SYNTHESIS AND BIOLOGICAL ACTIVITY OF SPERGUALIN ANALOGUES. I.

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Stable spergualin analogues were synthesized by substitutions of the α-hydroxyglycine residue of spergualin with various α- or ω-amino acids.

The antitumor activity of these analogues against L1210 and their immunosuppressive effects on delayed-type hypersensitivity and antibody formation was then examined. Analogues substituted with glycine and L-serine showed significant biological activity but were less potent than 15-deoxyspergualin.

Among the analogues synthesized so far, 10-[*N*-4-(4-guanidinophenyl)butyryl-L-seryl]-1,5,10-triazadecane has possessed the strongest antitumor and immunosuppressive activities.

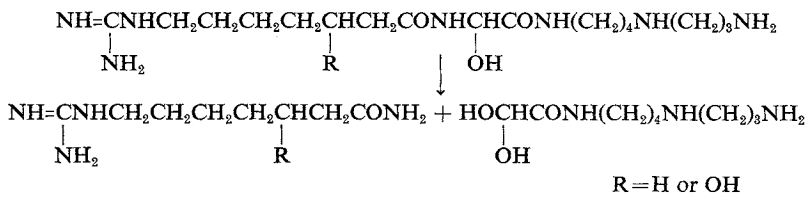
Spergualin, (−)-(15*S*)-1-amino-19-guanidino-11,15-dihydroxy-4,9,12-triazanonadecane-10,13-dione, is an antitumor antibiotic isolated from culture filtrates of *Bacillus laterosporus* BMG-162-aF2.[1,2] Recently, the immunosuppressive activity of spergualin has been reported.[3]

The total synthesis of spergualin has been accomplished.[4] The structure and antitumor activity relationships of spergualin analogues and derivatives against L1210 were also studied. Deoxyspergualin, the 15-deoxy analogue of spergualin, showed stronger antitumor[5] and immunosuppressive[6] activities than spergualin. This demonstrated that the 15-hydroxyl group was not essential for manifestation of these activities.

These analogues, however, were unstable due to an unusual hemiaminal structure at 10~12 position in the molecule. Consequently they hydrolyzed gradually into an ω-guanidino-fatty acid amide and a hydrate of glyoxyloylspermidine in an aqueous solution as shown in Scheme 1.

In this paper, the syntheses and structure activity of stable spergualin analogues are reported in which the α-hydroxyglycine residue corresponding to 10~12 position of deoxyspergualin is substituted with various α- or ω-amino acids.

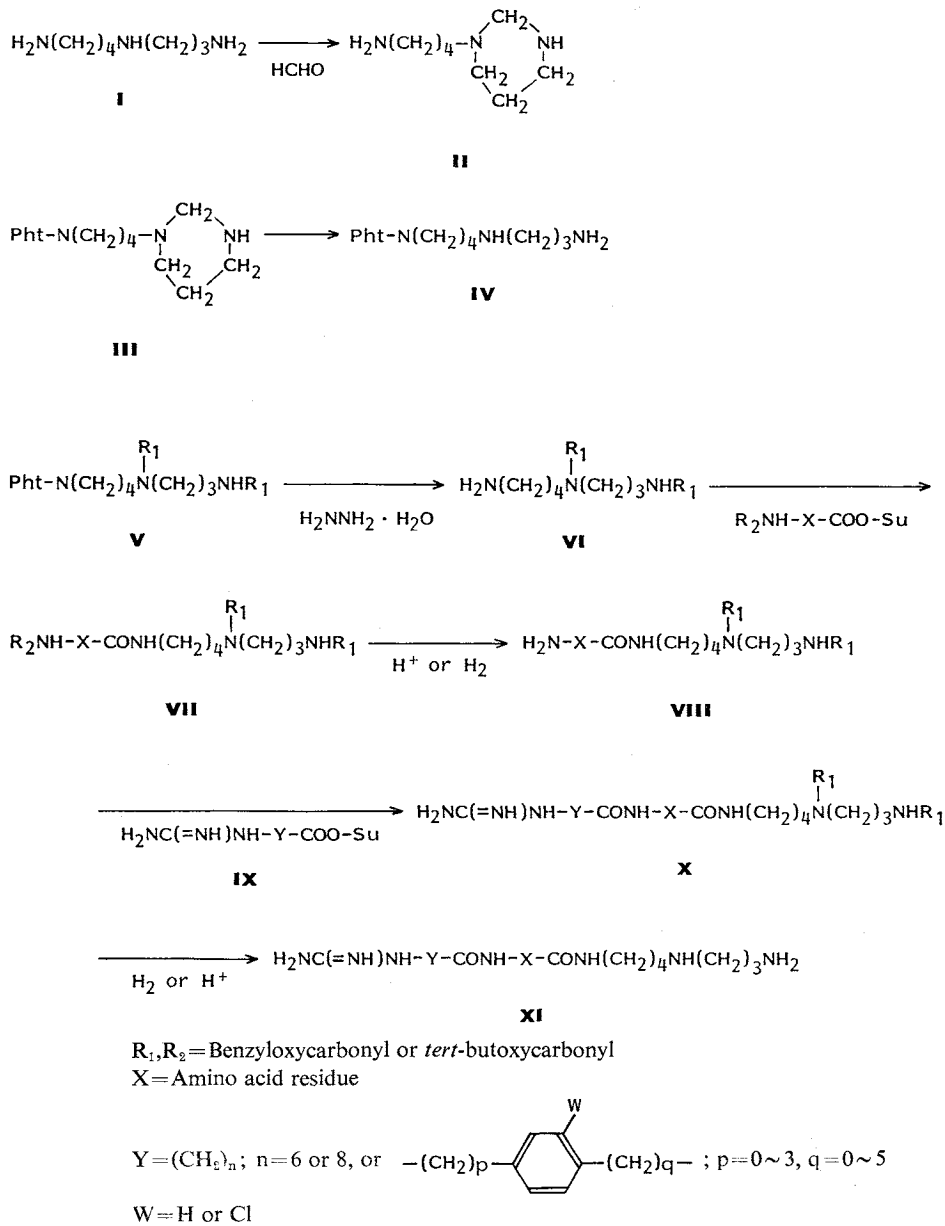
Scheme 1. Degradation of spergualins in an aqueous solution.



