1.00 g, 2.26 mmol) was dissolved in 1 M NaOH (15 mL) and MeOH (15 mL), and stirred for 1 h at 60 °C. The reaction mixture was neutralized with 1 M HCl, and MeOH was removed in vacuo. The aqueous solution was extracted with $CHCl_3$ (30 mL × 3), and the extract was washed with brine (20 mL × 3). The organic layer was dried over anhydrous $MgSO_4$, and then concentrated in vacuo to give colorless oil (0.851 g, 87.9%); the crude product was pure enough to use for the next reaction. ^{1}H NMR ($CDCl_3$) δ 1.52 (4H, m, C^6H_2, C^7H_2), 2.58 (2H, m, C^2H_2), 3.15 (2H, m, C^3H_2) 3.28 (2H, m, C^5H_2), 3.52 (2H, t, C^8H_2), 5.09 (2H, s, CH_2/Z), 5.12 (2H, s, CH_2/Z), 7.27–7.37 (10H, m, Ph/Z). MALDI TOF-MS: found m/z 451.2 $(M + Na)^+$ (calcd for $C_{23}H_{28}N_2O_6$ + Na: 451.2).

4,N^8-Bis(benzyloxycarbonyl)-8-amino-4-azaoctanoic Acid Succinimidyl Ester [Z-Pua(Z)-ONSu] (18). To a solution of **17** (0.300 g, 0.700 mmol) in DMF (15 mL) were added HONSu (0.148 g, 12.5 mmol) and EDC·HCl (0.0763 g, 0.770 mmol), and the mixture was stirred overnight at r. t. The reaction mixture was concentrated in vacuo, and the residue was dissolved in AcOEt (30 mL). The solution was washed with 10% citric acid (5 mL × 3), saturated aqueous $NaHCO_3$ (5 mL × 3), and brine (5 mL × 3). The organic layer was dried over anhydrous $MgSO_4$, and then concentrated in vacuo to give **18** (0.295 g, 80.2%) as colorless oil. The thus-obtained crude product was used for the next reaction without further purification.

Methyl 7-Azido-4-benzyloxycarbonyl-4-azaheptanoate (21). Methyl acrylate (3.78 g, 43.9 mmol) was added to 3-amino-1-propanol (**19**) (3.00 g, 39.9 mmol), and the mixture was stirred for 1 h at 0 °C and additionally for 32 h at r. t. To a solution of the thus-ob-

tained methyl 7-hydroxy-4-azaheptanoate in THF (100 mL) was added TEA (4.50 g, 44.5 mmol), and was then added dropwise ZCl (8.17 g, 47.9 mmol) in THF (50 mL) at r. t. over a 30-minute period. The mixture was stirred overnight at r. t., and worked up in a similar manner as described in the preparation of **14**. The thus-obtained crude product was purified by silica-gel column chromatography (Art. 7734, 180 g, 2 × 70 cm, $CHCl_3$:MeOH = 50:1). The fractions containing the desired product were combined, and concentrated in vacuo to give methyl 4-benzyloxycarbonyl-7-hydroxy-4-azaheptanoate (**20**) as colorless oil (11.3 g, 95.8%). ^{1}H NMR ($CDCl_3$) δ 1.71 (2H, m, C^6H_2), 2.59 (2H, m, C^2H_2), 3.38 (2H, m, C^5H_2), 3.46 (2H, m, C^3H_2), 3.55 (2H, m, C^7H_2), 3.65 (3H, s, CH_3), 5.16 (2H, s, CH_2/Z), 7.30–7.40 (5H, m, Ph/Z). MALDI TOF-MS: found m/z 296.2 $[M + H]^+$ (calcd for $C_{15}H_{21}NO_5$ + H: 296.2).

To a solution of the thus-obtained **20** (11.0 g, 37.4 mmol) in toluene (160 mL) were added TEA (4.15 g, 41.0 mmol) and MsCl (4.28 g, 37.4 mmol) at 0 °C, and the solution was stirred for 2 h at 0 °C. To the reaction mixture were added NaN_3 (19.5 g, 299 mmol) and TBAB (3.58 g, 11.2 mmol) in H_2O (160 mL) under heating at 80 °C. After stirring overnight at 80 °C, the aqueous layer was removed from the mixture, and the organic layer was washed with 10% citric acid (20 mL × 3), saturated aqueous $NaHCO_3$ (20 mL × 3), and brine (20 mL × 3). The organic layer was dried over anhydrous $MgSO_4$, and the solvent was then removed in vacuo. The thus-obtained crude product was purified by silica-gel column chromatography (Art. 7734, 180 g, 2 × 70 cm, hexane:AcOEt = 4:1). The fractions containing the desired product were combined and concentrated in vacuo to give **21** as colorless oil (7.94 g, 66.3%). ^{1}H NMR ($CDCl_3$) δ 1.81 (2H, m, C^6H_2), 2.60 (2H, m, C^2H_2), 3.31 (2H, m, C^5H_2), 3.37 (2H, t, C^3H_2), 3.55 (2H, t, C^7H_2), 3.67 (3H, s, CH_3), 5.13 (2H, s, CH_2/Z), 7.26–7.40 (5H, m, Ph/Z). MALDI TOF-MS: found m/z 293.3 $(M - N_2 + H)^+$ (calcd for $C_{15}H_{20}N_4O_4 - N_2$ + H: 293.1).

Methyl 4,N^7-Bis(benzyloxycarbonyl)-7-amino-4-azaheptanoate (22). To a solution of **21** (5.28 g, 16.5 mmol) in THF (40 mL) and H_2O (4 mL) was added PPh_3 (4.76 g, 18.1 mmol) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and additionally for 30 h at r. t., and concentrated in vacuo. The residue was dissolved in diethyl ether (150 mL), and extracted with 1 M HCl (10 mL × 3). The acidic aqueous extract was lyophilized, and the residue was dissolved in THF (20 mL). To a solution basified with TEA (1.87 g, 18.4 mmol) was added dropwize ZCl (3.09 g, 18.1 mmol) over a 30 minute-period. The reaction mixture was stirred overnight at r. t., and worked up in a similar manner as described in the preparation of **16**. The crude product was purified by silica-gel column chromatography (Art. 7734, 38 g, 1 × 40 cm, hexane:AcOEt = 5:1). The fractions containing the desired product were combined, and concentrated in vacuo to give **22** as colorless oil (5.08 g, 71.8%). ^{1}H NMR ($CDCl_3$) δ 1.70 (2H, m, C^6H_2), 2.56 (2H, m, C^2H_2), 3.15 (2H, m, C^5H_2), 3.35 (2H, m, C^3H_2), 3.51 (2H, t, C^7H_2), 3.65 (3H, s, CH_3), 5.09 (2H, s, CH_2/Z), 5.12 (2H, s, CH_2/Z), 7.25–7.40 (10H, m, Ph/Z). MALDI TOF-MS: found m/z 443.5 $(M + H)^+$ (calcd for $C_{24}H_{30}N_2O_6$ + H: 443.2), 465.4 $(M + Na)^+$ (calcd for $C_{24}H_{30}N_2O_6$ + Na: 465.2).

4,N^7-Bis(benzyloxycarbonyl)-7-amino-4-azaheptanoic Acid Succinimidyl Ester [Z-Apa(Z)-ONSu] (24). Methyl 4,N^7-bis(benzyloxycarbonyl)-7-amino-4-azaoctanoate (**22**) (4.70 g, 11.0 mmol) was dissolved in 1 M NaOH (15 mL) and MeOH (15 mL), and the mixture was stirred for 1.5 h at 60 °C. The reaction mixture was worked up in a similar manner as described in the preparation of **17** to give of 4,7-bis(benzyloxycarbonyl)-7-amino-4-azaheptanoic acid (**23**) in a quantitative yield.

To a solution of **23** obtained above in DMF (70 mL) were added HONSu (1.43 g, 12.5 mmol) and EDC·HCl (2.40 g, 12.5 mmol), and the mixture was stirred overnight at r. t. The reaction mixture was worked up in a similar manner as described in the preparation of **18** to give **24** (4.53 g, 80.2%) as colorless oil. The thus-obtained crude product was used for the next reaction without further purification.