Synthesis

The general method for analogue synthesis is shown in Scheme 2. The main problem in this strategy is selective protection of the three amino groups of spermidine (I). I easily gives 1-(4-aminobutyryl)hexahydropyrimidine (II) after treating with paraformaldehyde. The resulting primary amino group can be protected selectively. But an acid sensitive protecting group cannot be applied, because an acid catalyzed ring opening of the hexahydropyrimidine is caused. The phthaloyl group was selected to protect the amino group at position-4 of II. It was treated with *N*-ethoxycarbonylphthalimide to give 1-(4-*N*-phthaloylaminobutyryl)hexahydropyrimidine (III) (not isolated), which was followed by

Scheme 2. General synthetic scheme for spergualin analogues.



R_1, R_2 = Benzyloxycarbonyl or *tert*-butoxycarbonyl

X = Amino acid residue

Y = $(CH_2)_n$; n = 6 or 8, or ; p = 0~3, q = 0~5

W = H or Cl

glacial acetic acid treatment to yield 10-*N*-phthaloyl-1,5,10-triazadecane (**VI**) in a 38.5%-yield from **II**. The two amino groups of **VI** were protected by benzyl or *tert*-butoxycarbonyl (BOC) groups using benzyl- or *tert*-butyl *S*-4,6-dimethylpyrimidin-2-yl thiolcarbonate, respectively. Then the phthaloyl group was removed by hydrazine, and the obtained 1,5-diprotected-1,5,10-triazadecane was coupled with protected amino acid *N*-hydroxysuccinimide ester (except benzyloxycarbonyl-DL-homoserine lactone in case of analogue **14**) to yield 10-(*N*-protected aminoacyl)-1,5-diprotected-1,5,10-triazadecane (**VII**). The amino acid protecting group in **VII** was selectively removed to give 10-aminoacyl-1,5-diprotected-1,5,10-triazadecane (**VIII**), which was subjected to reaction with ω-guanidino-fatty acid *N*-hydroxysuccinimide ester (**IX**) to give 10-[*N*-(ω-guanidino-fattyacyl)aminoacyl]-1,5-diprotected-1,5,10-triazadecane (**X**). Removal of two protecting groups from **X** by hydrogenolysis or acid treatment gave crude 10-[(*N*-(ω-guanidino-fattyacyl)aminoacyl]-1,5,10-triazadecane (**XI**). Purification of the crude product was performed by column chromatography using CM-Sephadex C-25 and Sephadex LH-20. The purity of the final compounds were confirmed to be over 99% by HPLC analysis.

Structure Activity Studies

Antitumor Activity against L1210 (IMC) Mouse Leukemia[1]

In order to determine the antitumor activity of these synthesized analogues, the life span prolongation of L1210 (IMC) bearing mice treated with the analogues for 9 days starting the day after tumor innoculation was assessed.

The antitumor activity of the analogues is summarized in Tables 1 and 2. The replacement of the

Table 1. Antitumor and immunosuppressive activities.

$H_2NC(=NH)NH$–Y–$CONH$–X–$CONH(CH_2)_4NH(CH_2)_3NH_2$

No.	Compound Y	Compound X	Antitumor activity[a] dose range over T/C=120%	DTH Dose (mg/kg/day) 6.25	DTH Dose (mg/kg/day) 12.5	DTH Dose (mg/kg/day) 25	PFC Dose (mg/kg/day) 6.25	PFC Dose (mg/kg/day) 12.5
1	Deoxyspergualin		0.20~25	85		92		89.4
2	$(CH_2)_6$	Gly	0.39~25	66		92		89.4
3	$(CH_2)_6$	L-Ala	NA		7		35.8	
4	$(CH_2)_6$	L-Leu	NA	5			0	
5	$(CH_2)_6$	L-Phe	NA	24			NT	
6	$(CH_2)_6$	L-Asp	NA		1			10
7	$(CH_2)_6$	L-Gln	NA		−14		NT	
8	$(CH_2)_6$	L-Pro	NA		4			5.8
9	$(CH_2)_6$	L-His	NA		10			41.0
10	$(CH_2)_6$	L-Arg	NA		22		NT	
11	$(CH_2)_6$	L-Ser	0.39~25		76			89.4
12	$(CH_2)_6$	D-Ser	NA	8	21			38.8
13	$(CH_2)_6$	L-Thr	NA		−8		NT	
14	$(CH_2)_6$	DL-Hos	NA		NT		NT	
15	$(CH_2)_6$	β-Ala	25		15		NT	
16	$(CH_2)_6$	γ-Aba	3.13~25		−3			72.7
17	$(CH_2)_6$	DL-HABA	0.78~12.5		50		53.6	
18	$(CH_2)_8$	L-Ser	0.78~6.25		NT		NT	

[a] Against L1210 (IMC) mouse leukemia (dose; mg/kg/day).

DTH: Delayed type hypersensitivity assay, PFC: plaque forming cell assay, Hos: homoserine, Aba: γ-aminobutyric acid, HABA: α-hydroxy-γ-aminobutyric acid.

NA: Not active, NT: not tested.

Table 2. Antitumor and immunosuppressive activities.

$H_2NC(=NH)NH$–Y–CONH–X–$CONH(CH_2)_4NH(CH_2)_3NH_2$

No.	Compound Y	X	Antitumor activity[a] dose range over T/C=120%	DTH Dose(mg/kg/day) 6.25	12.5	25	PFC Dose (mg/kg/day) 6.25	12.5
19	*p*-$C_6H_4(CH_2)_3$-	Gly	0.78~25			67		71.7
20	*p*-$C_6H_4(CH_2)_3$-	L-Ser	0.39~25	46		52		92.9
21	*p*-$(CH_2)_3C_6H_4$-	Gly	NA		NT			6.8
22	*p*-$(CH_2)_3C_6H_4$-	L-Ser	NA		NT		0	
23	*p*-$CH_2C_6H_4(CH_2)_2$-	L-Ser	6.25~12.5		NT			9.1
24	*m*-$CH_2C_6H_4(CH_2)_2$-	L-Ser	NA		NT			6.0
25	*p*-$C_6H_4(CH_2)_4$-	L-Ser	3.13~12.5		48			19.0
26	*p*-$C_6H_4(CH_2)_5$-	L-Ser	NA		NT			10.7
27	*p*-$CH_2C_6H_4$(*o*-Cl)$(CH_2)_2$-	L-Ser	NA		NT			NT

[a] Against L1210 (IMC) mouse leukemia (dose; mg/kg/day).
DTH: Delayed type hypersensitivity assay, PFC: plaque forming cell assay.
NA: Not active, NT: not tested.

α-hydroxyglycine moiety of deoxyspergualin (**1**) by amino acids was clearly successful. The analogues substituted with glycine (**2**), which is the 11-deoxy analogue of **1**, exhibited remarkable activity but their effective dose range was slightly narrower than that of **1**.

Substitution by L-Ala (analogue **3**), L-Leu (**4**), L-Phe (**5**), which are neutral amino acids, resulted in no activity. Analogues substituted with polar amino acids such as L-Asp(**6**), L-His (**9**), L-Arg (**10**) were also inactive.

Thus, the antitumor activity of the analogues substituted with α-amino acids having polar groups and bulky groups in the side chain was dramatically decreased. While L-Ser has a larger side chain than L-Ala, the analogue **11** expressed unexpectedly significant antitumor activity. L-Ser is considered to be the one methylene unit-inserted analogue of α-hydroxyglycine, so analogues with two more methylenes (analogues **13** and **14**) were synthesized, however they were inactive. Considering this, a hydrogen, hydroxyl or a hydroxymethyl group at position 11 in the molecular structure seems to be very important for the exhibition of strong antitumor activity.

When D-Ser (**12**) was introduced in place of L-Ser to investigate the role of stereochemistry at position 11, this analogue showed no activity. Analogue **12** bearing D-Ser showed a (+)-value for specific rotation, however, analogue **11** having L-Ser, **1** and spergualin,[7] which expressed strong activity, had a (−)-value. This result agrees well with the observation of UMEDA *et al.*[8] Because of difficulties in directly determining the absolute configuration of spergualin and **1**, our result was considered to be an alternative proof that an *S*-configuration of the stereochemistry of position 11 in these analogues is required for antitumor activity.