4-Benzyloxycarbonylamino-1-butanol (25). To a solution of 4-amino-1-butanol (**13**) (5.00 g, 56.1 mmol) in acetone (25 mL) were added TEA (6.81 g, 67.3 mmol) and ZONSu (14.4 g, 61.7 mmol). The mixture was stirred for 7 h at r. t., and the insoluble material was filtered off. The filtrate was concentrated in vacuo, and the residue was dissolved in AcOEt (100 mL). The solution was washed with 1 M HCl (20 mL × 3), saturated aqueous $NaHCO_3$ (20 mL × 3), and brine (30 mL × 3). The organic layer was dried over anhydrous $MgSO_4$, and then concentrated in vacuo. The thus-obtained crude crystalline product was recrystallized from AcOEt and hexane to give **25** as colorless crystals (11.6 g, 92.8%); mp 78–79 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.59 (4H, m, C^2H_2, C^3H_2), 3.23 (2H, m, C^4H_2), 3.66 (2H, m, C^1H_2), 5.09 (2H, s, CH_2/Z), 7.34 (5H, m, Ph/Z). MALDI TOF-MS: found m/z 224.1 $(M + H)^+$ (calcd for $C_{12}H_{17}NO_3$ + H: 224.1), 246.1 $(M + Na)^+$ (calcd for $C_{12}H_{16}NO_3$ + Na: 246.1).

4-Azido-*N*-benzyloxycarbonylbutylamine (26). To a solution of **25** (4.00 g, 17.9 mmol) in toluene (100 mL) were added TEA (1.99 g, 19.7 mmol) and MsCl (2.05 g, 17.9 mmol) at 0 °C, and the mixture was stirred for 2 h at 0 °C. To the solution were added NaN_3 (4.65 g, 71.6 mmol) and TBAB (0.577 g, 1.79 mmol) in H_2O (45 mL) under heating at 70 °C. After the mixture was additionally stirred for 24 h at 70 °C, the organic layer separated from aqueous layer was washed with 10% citric acid (15 mL × 3), saturated aqueous $NaHCO_3$ (15 mL × 3), and brine (15 mL × 3). The organic layer was dried over anhydrous $MgSO_4$, and then concentrated in vacuo. The thus-obtained crude product was purified by silica-gel column chromatography (Art. 7734, 220 g, 4 × 100 cm, hexane:AcOEt = 4:1). The fractions containing the desired product were combined, and concentrated in vacuo to give **26** as colorless oil (4.24 g, 95.6%). ^{1}H NMR ($CDCl_3$) δ 1.60 (4H, m, C^2H_2, C^3H_2), 3.22 (2H, m, C^4H_2), 3.30 (2H, m, C^1H_2), 5.10 (2H, s, CH_2/Z), 7.34 (5H, m, Ph/Z). MALDI TOF-MS: found m/z 271.3 $(M + Na)^+$ (calcd for $C_{12}H_{16}N_4O_2$ + Na: 271.1).

***N*-Benzyloxycarbonyl-1,4-butanediamine Hydrochloride (27).** To a solution of **26** (4.00 g, 29.7 mmol) in THF (30 mL) and H_2O (2 mL) was added PPh_3 (5.06 g, 19.3 mmol) at 0 °C. The reaction mixture was stirred for 2 h at 0 °C and additionally for 20 h at r. t., and then concentrated in vacuo. The residue was dissolved in AcOEt (100 mL), and the solution was extracted with 10% citric acid (30 mL × 3). The extract was washed with diethyl ether (20 mL × 3), and then basified with 2 M NaOH; the basic aqueous solution was extracted with $CHCl_3$ (20 mL × 6). The extract was dried over anhydrous $MgSO_4$, and then concentrated in vacuo. The residue was dissolved in 1 M HCl, and the solution was concentrated in vacuo to give **25** as colorless crystals (3.57 g, quant.) that were used for the next reaction without further purification; mp 188–190 °C (decomp.). ^{1}H NMR (CD_3OD) δ 1.41 (4H, m, C^2H_2, C^3H_2), 2.58 (2H, m, C^4H_2), 3.01 (2H, m, C^1H_2), 4.78 (2H, s, CH_2/Z), 7.20 (each 1H, brs, CH/Z). MALDI TOF-MS: found m/z 223.0 $(M + H)^+$ (calcd for $C_{12}H_{18}N_2O_2$ + H: 223.1).

3,N^7-Bis(benzyloxycarbonyl)-7-amino-3-azaheptanoic Acid [Z-Abg(Z)-OH] (28). *N*-Benzyloxycarbonyl-1,4-butanediamine hydrochloride (**27**) (1.00 g, 3.86 mmol) in CH_2Cl_2 (30 mL) and

MeOH (10 mL) was added to glyoxylic acid monohydrate (0.237 g, 2.57 mmol), and the mixture was stirred for 15 min at r. t. To the solution was added dropwise $NaB(OAc)_3H$ (1.69 g, 7.72 mmol) in CH_2Cl_2 (20 mL) at r. t. over a 30-minute period, and the mixture was additionally stirred for 5.5 h. The reaction mixture was concentrated in vacuo, and the residue was dissolved in saturated aqueous $NaHCO_3$ (60 mL); the solution was washed with CH_2Cl_2 (20 mL × 3). To the basic aqueous solution was added ZONSu (0.599 g, 2.57 mmol) in MeOH (20 mL), and the mixture was stirred for 23 h at r. t. The reaction mixture was acidified with 1 M HCl, and the acidic solution was extracted with AcOEt (30 mL × 3). The organic layer was dried over anhydrous $MgSO_4$, and then concentrated in vacuo. The crude product was purified by silica-gel column chromatography (Art. 7734, 100 g, 2 × 70 cm, $CHCl_3$:MeOH = 9:1). The fractions containing the desired product were combined, and concentrated in vacuo to give **28** as colorless oil (0.422 g, 39.3%). ^{1}H NMR ($CDCl_3$) δ 1.52 (4H, m, C^5H_2, C^6H_2), 3.15 (2H, m, C^7H_2), 3.35 (2H, m, C^4H_2), 3.96 and 4.00 (each 1H, brs, C^1H_2), 5.07 (2H, s, CH_2/Z), 5.11 and 5.14 (each 1H, brs, CH_2/Z), 7.21–7.38 (10H, m, Ph/Z). MALDI TOF-MS: found m/z 437.6 $(M + Na)^+$ (calcd for $C_{22}H_{26}N_2O_6$ + Na: 437.2).

3,N^7-Bis(benzyloxycarbonyl)-7-amino-3-azaheptanoic Acid Succinimidyl Ester [Z-Abg(Z)-ONSu] (29). To a solution of **28** (0.533 g, 1.29 mmol) in DMF (15 mL) were added HONSu (0.140 g, 1.42 mmol) and EDC·HCl (0.273 g, 1.42 mmol), and the mixture was stirred for 11 h at r. t. The reaction mixture was worked up in a similar manner as described in the preparation of **18** to give **29** as colorless crystals (0.530 g, 80.0%). The thus-obtained crude product was used for the next reaction without further purification. ^{1}H NMR ($CDCl_3$) δ 1.55 (4H, m, C^5H_2, C^6H_2), 2.79 (4H, m, CH_2/ONSu), 3.16 and 3.20 (each 1H, m, C^7H_2), 3.37 (2H, m, C^4H_2), 4.30 and 4.37 (each 1H, brs, C^1H_2), 5.08 (2H, s, CH_2/Z), 5.15 and 5.17 (each 1H, brs, CH_2/Z), 7.30–7.36 (10H, m, Ph/Z). MALDI TOF-MS: found m/z 534.4 $(M + Na)^+$ (calcd for $C_{26}H_{29}N_3O_8$ + Na: 534.5).