Among analogues replaced with ω-amino acids, the β-Ala analogue (**15**) showed no activity, while γ-aminobutyric acid (γ-Aba, **16**) exhibited moderate antitumor activity. An analogue substituted with α-hydroxy-γ-aminobutyric acid (HABA, **17**), which is the α-hydroxy analogue of **16**, showed stronger antitumor activity than **16**.

UMEDA *et al.*[5] reported that 1-amino-21-guanidino-11-hydroxy-4,9,12-triazauneicosane-10,13-dione, having two more methylene groups in the guanidino-fatty acid residue than **1**, showed a strong activity similar to **1**. Therefore, an analogue **18** with two more methylene groups than analogue **11**

was synthesized and tested for antitumor activity. As expected, analogue **18** revealed almost the same activity as analogues **2** and **11**.

Although, analogues **16** and **17** have two more methylene groups than analogue **2**, but not in the guanidino-fatty acid residue, these analogues, especially analogue **17**, had significant antitumor activity. These results indicate that not guanidino-fattyacyl but the guanidino-fattyacylaminoacyl moiety is strictly limited in the molecular length required to exhibit activity. Analogue **17** has a hydroxyl group at the 11 position in the molecule and exhibited a moderate antitumor activity. Thus, the importance of the hydroxyl group at this position was confirmed once again.

From these results, it is concluded that the α-hydroxylglycine residue could be replaced with a glycine or serine residue.

In the following experiments, the straight chain (position 14~19) of the guanidinoheptanoic acid moiety was substituted with aralkyl groups to examine the role of the 7 straight methylenes in antitumor activity. As shown in Table 2, three methylene units could be replaced with a benzene ring. Analogues **19** and **20**, bearing a 4-guanidinophenylbutyric acid expressed stronger activity than **2** and **11**.

When the benzene ring was migrated on the butyryl skeleton, the activity level was significantly changed. 4-Guanidinopropylbenzoyl analogues (**21** and **22**) showed no activity. However, 4-guanidinomethylphenylpropionyl analogue (**23**) had slight activity. In addition, the introduction of a chlorine atom in the ring (**27**) and a change of the substituted position (**24**) resulted in the loss of activity. As mentioned above about the analogues having guanidinoheptanoic acid, analogues with two more methylenes demonstrate a strong activity. In contrast, 4-guanidinophenylpentanoic acid (**25**) and 4-guanidinophenylhexanoic acid (**26**) analogues showed little or no antitumor activity. This difference seems to indicate that the conformation of **25** or **26** differs from that of **2** or **11** by introducing the benzene ring and the flexibility around the position 13~16 is indispensable for expression of the activity.

In conclusion, among the analogues so far synthesized, analogue **20** was the strongest in the antitumor activity.

Immunosuppressive Activity

Effects of the analogues on both cell-mediated (delayed type hypersensitivity, DTH[9]) and humoral (antibody formation, PFC[10]) were examined (Tables 1 and 2).

Analogues **2**, **11**, **19** and **20**, which had strong antitumor activity also showed the both immunosuppressive activities. Whereas **2** and **11** exhibited almost the same DTH inhibitory activity as **1**, they showed less PFC inhibition activity. Strong suppressions of both the DTH reaction and antibody formation were observed in **1**, but analogues **2**, **11**, **19** and **20** were weaker in the suppression of antibody formation than suppression of DTH.

The analogues synthesized in this study could be classified into three categories. Group A (**2**, **11**, **16**, **17**, **19** and **20**) had both antitumor and DTH-suppressive activity, group B had neither activity and group C (**25**) showed weak antitumor activity and stronger suppression of the DTH reaction than PFC. It was interesting that analogue **25** suppressed the DTH reaction more than the antibody formation as shown in Table 2.

The structure-activity relationships in the antitumor activity could be also applied to that of the immunological activities. This suggests that the mechanism of action of these compounds for these activities may be closely related.

Experimental

MP's were determined by a Shibata mp apparatus and were uncorrected. Optical rotations were measured by a Perkin-Elmer 141 automatic polarimeter. NMR spectroscopy was carried out on a Jeol-PMX-60 spectrometer. The abbreviations s, d, dd, m and br indicate singlet, doublet, double doublet, multiplet and broad, respectively. TLC was used routinely for monitoring the reactions; Merck precoated Silica gel plates (Art. 5715) were used and detection was carried out with UV absorption, or visualization with iodine, ninhydrin or Sakaguchi reagent. IR spectra were measured on a Model 260-30 Hitachi IR Spectrophotometer. Field desorption mass spectrometry (FD-MS) was carried out on a Jeol DX-300 spectrometer equipped with JMS-2000S Mass Data Analysis System; emitter current 15~20 mA and acceleration voltage 3 kV.

10-[*N*-(7-Guanidinoheptanoyl)-Gly]-1,5,10-triazadecane Trihydrochloride (Analogue **2**)

1) 10-Phthaloyl(Pht)-1,5,10-triazadecane Dihydrochloride (**IV**): 1-(4-Aminobutyl)hexahydropyrimidine (**II**) (55.0 g, 0.35 mol) and ethoxycarbonylphthalimide (92.0 g, 0.42 mol) were dissolved in 580 ml of DMSO. To the solution was added 42.0 g (0.4 mol) of AcOH and the mixture was allowed to react overnight at room temperature. The solution was concentrated under reduced pressure. The resulting residue was dissolved in 200 ml of water, then the pH of the solution was adjusted to 1.0 by 1 N HCl, followed by concentrating the solution. The residue obtained was recrystallized from EtOH to provide 46.9 g of 10-Pht-1,5,10-triazadecane dihydrochloride (**IV**) in a yield of 38.5%: MP 244~246°C; ^{1}H NMR (D_2O) δ 1.5~2.0 (4H, br), 2.0~2.5 (2H, m), 2.9~3.5 (6H, br), 3.5~3.9 (2H, br), 7.76 (4H, s).

2) 10-Pht-1,5-di-Z-1,5,10-triazadecane (**V**, R_1=Z): **IV** (27.9 g, 80.0 mmol) was benzyloxycarbonylated using 43.9 g (160 mmol) of benzyl *S*-4,6-dimethylpyrimidin-2-ylthiolcarbonate and 17.8 g (176 mmol) of Et_3N in 300 ml of $CHCl_3$. The reaction mixture was subjected to react for 6 hours at room temperature. The resulting solution was washed with 1 N HCl and satd NaCl aqueous solution and dried over anhydrous Na_2SO_4. Evaporation of $CHCl_3$ gave 43.1 g of oily **V** in a quantitative yield: ^{1}H NMR ($CHCl_3$) δ 1.3~2.1 (6H, br), 1.9~3.9 (8H, m), 5.1 (4H, s), 7.30 (5H, s), 7.33 (5H, s), 7.73 (4H, m).

3) 1,5-Di-Z-1,5,10-triazadecane Dihydrochloride (**VI**, R_1=Z): **V** (R_1=Z, 31.3 g, 57.6 mmol) was treated overnight with 18.2 g (0.291 mol) of 80% hydrazine hydrate in 600 ml of EtOH under reflux. The precipitates were removed by filtration, and the filtrate was concentrated under reduced pressure. The residue was taken up by 300 ml of EtOAc, then **VI** was extracted with dil HCl. The aqueous layer was washed with EtOAc, then Na_2CO_3 was added to bring the pH of the solution to 10. An oily deposit was extracted with 500 ml of EtOAc. The EtOAc layer was washed with satd NaCl aqueous solution, then dried over anhydrous Na_2SO_4. The filtrate was concentrated under reduced pressure, providing 20.1 g of **VI** (R_1=Z) in a yield of 84.8%: ^{1}H NMR ($CHCl_3$) δ 1.0~2.3 (8H, br), 2.3~2.9 (2H, br), 2.9~3.5 (6H, m), 5.05 (2H, s), 5.07 (2H, s), 5.1~6.1 (1H, br), 7.30 (10H, s).

4) 10-(*N*,*N*-Pht-Gly)-1,5-di-Z-1,5,10-triazadecane (**VII**, R_1=Z, R_2=Pht, X=Gly): **VI** (R_1=Z, 12.4 g, 30 mmol), 10.6 g (35 mmol) of Pht-Gly-O-succinimide (Su) and 4.9 ml (35 mmol) of Et_3N was treated in 200 ml of THF at room temperature overnight. The resulting solution was condensed under reduced pressure, then the residue was dissolved in 1,200 ml of EtOAc. The layer was washed with 5% $NaHCO_3$ solution, 0.5 N HCl and NaCl aqueous solution, successively and dried over anhydrous Na_2SO_4. The filtrate was concentrated under reduced pressure. The resulting residue was crystallized from EtOAc and ether to yield 14.6 g of **VII** in a yield of 81.0%: MP 102~104°C; ^{1}H NMR (DMSO-d_6) δ 1.0~2.2 (6H, br), 2.7~3.6 (8H, br), 4.20 (2H, s), 5.01 (2H, s), 5.03 (2H, s), 6.8~8.4 (2H, br), 7.30 (10H, s), 7.84 (4H, s); Rf 0.4 ($CHCl_3$ - MeOH - AcOH, 95 : 5 : 3).