4,8-Bis(benzyloxycarbonyl)-N^{12}-(N^{α}-*t*-butoxycarbonylasparaginyl)-N^1-(2,2,2-trichloroethoxycarbonyl)-4,8-diaza-1,12-dodecanediamine (31). To a solution of 4,8-bis(benzyloxycarbonyl)-N^1-(2,2,2-trichloroethoxycarbonyl)-N^{12}-(*t*-butoxycarbonyl)-4,8-diaza-1,12-dodecanediamine (**30**)[17] (2.66 g, 3.57 mmol) in CH_2Cl_2 (10 mL) was added TFA (10 mL), and the solution was stirred for 30 min at r. t. The reaction mixture was concentrated in vacuo, and the residue was dissolved in DMF (30 mL); the solution was neutralized with TEA. To the solution were added TEA (0.719 g, 7.14 mmol) and Boc-Asn-ONp (1.52 g, 4.30 mmol), and the mixture was stirred for 1 h at r. t. The reaction mixture was concentrated in vacuo, and the residue was dissolved in AcOEt (50 mL). The solution was washed with 10% citric acid (10 mL × 3), saturated aqueous $NaHCO_3$ (10 mL × 6), and brine (10 mL × 3). The organic layer was dried over anhydrous $MgSO_4$, and then concentrated in vacuo. The crude product was purified by silica-gel column chromatography (Art. 7734, 80 g, 2 × 47 cm, AcOEt and then $CHCl_3$:MeOH = 24:1). The fractions containing the desired product were combined, and concentrated in vacuo to give **31** as colorless crystals (2.77 g, 90.3%); mp 83–84 °C. ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s, $(CH_3)_3$C/Boc), 1.45–1.74 (8H, m, {C^2H_2, C^6H_2, $C^{10}H_2$, $C^{11}H_2$}/Dada), 2.52 and 2.91 (each 1H, dd, β-CH_2/Asn, J = 15.6, 6.6 Hz), 3.20 (12H, m, {C^1H_2, C^3H_2, C^5H_2, C^7H_2, C^9H_2, $C^{12}H_2$}/Dada), 4.43 (1H, m, α-CH/Asn), 4.71 (2H, s, CH_2/Troc), 5.11 (4H, s, CH_2/Z), 7.27–7.37 (10H, m, Ph/Z). HRFAB-MS: found m/z 859.2934 $(M + H)^+$ (calcd for $C_{38}H_{53}Cl_3N_6O_{10}$ + H: 859.2967).

4,8-Bis(benzyloxycarbonyl)-N^{12}-{N^{α}-[2,4-bis(benzyloxy)-phenylacetyl]asparaginyl}-N^1-(2,2,2-trichloroethoxycarbonyl)-4,8-diaza-1,12-dodecanediamine (32). To a solution of **31** (1.00 g, 2.91 mmol) in CH_2Cl_2 (10 mL) was added TFA (10 mL), and the solution was stirred for 1.5 h at r. t. The reaction mixture was concentrated in vacuo, and the residue was dissolved in DMF (50 mL); the solution was neutralized with TEA. To the solution were added TEA (0.586 g, 5.82 mmol) and 2,4-bis(benzyloxy)phenylacetic acid succinimidyl ester [Dhpa(Bzl)$_2$-ONSu][20,23,24] (1.24 g, 2.91 mmol). The reaction mixture was stirred for 22 h at r. t., and then concentrated in vacuo. The residue was dissolved in $CHCl_3$ (250 mL), and the solution was washed with 10% citric acid (50 mL × 3) and saturated aqueous $NaHCO_3$ (50 mL × 2). The organic layer was dried over anhydrous $MgSO_4$, and then concentrated in vacuo. The thus-obtained crude product was purified by silica-gel column chromatography (Art. 9385, 208 g, 3.3 × 50 cm, $CHCl_3$:MeOH = 97:3). The fractions containing the desired product were combined, and concentrated in vacuo to give **32** as colorless crystals (3.10 g, 97.8%); mp 139–140 °C. ^{1}H NMR ($CDCl_3$) δ 1.28 (2H, m, $C^{10}H_2$/Dada), 1.38 (2H, m, $C^{11}H_2$/Dada), 1.56–1.80 (4H, m, {C^2H_2, C^6H_2}/Dada), 2.24 and 2.73 (each 1H, dd, β-CH_2/Asn, J = 15.6, 6.6 Hz), 3.17 (12H, m, {C^1H_2, C^3H_2, C^5H_2, C^7H_2, C^9H_2, $C^{12}H_2$}/Dada), 3.58 (2H, s, CH_2/Dhpa), 4.61 (1H, m, α-CH/Asn), 4.71 (2H, s, CH_2/Troc), 5.01 (2H, s, CH_2/Z), 5.07 and 5.09 (each 1H, s, CH_2/Z), 6.61 (1H, dd, C^3H/Dhpa, J = 8.4, 2.4 Hz), 6.64 (1H, d, C^5H/Dhpa, J = 2.4 Hz), 7.13 (1H, d, C^6H/Dhpa, J = 8.4 Hz), 7.20–7.42 (20H, m, Ph/Z and Ph/Bzl). HRFAB-MS: found m/z 1089.3655 $(M + H)^+$ (calcd for $C_{55}H_{63}Cl_3N_6O_{11}$ + H: 1089.3699).

N^{12}-[N^{α}-(2,4-Dihydroxyphenylacetyl)asparaginyl]-4,8-diaza-1,12-dodecanediamine [Des-Lys-NPTX-594] Tris-trifluoroacetate (2). To a solution of **32** (0.700 g, 0.640 mmol) in 90% AcOH (50 mL) was added Zn dust (1.28 g, 19.1 mmol), and the suspension was stirred for 5.5 h at r. t. The insoluble materials were filtered off, and the filtrate was concentrated in vacuo; the residue was dissolved in MeOH (15 mL) and AcOH (30 mL). To the solution was added Pd-black (200 mg), and hydrogen was gently introduced into the suspension under stirring for 7 h at r. t. After the catalyst was filtered off, the filtrate was concentrated in vacuo. The residue was finally purified by preparative RPHPLC (10–40% CH_3CN containing 0.1% TFA–H_2O containing 0.1% TFA; flow rate: 8.0 mL min^{-1}). The fractions containing the desired compound were combined, and lyophilized to give **2** as a powdery 3TFA salt (128 mg, 43.2%). ^{1}H NMR (D_2O) δ 1.14–1.37 (4H, m, {$C^{10}H_2$, $C^{11}H_2$}/Dada), 1.94 (4H, m, {C^2H_2, C^6H_2}/Dada), 2.58 and 2.65 (each 1H, dd, β-CH_2/Asn, J = 15.5, 7.5 Hz), 2.75–3.21 (12H, m, {C^1H_2, C^3H_2, C^5H_2, C^7H_2, C^9H_2, $C^{12}H_2$}/Dada), 3.36 and 3.44 (each 1H, d, CH_2/Dhpa, J = 15.6 Hz), 3.86 (1H, dd, α-CH/Asn, J = 7.5, 7.5 Hz), 6.31 (2H, m, Ph-{C^3H, C^5H}/Dhpa, J = 8.7 Hz), 6.95 (1H, d, Ph-C^6H/Dhpa, J = 8.7 Hz). HRFAB-MS: found m/z 467.2970 $(M + H)^+$ (calcd for $C_{22}H_{38}N_6O_5$ + H: 467.2982).

4,8-Bis(benzyloxycarbonyl)-N^{12}-{N^{α}-[2,4-bis(benzyloxy)-phenylacetyl]asparaginyl}-4,8-diaza-1,12-dodecanediamine Acetate (33). To a solution of **32** (1.50 g, 1.38 mmol) in 90% AcOH (50 mL) was added Zn dust (2.69 g, 41.1 mmol), and the suspension was stirred for 24 h at r. t. H_2S gas generated from solid H_2S (12.6 g, 14.1 mmol) was introduced to the suspension under stirring for 1.5 h at r. t. After releasing excess H_2S by introducing CO_2 generated from dry ice, the insoluble materials were filtered off through celite 545®, and the filtrate was concentrated in vacuo. The residue was dissolved in 1,4-dioxane (100 mL), and the solu-

tion was lyophilized to give **33** as a powdery monoacetate (1.28 g, 96.2%). The thus-obtained crude product was used for the next reaction without further purification.