5) 10-Gly-1,5-di-Z-1,5,10-triazadecane (**VIII**, R_1=Z, X=Gly): When **VII** (14.4 g, 24 mmol) and 6.0 g of 80% hydrazine hydrate was treated in 370 ml of EtOH in a similar manner to **VI**, **VIII** was obtained as an oil in a quantitative yeild: ^{1}H NMR ($CDCl_3$) δ 0.8~2.1 (6H, br), 2.8~3.5 (10H, br), 5.0~6.1 (2H, br), 5.06 (2H, s), 5.10 (2H, s), 7.33 (10H, s); Rf 0.1 ($CHCl_3$ - MeOH - AcOH, 95 : 5 : 3).

6) 7-Guanidinoheptanoic Acid *N*-Hydroxysuccinimide Ester Hydrochloride (**IX**, Y=$(CH_2)_6$): 7-Guanidinoheptanoic acid (1.87 g, 10 mmol)[5] was dissolved in 1 N HCl, and the solution was con-

centrated to dryness under reduced pressure to obtain 2.24 g of 7-guanidinoheptanoic acid hydrochloride. This was dissolved in 10 ml of DMF, and 2.06 g of dicyclohexylcarbodiimide (DCCD) and 1.00 g (12 mmol) of *N*-hydroxysuccinimide (HOSu) were added to this solution under ice cooling. The solution was stirred for 30 minutes at 0°C, followed by standing at room temperature overnight. The solution was evaporated after filtrating the precipitates. Washing with petroleum ether and drying under reduced pressure of the resulting oily material gave 3.65 g of **IX**: ^{1}H NMR (DMSO-d_6) δ 1.1~2.0 (8H, br), 2.67 (2H, t, J=6 Hz), 2.84 (4H, s), 3.1 (2H, m), 7.3 (1H, br), 8.0 (4H, br).

7) 10-[*N*-(7-Guanidinoheptanoyl)-Gly]-1,5-di-Z-1,5,10-triazadecane Hydrochloride (**X**, R_1=Z, X=Gly, Y=$(CH_2)_6$): **VIII** (12.5 g, 24 mmol), 11.3 g of 7-guanidinoheptanoic acid *N*-hydroxysuccinimide ester hydrochloride (**IX**) and 4.0 ml (29 mmol) of Et_3N was dissolved in 120 ml of THF and 50 ml of DMF. The resulting solution was stirred overnight at room temperature. Concentration of the reaction mixture gave an oily residue, which was taken up with 700 ml of EtOAc. The EtOAc layer was washed with 0.5 N HCl saturated with NaCl and satd NaCl aqueous solution. An oily material which precipitated during these washings was dissolved by addition of a small amount of EtOH. Then the EtOAc layer was dried over anhydrous Na_2SO_4. The filtrate was evaporated to obtain 20.8 g of **X** in a quantitative yield as an oil: ^{1}H NMR ($CDCl_3$) δ 0.8~1.9 (14H, br), 1.9~2.4 (2H, br), 2.7~3.5 (10H, br), 3.5~4.0 (2H, br), 5.04 (4H, s), 6.2~8.0 (8H, br), 7.27 (10H, s); Rf 0.3 (BuOH - AcOH - H_2O, 4:1:1).

8) 10-[*N*-(7-Guanidinoheptanoyl)-Gly]-1,5,10-triazadecane Trihydrochloride (**XI**, X=Gly, Y=$(CH_2)_6$) (Analogue **2**): **X** (20.8 g, 24 mmol) was dissolved in 300 ml of MeOH and 10 ml of AcOH, and hydrogenated over 0.5 g of palladium black for 3 hours at room temperature. The filtrate was concentrated under reduced pressure, which was dissolved in 70 ml of water. The solution was passed through a column packed with 1,500 ml of CM-Sephadex C-25 (Na^+). Active fractions were collected by eluting the column in the mode of gradient between 7,500 ml of water and 7,500 ml of 1 N NaCl solution. After the collected fractions were concentrated to dryness, MeOH was added to the residue, and insoluble materials were filtered off. In order to remove the small amount of NaCl, the resulting oily material was dissolved in 50 ml of MeOH. The solution was chromatographed on a column packed with 400 ml of Sephadex LH-20 to obtain 5.30 g of **XI**. Lyophilization gave purified analogue **2** in a yield of 45.1%: MP 163~165°C; ^{1}H NMR (DMSO-d_6) δ 0.9~1.8 (12H, br), 1.8~2.4 (4H, br), 2.6~3.3 (10H, br), 3.63 (2H, d, J=5 Hz), 6.9~9.2 (12H, br); IR ν_{max} (KBr) cm^{-1} 3410, 3310, 3150, 2930, 1640, 1520, 1470, 1410, 1160, 965; Rf 0.4 (PrOH - pyridine - H_2O - AcOH, 6:4:3:2); FD-MS *m/z* 372 (M+1).