4,8-Bis(benzyloxycarbonyl)-N^1-[4,N^8-bis(benzyloxycarbonyl)-8-amino-4-azaoctanoyl]-N^{12}-{N^{α}-[2,4-bis(benzyloxy)phenylacetyl]asparaginyl}-4,8-diaza-1,12-dodecanediamine (41). (**General procedure 1**: Coupling of the terminal amino acid residue by the active ester method). To a solution of **33** (0.300 g, 0.308 mmol) in DMF (15 mL) were added TEA (0.0854 g, 0.616 mmol) and Z-Pua(Z)-ONSu (**18**) (0.203 g, 0.370 mmol), and the mixture was stirred for 18 h at r. t. The reaction mixture was concentrated in vacuo, and the residue was dissolved in $CHCl_3$ (300 mL). The solution was washed with 10% citric acid (30 mL × 3) and saturated aqueous $NaHCO_3$ (50 mL × 3). The organic layer was dried over anhydrous $MgSO_4$, and then the solvent was removed in vacuo. The crude product was purified by silica-gel column chromatography (Art. 9385, 23.5 g, 1 × 45 cm, $CHCl_3$ to $CHCl_3$:MeOH = 91:9). The fractions containing the desired product were combined, and concentrated in vacuo to give **41** as colorless crystals (0.368 g, 90.1%); mp 112–115 °C. ^{1}H NMR ($CDCl_3$) δ 1.26 (4H, m, {$C^{10}H_2$, $C^{11}H_2$}/Dada), 1.43 (2H, m, C^6H_2/Pua), 1.53 (2H, m, C^7H_2/Pua), 1.73 (4H, m, {C^2H_2, C^6H_2}/Dada), 2.45 (2H, m, C^2H_2/Pua), 2.25 and 2.71 (each 1H, dd, β-CH_2/Asn, J = 15.6, 6.6 Hz), 2.85–3.38 (16H, m, {C^1H_2, C^3H_2, C^5H_2, C^7H_2, C^9H_2, $C^{12}H_2$}/Dada, {C^3H_2, C^5H_2}/Pua), 3.51 (2H, m, C^8H_2/Pua), 3.57 (2H, s, CH_2/Dhpa), 4.59 (1H, m, α-CH/Asn), 5.01 (2H, s, CH_2/Z), 5.08 (10H, m, CH_2/Z and CH_2/Bzl), 6.56 (1H, dd, Ph-C^3H/Dhpa, J = 8.1, 2.1 Hz), 6.64 (1H, d, Ph-C^5H/Dhpa, J = 2.1 Hz), 7.12 (1H, d, Ph-C^6H/Dhpa, J = 8.1 Hz), 7.26–7.43 (30H, m, Ph/Z and Ph/Bzl). HRFAB-MS: found m/z 1325.6434 $(M + H)^+$ (calcd for $C_{75}H_{88}N_8O_{14}$ + H: 1325.6498).

N^1-(8-Amino-4-azaoctanoyl)-N^{12}-[N^{α}-(2,4-dihydroxyphenylacetyl)asparaginyl]-4,8-diaza-1,12-dodecanediamine {Pua-[des-Lys-(NPTX-594)]} Tetrakis-trifluoroacetate (3). (**General procedure 2**: Deprotection and purification of final compounds). To a solution of **41** (30 mg, 22.6 μmol) in MeOH (1 mL) and AcOH (2 mL) was added 10% $Pd(OH)_2$-C (35 mg) as a catalyst, and the suspension was stirred under an atmosphere of hydrogen for 24 h at r. t., and the catalyst was filtered off, and the filtrate was concentrated in vacuo. The residue was finally purified by preparative RPHPLC (10–40% CH_3CN containing 0.1% TFA–H_2O containing 0.1% TFA; flow rate: 8.0 mL min^{-1}). The fractions containing the desired compound were combined and lyophilized to give **3** as a white powdery substance (4.00 mg, 16.6%). ^{1}H NMR (D_2O) δ 1.35 (4H, m, {$C^{10}H_2$, $C^{11}H_2$}/Dada), 1.58 (4H, m, {C^6H_2, C^7H_2}/Pua), 1.71 (2H, m, C^6H_2/Dada), 1.86 (2H, m, C^2H_2/Dada), 2.53 (2H, m, C^2H_2/Pua), 2.51 and 2.61 (2H, dd, β-CH_2/Asn, J = 15.6, 6.0 Hz), 2.70–3.02 (14H, m, {C^3H_2, C^5H_2, C^7H_2, C^9H_2}/Dada, {C^3H_2, C^5H_2, C^8H_2}/Pua), 3.08–3.15 (4H, m, {C^1H_2, $C^{12}H_2$}/Dada), 3.31 and 3.40 (each 1H, d, CH_2/Dhpa, J = 15.9 Hz), 4.40 (1H, dd, α-CH/Asn, J = 6.0, 6.0 Hz), 6.27 (2H, m, Ph-{C^3H, C^5H}/Dhpa), 6.91 (1H, m, Ph-C^6H/Dhpa). HRFAB-MS: found m/z 609.4061 $(M + H)^+$ (calcd for $C_{29}H_{53}N_8O_6$ + H: 609.4088).

4,8-Bis(benzyloxycarbonyl)-N^1-[4,N^7-bis(benzyloxycarbonyl)-7-amino-4-azaheptanoyl]-N^{12}-{N^{α}-[2,4-bis(benzyloxy)phenylacetyl]asparaginyl}-4,8-diaza-1,12-dodecanediamine (42). To a solution of **33** (0.894 g, 0.917 mmol) in DMF (30 mL) were added TEA (0.185 g, 1.83 mmol) and Z-Apa(Z)-ONSu (**24**) (0.469g 0.917 mmol), and the mixture was worked up in a similar manner as described in General procedure 1. The crude product was purified by silica-gel column chromatography (Art. 9385, 150 g, 3 × 50 cm, $CHCl_3$:MeOH = 97:3) to give **42** as colorless crystals (0.895 g, 74.6%); mp 125–128 °C. ^{1}H NMR ($CDCl_3$) δ 1.22–1.82 (12H, m, {C^2H_2, C^6H_2, $C^{10}H_2$, $C^{11}H_2$}/Dada, {C^2H_2, C^6H_2}/Apa), 2.25 and 2.71 (each 1H, dd, β-CH_2/Asn, J = 15.5, 6.0 Hz), 2.89–3.41 (18H, m, {C^1H_2, C^3H_2, C^5H_2, C^7H_2, C^9H_2, $C^{12}H_2$}/Dada, {C^3H_2, C^5H_2, C^7H_2}/Apa), 3.57 (2H, s, CH_2/Dhpa), 4.60 (1H, m, α-CH/Asn), 5.01 (2H, s, CH_2/Z), 5.07 (10H, m, CH_2/Z and CH_2/Bzl), 6.56 (1H, dd, Ph-C^3H/Dhpa, J = 8.4, 2.1 Hz), 6.64 (1H, d, Ph-C^5H/Dhpa, J = 2.1 Hz), 7.12 (1H, d, Ph-C^6H/Dhpa, J = 8.4 Hz), 7.26–7.43 (30H, m, Ph/Z and Ph/Bzl). HRFAB-MS: found m/z 1311.6293 $(M + H)^+$ (calcd for $C_{74}H_{86}N_8O_{14}$ + H: 1311.6342).

N^1-(7-Amino-4-azaheptanoyl)-N^{12}-[N^{α}-(2,4-dihydroxyphenylacetyl)asparaginyl]-4,8-diaza-1,12-dodecanediamine {Apa-[des-Lys-(NPTX-594)]} Tetrakis-trifluoroacetate (4). Compound **42** (200 mg, 0.152 mmol) in MeOH (15 mL) and AcOH (30 mL) was hydrogenated in the presence of Pd-black (0.200 g) as a catalyst, and worked up in a similar manner as described in General procedure 2 to give **4** as white powdery substance (67.7 mg, 62.7%). ^{1}H NMR (D_2O) δ 1.35 (4H, m, {$C^{10}H_2$, $C^{11}H_2$}/Dada), 1.80 (2H, m, C^2H_2), 1.95 (4H, m, C^6H_2/Dada, C^6H_2/Apa), 2.62 (2H, m, C^2H_2/Apa), 2.64 (2H, d, β-CH_2/Asn, J = 6.6 Hz), 2.80–3.14 (14H, m, {C^3H_2, C^5H_2, C^7H_2, C^9H_2}/Dada, {C^3H_2, C^5H_2, C^7H_2}/Apa), 3.12–3.23 (4H, m, {C^1H_2, $C^{12}H_2$}/Dada), 3.39 and 3.47 (each 1H, d, CH_2/Dhpa, J = 15.3 Hz), 4.48 (1H, t, α-CH/Asn, J = 6.6 Hz), 6.35 (2H, m, Ph-{C^3H, C^5H}/Dhpa), 6.99 (1H, m, Ph-C^6H/Dhpa). HRFAB-MS: found m/z 595.3928 $(M + H)^+$ (calcd for $C_{28}H_{51}N_8O_6$ + H: 595.3932).