10-[*N*-(7-Guanidinoheptanoyl)-L-Ser]-1,5,10-triazadecane Trihydrochloride (**XI**, X=Ser, Y=$(CH_2)_6$) (Analogue **11**)

1) 10-(BOC-OBzl-L-Ser)-1,5-di-Z-1,5,10-triazadecane (**VII**, R_1=Z, R_2=BOC, X=OBzl-L-Ser): **VI** (4.76 g, 11.5 mmol), 1.04 g (10.3 mmol) of Et_3N and 5.87 g (15 mmol) of BOC-OBzl-L-Ser-OSu were treated in a similar manner as the preparation of **VII** (R_1=Z, R_2=Pht, X=Gly). Yield 8.34 g (quantitative): ^{1}H NMR ($CDCl_3$) δ 0.8~2.2 (6H, br), 1.46 (9H, s), 2.7~3.5 (8H, br), 3.66 (2H, m), 3.9~4.4 (1H, br), 4.5 (2H, s), 5.11 (4H, s), 5.1~5.4 (2H, br), 6.1~6.8 (1H, br), 7.33 (15H, s); Rf 0.8 ($CHCl_3$ - MeOH, 9:1).

2) 10-(OBzl-L-Ser)-1,5-di-Z-1,5,10-triazadecane (**VIII**, R_1=Z, X=OBzl-L-Ser): 10-(BOC-OBzl-L-Ser)-1,5-di-Z-1,5,10-triazadecane 8.00 g (11.5 mmol) was treated with 8 ml of TFA for 3 hours at room temperature.

The reaction mixture was concentrated to give an oil, which was dissolved in 200 ml of EtOAc. The EtOAc layer was washed with 5% $NaHCO_3$ aqueous solution and water, then dried over anhydrous Na_2SO_4. The filtrate was evaporated to provide 6.82 g of 10-(OBzl-L-Ser)-1,5-di-Z-1,5,10-triazadecane: ^{1}H NMR ($CDCl_3$) δ 1.2~2.0 (6H, br), 1.74 (2H, s), 2.8~3.5 (8H, br), 3.64 (3H, m), 4.51 (2H, s), 5.12 (4H, s), 4.6~6.0 (2H, br), 7.33 (15H, s); Rf 0.5 ($CHCl_3$ - MeOH, 9:1).

3) 10-[*N*-(7-Guanidinoheptanoyl)-OBzl-L-Ser]-1,5-di-Z-1,5,10-triazadecane Hydrochloride (**X**, R_1=Z, X=OBzl-L-Ser, Y=$(CH_2)_6$): 10-(OBzl-L-Ser)-1,5-di-Z-1,5,10-triazadecane 3.56 g (6.03 mmol) and 6.14 g (7 mmol) of 7-guanidinoheptanoic acid *p*-nitrophenyl ester were treated in a similar manner

as the preparation of **X** (R_1=Z, X=Gly, Y=$(CH_2)_6$) to give 4.77 g of 10-[*N*-(7-guanidinoheptanoyl)-OBzl-L-Ser]-1,5-di-Z-1,5,10-triazadecane hydrochloride: ^{1}H NMR (DMSO-d_6) δ 1.0~2.0 (14H, br), 2.0~2.4 (2H, br), 2.7~3.5 (10H, br), 3.58 (2H, d, J=5 Hz), 4.2~4.8 (1H, br), 4.47 (2H, s), 5.01 (2H, s), 5.04 (2H, s), 6.8~8.3 (8H, br), 7.27 (5H, s), 7.30 (10H, s); Rf 0.2 ($CHCl_3$ - MeOH - AcOH, 9 : 1 : 0.3).

10-[*N*-(7-Guanidinoheptanoyl)-L-Ser]-1,5,10-triazadecane Trihydrochloride (**11**)

10-[*N*-(7-Guanidinoheptanoyl)-OBzl-L-Ser]-1,5-di-Z-1,5,10-triazadecane hydrochloride 2.50 g (2.65 mmol) was treated in a similar manner as the preparation of analogue **2**. Analogue **11** (0.50 g) was obtained in a yield of 36.6%: ^{1}H NMR (DMSO-d_6) δ 0.8~1.8 (12H, br), 1.8~2.4 (4H, br), 2.5~3.4 (10H, br), 3.57 (2H, d, J=5 Hz), 4.18 (1H, m), 5.5~6.5 (1H, br), 6.7~9.5 (12H, br); IR ν_{max} (KBr) cm^{-1} 3350, 2940, 1640, 1535, 1465, 1375, 1160, 1060, 965; Rf 0.3 (PrOH - pyridine - H_2O - AcOH, 6 : 4 : 3 : 2); $[\alpha]_D^{27}$ −15.2° (*c* 1.0, H_2O); FD-MS *m/z* 402 (M+1).

Procedures similar to that used for analogue **11** were followed in the preparations of analogues **3**~**10** and **12**~**15**.