4,8-Bis(benzyloxycarbonyl)-N^1-[3,N^7-bis(benzyloxycarbonyl)-7-amino-3-azaheptanoyl]-N^{12}-{N^{α}-[2,4-bis(benzyloxy)phenylacetyl]asparaginyl}-4,8-diaza-1,12-dodecanediamine (43). To a solution of **33** (0.120 g, 0.117 mol) in DMF (30 mL) were added TEA (0.185 g, 1.83 mmol) and Z-Abg(Z)-ONSu (**29**) (0.0663 g, 0.129 mmol), and the mixture was worked up in a similar manner as described in General procedure 1. The crude product was purified by silica-gel column chromatography (Art. 9385, 14 g, 1 × 25 cm, $CHCl_3$:MeOH = 20:1) to give **43** as colorless crystals (0.106 g, 69.9%); mp 119–122 °C. ^{1}H NMR ($CDCl_3$) δ 1.26–1.82 (12H, m, {C^2H_2, C^6H_2, $C^{10}H_2$, $C^{11}H_2$}/Dada, {C^5H_2, C^6H_2}/Abg), 2.25 and 2.71 (each 1H, dd, β-CH_2/Asn, J = 15.9, 7.2 Hz), 2.83–3.41 (18H, m, {C^1H_2, C^3H_2, C^5H_2, C^7H_2, C^9H_2, $C^{12}H_2$}/Dada, {C^2H_2, C^4H_2, C^7H_2}/Abg), 3.57 (2H, s, CH_2/Dhpa), 3.87 (1H, m, α-CH/Lys), 4.60 (1H, m, α-CH/Asn), 5.01 (2H, s, CH_2/Z), 5.07 (10H, m, CH_2/Z and CH_2/Bzl), 6.56 (1H, dd, Ph-C^3H/Dhpa, J = 8.4, 2.1 Hz), 6.64 (1H, d, Ph-C^5H/Dhpa, J = 2.1 Hz), 7.13 (1H, d, Ph-C^6H/Dhpa, J = 8.4 Hz), 7.31–7.42 (30H, m, Ph/Z and Ph/Bzl). HRFAB-MS: found m/z 1311.6307 $(M + H)^+$ (calcd for $C_{74}H_{86}N_8O_{14}$ + H: 1311.6342).

N^1-(7-Amino-3-azaheptanoyl)-N^{12}-[N^{α}-(2,4-dihydroxyphenylacetyl)asparaginyl]-4,8-diaza-1,12-dodecanediamine {Abg-[des-Lys-(NPTX-594)]} Tetrakis-trifluoroacetate (5). Compound **43** (21.1 mg, 16.1 μmol) in MeOH (1 mL) and AcOH (2 mL) was hydrogenated in the presence of 10% $Pd(OH)_2$-C (20 mg) as a catalyst, and worked up in a similar manner as described in General procedure 2 to give **5** as white powdery 4TFA salt (6.30 mg, 37.3%). ^{1}H NMR (D_2O) δ 1.35 (4H, m, C^5H_2/Abg, C^6H_2/Abg), 1.60 (4H, m, {$C^{10}H_2$, $C^{11}H_2$}/Dada), 1.73 (2H, m, C^6H_2/Dada), 1.86 (2H, m, C^2H_2/Dada), 2.52 and 2.61 (each 1H, dd, β-CH_2/Asn, J = 15.9, 7.2 Hz), 2.72–3.04 (14H, m, {C^3H_2, C^5H_2, C^7H_2, C^9H_2}/Dada, {C^2H_2, C^4H_2, C^7H_2}/Abg), 3.15 (4H, m, {C^1H_2, $C^{12}H_2$}/Dada), 3.31 and 3.39 (each 1H, d, CH_2/Dhpa, J = 15.3 Hz), 3.70 (1H, m, α-CH/Lys), 4.40 (1H, dd, α-CH/Asn,

J = 7.2, 7.2 Hz), 6.56 (2H, m, Ph-{C^3H, C^5H}/Dhpa), 6.91 (1H, m, Ph-C^6H/Dhpa). HRFAB-MS: found m/z 595.3903 $(M + H)^+$ (calcd for $C_{28}H_{51}N_8O_6$ + H: 595.3932).

4,8-Bis(benzyloxycarbonyl)-N^1-[N^α,N^g,N^g-tris(benzyloxycarbonyl)arginyl]-N^{12}-{N^α-[2,4-bis(benzyloxy)phenylacetyl]-asparaginyl}-4,8-diaza-1,12-dodecanediamine (44). To a solution of **33** (100 mg, 0.103 mmol) in DMF (5 mL) were added TEA (23.0 mg, 0.227 mmol) and N^α,N^g,N^g-tris(benzyloxycarbonyl)-arginine [Z-Arg(Z)$_2$-ONSu] (**34**) (76.1 mg, 0.370 mmol), and the mixture was worked up in a similar manner as described in General procedure 1. The crude product was purified by silica-gel column chromatography (Art. 9385, 24 g, 1 × 45 cm, $CHCl_3$ to $CHCl_3$:MeOH = 97:3) to give **44** as colorless crystals (0.107 g, 70.4%); mp 132–136 °C. ^{1}H NMR ($CDCl_3$) δ 1.25–1.43 (4H, m, {$C^{10}H_2$, $C^{11}H_2$}/Dada), 1.68 (8H, m, {C^2H_2, C^6H_2}/Dada, β-CH_2/Arg, γ-CH_2/Arg), 2.23 and 2.70 (each 1H, dd, β-CH_2/Asn, J = 15.0, 6.0 Hz), 2.93–3.33 (14H, m, {C^1H_2, C^3H_2, C^5H_2, C^7H_2, C^9H_2, $C^{12}H_2$}/Dada, δ-CH_2/Arg), 3.56 (2H, s, CH_2/Dhpa), 3.97 (1H, m, α-CH/Arg), 4.59 (1H, m, α-CH/Asn), 5.02–5.21 (14H, m, CH_2/Z × 5 and CH_2/Bzl × 2), 6.55 (1H, dd, Ph-C^3H/Dhpa, J = 8.1, 2.1 Hz), 6.63 (1H, d, Ph-C^5H/Dhpa, J = 2.1 Hz), 7.13 (1H, d, Ph-C^6H/Dhpa, J = 8.1 Hz), 7.20–7.42 (35H, m, Ph/Z and Ph/Bzl). HRFAB-MS: found m/z 1473.6799 $(M + H)^+$ (calcd for $C_{82}H_{93}N_{10}O_{16}$ + H: 1473.6771).

N^1-Arginyl-N^{12}-[N^α-(2,4-dihydroxyphenylacetyl)asparaginyl]-4,8-diaza-1,12-dodecanediamine {Arg-[des-Lys-(NPTX-594)]} Tetrakis-trifluoroacetate (6). Compound **44** (125 mg, 22.6 μmol) in MeOH (10 mL) and AcOH (20 mL) was hydrogenated in the presence of Pd-black (50 mg) as a catalyst, and worked up in a similar manner as described in General procedure 2 to give **6** as a white powdery 4TFA salt (14.8 mg, 14.7%). ^{1}H NMR (D_2O) δ 1.34 (4H, m, {$C^{10}H_2$, $C^{11}H_2$}/Dada), 1.46 (2H, m, γ-CH_2/Arg), 1.73 (4H, m, β-CH_2/Arg, C^2H_2/Dada), 1.86 (2H, m, C^6H_2/Dada), 2.53 and 2.61 (2H, dd, β-CH_2/Asn, J = 14.4, 5.7 Hz), 2.70–2.93 (8H, m, {C^3H_2, C^5H_2, C^7H_2, C^9H_2}/Dada), 3.00–3.28 (6H, m, {C^1H_2, $C^{12}H_2$}/Dada, δ-CH_2/Arg), 3.31 and 3.39 (each 1H, d, CH_2/Dhpa, J = 15.6 Hz), 3.78 (1H, t, α-CH/Arg), 4.38 and 4.41 (1H, t, α-CH/Asn, J = 5.7 Hz), 6.26 (2H, m, Ph-{C^3H, C^5H}/Dhpa), 6.90 (1H, m, C^6H/Dhpa). HRFAB-MS: found m/z 623.3961 $(M + H)^+$ (calcd for $C_{28}H_{50}N_{10}O_6$ + H: 623. 3993).

4,8-Bis(benzyloxycarbonyl)-N^1-[6-(benzyloxycarbonylamino)caproyl]-N^{12}-{N^α-[2,4-bis(benzyloxy)phenylacetyl]asparaginyl}-4,8-diaza-1,12-dodecanediamine (45). To a solution of **33** (120 mg, 0.117 mmol) in DMF (5 mL) were added TEA (26.0 mg, 0.257 mmol) and 6-(benzyloxycarbonylamino)caproic acid succinimidyl ester [Z-Acp-ONSu][18] (**35**) (46.6 mg, 0.129 mmol), and the mixture was worked up in a similar manner as described in General procedure 1. The crude product was purified by silica-gel column chromatography (Art. 9385, 14 g, 1 × 25 cm, $CHCl_3$ to $CHCl_3$:MeOH = 20:1) to give **45** as colorless crystals (0.106 g, 77.9%); mp 126–130 °C. ^{1}H NMR ($CDCl_3$) δ 1.20–1.84 (14H, m, {C^2H_2, C^6H_2, $C^{10}H_2$, $C^{11}H_2$}/Dada, {C^3H_2, C^4H_2, C^5H_2}/Acp), 2.15 (2H, m, C^2H_2/Acp), 2.23 and 2.71 (each 1H, dd, β-CH_2/Asn, J = 15.3, 6.0 Hz), 2.87–3.33 (14H, m, {C^1H_2, C^3H_2, C^5H_2, C^7H_2, C^9H_2, $C^{12}H_2$}/Dada, C^6H_2/Acp), 3.57 (2H, s, CH_2/Dhpa), 4.22 (1H, m, α-CH/Glu), 4.60 (1H, m, α-CH/Asn), 5.01 (2H, s, CH_2/Z), 5.08 (8H, m, CH_2/Z and CH_2/Bzl), 6.56 (1H, dd, Ph-C^3H/Dhpa, J = 8.1, 2.4 Hz), 6.64 (1H, d, Ph-C^5H/Dhpa, J = 2.4 Hz), 7.12 (1H, d, Ph-C^6H/Dhpa, J = 8.1 Hz), 7.22–7.43 (25H, m, Ph/Z and Ph/Bzl). HRFAB-MS: found m/z 1162.5859 $(M + H)^+$ (calcd for $C_{66}H_{80}N_7O_{12}$ + H: 1162.5865).

N^1-6-Aminocaproyl-N^{12}-[N^α-(2,4-dihydroxyphenylacetyl)-asparaginyl]-4,8-diaza-1,12-dodecanediamine {Acp-[des-Lys-(NPTX-594)]} Tris-trifluoroacetate (7). Compound **45** (60.0 mg, 56.0 μmol) in MeOH (5 mL) and AcOH (10 mL) was hydrogenated in the presence of Pd-black (50 mg) as a catalyst, and worked up in a similar manner as described in General procedure 2 to give **7** as a white powdery 3TFA salt (5.60 mg, 15.6%). ^{1}H NMR (D_2O) δ 1.19 (2H, m, C^4H_2/Acp), 1.26–1.56 (8H, m, {$C^{10}H_2$, $C^{11}H_2$}/Dada, {C^3H_2, C^5H_2}/Acp), 1.70 (2H, m, C^2H_2/Dada), 1.86 (2H, m, C^6H_2/Dada), 2.09 (2H, t, C^2H_2/Acp), 2.55 and 2.61 (2H, d, β-CH_2/Asn, J = 15.0, 7.5 Hz), 2.70–3.04 (10H, m, C^6H_2/Acp, {C^3H_2, C^5H_2, C^7H_2, C^9H_2}/Dada), 3.10 (2H, m, $C^{12}H_2$/Dada), 3.31 and 3.39 (each 1H, d, CH_2/Dhpa, J = 15.6 Hz), 3.46 and 3.54 (each 1H, m, C^1H_2/Dada), 4.40 (1H, dd, α-CH/Asn, J = 7.5, 7.5 Hz), 6.27 (2H, m, Ph-{C^3H, C^5H}/Dhpa), 6.91 (1H, m, Ph-C^6H/Dhpa). HRFAB-MS: found m/z 580.3823 $(M + H)^+$ (calcd for $C_{28}H_{49}N_7O_6$ + H: 580.3823).

4,8-Bis(benzyloxycarbonyl)-N^1-(N^α-benzyloxycarbonylglycyl)-N^{12}-{N^α-[2,4-bis(benzyloxy)phenylacetyl]asparaginyl}-4,8-diaza-1,12-dodecanediamine (46). To a solution of **33** (120 mg, 0.117 mmol) in DMF (15 mL) were added TEA (26.0 mg, 0.257 mmol) and *N*-benzyloxycarbonylglycine succinimidyl ester [Z-Gly-ONSu] (**36**) (39.5 mg, 0.129 mmol), and the mixture was worked up in a similar manner as described in General procedure 1. The crude product was purified by silica-gel column chromatography (Art. 9385, 14 g, 1 × 25 cm, $CHCl_3$ to $CHCl_3$:MeOH = 20:1) give **46** as colorless crystals (89.5 mg, 69.4%), mp 112–116 °C. ^{1}H NMR ($CDCl_3$) δ 1.18–1.43 (4H, m, {$C^{10}H_2$, $C^{11}H_2$}/Dada), 1.49–1.75 (4H, m, {C^2H_2, C^6H_2}/Dada), 2.26 and 2.70 (each 1H, dd, β-CH_2/Asn, J = 15.3, 6.9 Hz), 2.85–3.33 (12H, m, {C^1H_2, C^3H_2, C^5H_2, C^7H_2, C^9H_2, $C^{12}H_2$}/Dada), 3.56 (2H, s, CH_2/Dhpa), 3.82 (2H, s, CH_2/Gly), 4.59 (1H, m, α-CH/Asn), 5.01 (2H, s, CH_2/Z), 5.06 (8H, m, CH_2/Z and CH_2/Bzl), 6.55 (1H, dd, Ph-C^3H/Dhpa, J = 8.1, 2.4 Hz), 6.64 (1H, d, Ph-C^5H/Dhpa, J = 2.4 Hz), 7.12 (1H, d, Ph-C^6H/Dhpa, J = 8.1 Hz), 7.22–7.43 (25H, m, Ph/Z and Ph/Bzl). HRFAB-MS: found m/z 1106.5216 $(M + H)^+$ (calcd for $C_{62}H_{71}N_7O_{12}$ + H: 1106.5239).

N^{12}-[N^α-(2,4-Dihydroxyphenylacetyl)asparaginyl]-N^1-glycyl-4,8-diaza-1,12-dodecanediamine {Gly-[des-Lys-(NPTX-594)]} Tris-trifluoroacetate (8). Compound **46** (192 mg, 0.173 mmol) in MeOH (10 mL) and AcOH (20 mL) was hydrogenated in the presence of Pd-black (50 mg) as a catalyst, and worked up in a similar manner as described in General procedure 2 to give **8** as a white powdery 3TFA salt (66.4 mg, 60.4%). ^{1}H NMR (D_2O) δ 1.39 (4H, m, {$C^{10}H_2$, $C^{11}H_2$}/Dada), 1.77 (2H, m, C^2H_2/Dada), 1.91 (2H, m, C^6H_2/Dada), 2.60 and 2.68 (2H, dd, β-CH_2/Asn, J = 14.7, 7.8 Hz), 2.74–3.18 (10H, m, {C^3H_2, C^5H_2, C^7H_2, C^9H_2, $C^{12}H_2$}/Dada), 3.20 (2H, m, C^1H_2/Dada), 3.36 and 3.43 (each 1H, d, CH_2/Dhpa, J = 15.5 Hz), 3.66 (2H, s, α-CH_2/Gly), 4.40 (1H, dd, α-CH/Asn, J = 7.8, 7.8 Hz), 6.31 (2H, m, Ph-{C^3H, C^5H}/Dhpa), 6.95 (1H, m, Ph-C^6H/Dhpa). HRFAB-MS: found m/z 524.3187 $(M + H)^+$ (calcd for $C_{24}H_{42}N_7O_6$ + H: 524.3197).

N^1-(*O*-Benzyl-N^α-benzyloxycarbonylseryl)-4,8-bis(benzyloxycarbonyl)-N^{12}-{N^α-[2,4-bis (benzyloxy)phenylacetyl]asparaginyl}-4,8-diaza-1,12-dodecanediamine (47). To a solution of **33** (300 mg, 0.308 mmol) in DMF (10 mL) were added TEA (68.6 mg, 0.678 mmol) and *O*-benzyl-N^α-benzyloxycarbonylserine succinimidyl ester [Z-Ser(Bzl)-ONSu] (**37**) (145 mg, 0.339 mmol), and the mixture was worked up in a similar manner as described in General procedure 1. The crude product was purified by silica-gel

column chromatography (Art. 9385, 20 g, 1 × 40 cm, $CHCl_3$:MeOH = 97:3) to give **47** as colorless crystals (313 mg, 82.8%), mp 122–125 °C. ^{1}H NMR (DMSO-d_6) δ 1.14–1.42 (4H, m, {$C^{10}H_2$, $C^{11}H_2$}/Dada), 1.48–1.70 (4H, m, {C^2H_2, C^6H_2}/Dada), 2.22–2.56 (2H, m, β-CH_2/Asn), 2.80–3.20 (12H, m, {C^1H_2, C^3H_2, C^5H_2, C^7H_2, C^9H_2, $C^{12}H_2$}/Dada), 3.40 (2H, s, CH_2/Dhpa), 3.56 (1H, m, α-CH/Ser), 4.21 (1H, m, α-CH/Asn), 4.34 (2H, m, β-CH_2/Ser), 5.01–5.07 (12H, m, CH_2/Phe, CH_2/Z, and CH_2/Bzl), 6.51 (1H, d, Ph-C^3H/Dhpa, J = 8.1 Hz), 6.63 (1H, s, Ph-C^5H/Dhpa), 7.06 (1H, d, Ph-C^6H/Dhpa, J = 8.1 Hz), 7.20–7.43 (30H, m, Ph/Z and Ph/Bzl). HRFAB-MS: found m/z 1226.5757 $[M + H]^+$ (calcd for $C_{70}H_{79}N_7O_{13}$ + H: 1226.5814).

***N*^{12}-[*N*$^{\alpha}$-(2,4-Dihydroxyphenylacetyl)asparaginyl]-*N*^{1}-seryl-4,8-diaza-1,12-dodecanediamine {Ser-[des-Lys-(NPTX-594)]} Tris-trifluoroacetate (9).** Compound **47** (50 mg, 0.408 mmol) in MeOH (1 mL) and AcOH (2 mL) was hydrogenated in the presence of 10% $Pd(OH)_2$-C (50 mg) as a catalyst, and worked up in a similar manner as described in General procedure 2 to give **9** as a white powdery 3TFA salt (4.20 mg, 18.6%). ^{1}H NMR (D_2O) δ 1.35 (4H, m, {$C^{10}H_2$, $C^{11}H_2$}/Dada), 1.74 (2H, m, C^2H_2/Dada), 1.86 (2H, m, C^6H_2/Dada), 2.54 and 2.61 (each 1H, dd, β-CH_2/Asn, J = 15.9, 6.3 Hz), 2.68–3.24 (12H, m, {C^1H_2, C^3H_2, C^5H_2, C^7H_2, C^9H_2, $C^{12}H_2$}/Dada), 3.31 and 3.39 (each 1H, d, CH_2/Dhpa, J = 15.6 Hz), 3.75 (2H, m, β-CH_2/Ser), 3.86 (1H, m, α-CH/Ser), 4.38 and 4.41 (1H, t, α-CH/Asn, J = 6.3 Hz), 6.27 (2H, m, Ph-{C^3H, C^5H}/Dhpa), 6.91 (1H, m, Ph-C^6H/Dhpa). HRFAB-MS: found m/z 554.3278 $[M + H]^+$ (calcd for $C_{25}H_{43}N_7O_7$ + H: 554.3302).

4,8-Bis(benzyloxycarbonyl)-*N*^{1}-(*N*$^{\alpha}$-benzyloxycarbonylphenylalanyl)-*N*^{12}-{*N*$^{\alpha}$-[2,4-bis(benzyloxy)phenylacetyl]asparaginyl}-4,8-diaza-1,12-dodecanediamine (48). To a solution of **33** (300 mg, 0.308 mmol) in DMF (10 mL) were added TEA (68.6 mg, 0.678 mmol) and *N*$^{\alpha}$-benzyloxycarbonylphenylalanine succinimidyl ester [Z-Phe-ONSu] (**38**) (122 mg, 0.339 mmol), and it was worked up in a similar manner as described in General procedure 1. The crude product was purified by silica-gel column chromatography (Art. 9385, 20 g, 1 × 40 cm, $CHCl_3$:MeOH = 97:3) to give **48** as colorless crystals (238 mg, 64.7%); mp 117–120 °C. ^{1}H NMR (DMSO-d_6) δ 1.10–1.42 (4H, m, {$C^{10}H_2$, $C^{11}H_2$}/Dada), 1.46–1.72 (4H, m, {C^2H_2, C^6H_2}/Dada), 2.22–2.56 (2H, m, β-CH_2/Asn), 2.88–3.20 (12H, m, {C^1H_2, C^3H_2, C^5H_2, C^7H_2, C^9H_2, $C^{12}H_2$}/Dada), 3.40 (2H, s, CH_2/Dhpa), 4.16 (1H, m, α-CH/Phe), 4.50 (1H, m, α-CH/Asn), 4.92–5.07 (12H, m, CH_2/Phe, CH_2/Z, and CH_2/Bzl), 6.52 (1H, dd, Ph-C^3H/Dhpa, J = 8.6, 2.4 Hz), 6.67 (1H, d, Ph-C^5H/Dhpa, J = 2.4 Hz), 7.06 (1H, d, Ph-C^6H/Dhpa, J = 8.6 Hz), 7.20–7.43 (30H, m, Ph/Phe, Ph/Z, and Ph/Bzl). HRFAB-MS: found m/z 1196.5715 $(M + H)^+$ (calcd for $C_{69}H_{78}N_7O_{12}$ + H: 1196.5708).

***N*^{12}-[*N*$^{\alpha}$-(2,4-Dihydroxyphenylacetyl)asparaginyl]-*N*^{1}-phenylalanyl-4,8-diaza-1,12-dodecanediamine {Phe-[des-Lys-(NPTX-594)]} Tetrakis-trifluoroacetate (10).** Compound **48** (50 mg, 0.418 mmol) in MeOH (1 mL) and AcOH (2 mL) was hydrogenated in the presence of 10% $Pd(OH)_2$-C (50 mg) as a catalyst, and worked up in a similar manner as described in General procedure 2 to give **10** as a white powdery 3TFA salt (7.74 mg, 30.1%). ^{1}H NMR (D_2O) δ 1.36 (4H, m, {$C^{10}H_2$, $C^{11}H_2$}/Dada), 1.53 (2H, m, C^2H_2/Dada), 1.85 (2H, m, C^6H_2/Dada), 2.58 (2H, m, β-CH_2/Asn), 2.70–3.18 (14H, m, {C^1H_2, C^3H_2, C^5H_2, C^7H_2, C^9H_2, $C^{12}H_2$}/Dada, β-CH_2/Phe), 3.30 and 3.39 (each 1H, d, CH_2/Dhpa, J = 15.9 Hz), 3.97 (1H, t, α-CH/Phe), 4.40 (1H, t, α-CH/Asn), 6.27 (2H, m, Ph-{C^3H, C^5H/}Dhpa), 6.90 (1H, m, Ph-C^6H/Dhpa), 7.10 (2H, m, Ph-{C^3H, C^5H}/Phe), 7.19–7.26 (3H, m, Ph-C^2H/Phe, Ph-(C^4H)/Phe, Ph-C^6H/Phe). HRFAB-MS: found m/z 614.3664 $(M + H)^+$ (calcd for $C_{31}H_{47}N_7O_6$ + H: 614.3666).

***N*^{1}-(*O*$^{\gamma}$-Benzyl-*N*$^{\alpha}$-benzyloxycarbonylglutamyl)-4,8-bis(benzyloxycarbonyl)-*N*^{12}-{*N*$^{\alpha}$-[2,4-bis(benzyloxy)phenylacetyl]asparaginyl}-4,8-diaza-1,12-dodecanediamine (49).** To a solution of **33** (300 mg, 0.308 mmol) in DMF (15 mL) were added TEA (62.3 mg, 0.614 mmol) and *O*$^{\gamma}$-benzyl-*N*$^{\alpha}$-benzyloxycarbonylglutamic acid succinimidyl ester [Z-Glu(OBzl)-ONSu] (**39**) (173 mg, 0.370 mmol), and the reaction mixture was worked up in a similar manner as described in General procedure 1. The crude product was purified by silica-gel column chromatography (Art. 9385, 23 g, 1 × 40 cm, $CHCl_3$:MeOH = 98:2) to give **49** as colorless crystals (255 mg, 65.2%); mp 129–132 °C. ^{1}H NMR ($CDCl_3$) δ 1.26–1.38 (4H, m, {$C^{10}H_2$, $C^{11}H_2$}/Dada), 1.50–2.03 (6H, m, {C^2H_2, C^6H_2}/Dada, β-CH_2/Glu), 2.44 (2H, m, γ-CH_2/Glu), 2.24 and 2.71 (each 1H, dd, β-CH_2/Asn, J = 14.4, 6.3 Hz), 2.93–3.33 (12H, m, {C^1H_2, C^3H_2, C^5H_2, C^7H_2, C^9H_2, $C^{12}H_2$}/Dada), 3.57 (2H, s, CH_2/Dhpa), 4.22 (1H, m, α-CH/Glu), 4.60 (1H, m, α-CH/Asn,), 5.01 (2H, s, CH_2/Z), 5.06 (10H, m, CH_2/Z and CH_2/Bzl), 6.55 (1H, dd, Ph-C^3H/Dhpa, J = 8.6, 2.4 Hz), 6.63 (1H, d, Ph-C^5H/Dhpa, J = 2.4 Hz), 7.12 (1H, d, Ph-C^6H/Dhpa, J = 8.6 Hz), 7.22–7.43 (30H, m, Ph/Z and Ph/Bzl). HRFAB-MS: found m/z 1268.5916 $(M + H)^+$ (calcd for $C_{72}H_{81}N_7O_{14}$ + H: 1268.5920).

***N*^{12}-[*N*$^{\alpha}$-(2,4-Dihydroxyphenylacetyl)asparaginyl]-*N*^{1}-glutamyl-4,8-diaza-1,12-dodecanediamine {Glu-[des-Lys-(NPTX-594)]} Tris-trifluoroacetate (11).** Compound **49** (245 mg, 0.193 mmol) in MeOH (10 mL) and AcOH (20 mL) was hydrogenated in the presence of Pd-black (50 mg) as a catalyst, and worked up in a similar manner as described in General procedure 2 to give **11** as a white powdery 3TFA salt (53.2 mg, 29.4%). ^{1}H NMR (D_2O) δ 1.41 (4H, m, {$C^{10}H_2$, $C^{11}H_2$}/Dada), 1.79 (2H, m, C^2H_2/Dada), 1.91 (2H, m, C^6H_2/Dada), 2.04 (2H, dd, β-CH_2/Glu, J = 6.9, 7.8 Hz), 2.37 (2H, t, γ-CH_2/Glu, J = 7.8 Hz), 2.57 and 2.66 (2H, dd, β-CH_2/Asn, J = 15.0, 7.5 Hz), 2.75–3.20 (8H, m, {C^3H_2, C^5H_2, C^7H_2, C^9H_2}/Dada), 3.20–3.30 (4H, m, {C^1H_2, $C^{12}H_2$}/Dada), 3.36 and 3.43 (each 1H, d, CH_2/Dhpa, J = 15.6 Hz), 3.88 (1H, t, α-CH/Glu, J = 6.9 Hz), 4.44 (1H, t, α-CH/Asn, J = 7.5 Hz), 6.31 (2H, m, Ph-{C^3H, C^5H}/Dhpa), 6.95 (1H, m, Ph-C^6H/Dhpa). HRFAB-MS: found m/z 596.3372 $(M + H)^+$ (calcd for $C_{27}H_{45}N_7O_8$ + H: 596.3408).

***N*^{1}-(*O*$^{\beta}$-Benzyl-*N*$^{\alpha}$-benzyloxycarbonylaspartyl-4,8-bis(benzyloxycarbonyl)-*N*^{12}-{*N*$^{\alpha}$-[2,4-bis(benzyloxy)phenylacetyl]asparaginyl}-4,8-diaza-1,12-dodecanediamine (50).** To a solution of **33** (300 mg, 0.308 mmol) in DMF (10 mL) were added TEA (68.6 mg, 0.678 mmol) and *O*$^{\gamma}$-benzyl-*N*$^{\alpha}$-benzyloxycarbonylaspartic acid succinimidyl ester [Z-Asp(OBzl)-ONSu] (**40**) (154 mg, 0.339 mmol), and the reaction mixture was worked up in a similar manner as described in General procedure 1. The crude product was purified by silica-gel column chromatography (Art. 9385, 20 g, 1 × 40 cm $CHCl_3$:MeOH = 97:3) to give **50** as colorless crystal (0.338 mg, 87.6%); mp 121–125 °C. ^{1}H NMR (DMSO-d_6) δ 1.15–1.43 (4H, m, {$C^{10}H_2$, $C^{11}H_2$}/Dada), 1.45–1.73 (4H, m, {C^2H_2, C^6H_2}/Dada), 2.22–2.56 (4H, m, β-CH_2/Asn, β-CH_2/Asp), 2.88–3.20 (12H, m, {C^1H_2, C^3H_2, C^5H_2, C^7H_2, C^9H_2, $C^{12}H_2$}/Dada), 3.40 (2H, s, CH_2/Dhpa), 4.36 (1H, m, α-CH/Asp), 4.50 (1H, m, α-CH/Asn), 4.95–5.10 (12H, m, CH_2/Z and CH_2/Bzl), 6.51 (1H, dd, Ph-C^3H/Dhpa, J = 8.6, 2.4 Hz), 6.66 (1H, d, Ph-C^5H/Dhpa, J = 2.4 Hz), 7.05 (1H, d, Ph-C^6H/Dhpa, J = 8.6 Hz), 7.20–7.43 (30H, m, Ph/Z and Ph/Bzl). HRFAB-MS: found m/z 1254.5704 $(M + H)^+$ (calcd for $C_{71}H_{79}N_7O_{14}$ + H: 1254.5763).

N^1-Aspartyl-N^{12}-[N^{α}-(2,4-dihydroxyphenylacetyl)asparaginyl]-4,8-diaza-1,12-dodecanediamine {Asp-[des-Lys-(NPTX-594)]} Tris-trifluoroacetate (12). Compound **50** (30 mg, 23.9 μmol) in MeOH (1 mL) and AcOH (2 mL) was hydrogenated in the presence of 10% $Pd(OH)_2$-C (50 mg) as a catalyst, and worked up in a similar manner as described in General procedure 2 to give **12** as a white powdery 3TFA salt (5.99 mg, 27.0%). ^{1}H NMR (D_2O) δ 1.36 (4H, m, {$C^{10}H_2$, $C^{11}H_2$})Dada), 1.72 (2H, m, C^2H_2/Dada), 1.86 (2H, m, C^6H_2/Dada), 2.55 and 2.61 (2H, dd, β-CH_2/Asn, J = 15.0, 7.5 Hz), 2.70 (2H, d, β-CH_2/Asp, J = 6.0 Hz), 2.72–3.04 (10H, m, {C^3H_2, C^5H_2, C^7H_2, C^9H_2, $C^{12}H_2$}/Dada), 3.09–3.28 (each 1H, m, C^1H_2/Dada), 3.31 and 3.39 (each 1H, d, CH_2/Dhpa, J = 15.6 Hz), 4.05 (1H, t, α-CH/Asp, J = 6.0 Hz), 4.40 (1H, d, α-CH/Asn, J = 7.5 Hz), 6.27 (2H, m, Ph-{C^3H, C^5H}/Dhpa), 6.96 (1H, m, Ph-C^6H/Dhpa). HRFAB-MS: found m/z 582.3240 $(M + H)^+$ (calcd for $C_{26}H_{43}N_7O_8$ + H: 582.3251).

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18 Abbreviations according to IUPAC-IUB commission, *Eur. J. Biochem.*, **138**, 9 (1984), are used. Abg: *N*-(4-aminobutyl)-glycine; Acp: 6-aminocaproic acid = 6-aminohexanoic acid; AcOEt: ethyl acetate; Apa: *N*-(3-aminopropyl)-β-alanine; Arg: L-arginine; Asn: L-asparagine; Boc: *t*-butoxycarbonyl; Bzl: benzyl; DMF: *N*,*N*-dimethylformamide; DMSO: dimethyl sulfoxide; EDC·HCl: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; Glu: L-glutamic acid; Gly: glycine; HRFAB-MS: high resolution fast atom bombardment mass spectrometry; HONSu: *N*-hydroxysuccinimide; Lys: L-lysine; MALDI TOF-MS: matrix assisted laser desorption ionization time of flight mass spectrometry; MsCl: methanesulfonyl chloride; Np: *p*-nitrophenyl (or 4-nitrophenyl); Phe: L-phenylalanine; Pua: *N*-(4-aminobutyl)-β-alanine; RPHPLC: reversed-phase high-performance liquid chromatography; Ser: L-serine; TBAB: tetrabutylammonium bromide; TEA: triethylamine; TFA: trifluoroacetic acid; THF: tetrahydrofuran; Troc: 2,2,2-trichloroethoxycarbonyl; TrocCl: 2,2,2-trichloroethoxycarbonyl chloride; Z: benzyloxycarbonyl; ZCl: benzyloxycarbonyl chloride.

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