10-[*N*-(7-Guanidinoheptanoyl)-L-Ala]-1,5-di-Z-1,5,10-triazadecane Hydrochloride (**X**, R_1=Z, X=L-Ala, Y=$(CH_2)_6$)

^{1}H NMR (DMSO-d_6) δ 0.9~2.0 (17H, br), 2.0~2.3 (2H, br), 2.3~3.5 (10H, br), 4.0~4.5 (1H, br), 4.99 (2H, s), 5.03 (2H, s), 6.3~8.7 (8H, br), 7.3 (10H, s); Rf 0.8 (PrOH - pyridine - H_2O - AcOH, 6 : 4 : 3 : 2).

10-[*N*-(7-Guanidinoheptanoyl)-L-Ala]-1,5,10-triazadecane Trihydrochloride (**XI**, X=L-Ala, Y=$(CH_2)_6$) (Analogue **3**)

^{1}H NMR (DMSO-d_6) δ 0.9~1.8 (15H, br), 1.8~2.4 (4H, br), 2.6~3.4 (10H, br), 3.9~4.5 (1H, br), 5.9~8.2 (12H, br); IR ν_{max} (KBr) cm^{-1} 3380, 2940, 1640, 1540, 1370, 1160, 965; Rf 0.4 (PrOH - pyridine - H_2O - AcOH, 6 : 4 : 3 : 2); $[\alpha]_D^{27}$ −21.6° (*c* 1.2, H_2O); FD-MS *m/z* 386 (M+1).

10-[*N*-(7-Guanidinoheptanoyl)-L-Leu]-1,5-di-Z-1,5,10-triazadecane Hydrochloride (**X**, R_1=Z, X=L-Leu, Y=$(CH_2)_6$)

^{1}H NMR (DMSO-d_6) δ 0.6~1.0 (6H, br), 1.1~1.9 (16H, br), 1.9~2.4 (3H, br), 2.7~3.5 (10H, br), 3.8~4.6 (1H, m), 5.06 (2H, s), 5.10 (2H, s), 6.8~8.3 (8H, br), 7.33 (s, 10H); Rf 0.5 (BuOH - AcOH - H_2O, 4 : 1 : 1).

10-[*N*-(7-Guanidinoheptanoyl)-L-Leu]-1,5,10-triazadecane Trihydrochloride (**XI**, X=L-Leu, Y=$(CH_2)_6$) (Analogue **4**)

^{1}H NMR (DMSO-d_6) δ 0.5~1.0 (6H, br), 1.0~1.9 (15H, br), 1.9~2.3 (4H, br), 2.6~3.5 (10H, br), 3.9~4.5 (1H, br), 6.8~9.1 (12H, br); IR ν_{max} (KBr) cm^{-1} 3400, 2945, 1645, 1535, 1465, 1370, 1165, 970; Rf 0.4 (PrOH - pyridine - H_2O - AcOH, 6 : 4 : 3 : 2); $[\alpha]_D^{27}$ −20.5° (*c* 1.4, H_2O); FD-MS *m/z* 428 (M+1).

10-[*N*-7-Guanidinoheptanoyl)-L-Phe]-1,5-di-Z-1,5,10-triazadecane Hydrochloride (**X**, R_1=Z, X=L-Phe, Y=$(CH_2)_6$)

^{1}H NMR (DMSO-d_6) δ 0.9~2.4 (16H, br), 2.7~3.7 (10H, br), 3.5 (2H, s), 4.3~4.7 (1H, br), 4.98 (2H, s), 5.02 (2H, s), 6.9~8.3 (8H, br), 7.17 (5H, s), 7.30 (10H, s); Rf 0.5 (BuOH - AcOH - H_2O, 4 : 1 : 1).

10-[*N*-(7-Guanidinoheptanoyl)-L-Phe]-1,5,10-triazadecane Trihydrochloride (**XI**, X=L-Phe, Y=$(CH_2)_6$) (Analogue **5**)

^{1}H NMR (DMSO-d_6) δ 0.9~1.8 (12H, br), 1.9~2.5 (4H, br), 2.7~3.5 (12H, br), 4.2~4.7 (1H, br), 6.9~9.3 (12H, br), 7.22 (5H, s); IR ν_{max} (KBr) cm^{-1} 3320, 2930, 1645, 1530, 1455, 1370, 965, 700; Rf 0.4 (PrOH - pyridine - H_2O - AcOH, 6 : 4 : 3 : 2); $[\alpha]_D^{27}$ +6.7° (*c* 1.2, H_2O); FD-MS *m/z* 462 (M+1).

10-[*N*-(7-Guanidinoheptanoyl)-β-Bzl-L-Asp]-1,5-di-Z-1,5,10-triazadecane Hydrochloride (**X**, R_1=Z, X=β-Bzl-L-Asp, Y=$(CH_2)_6$)

^{1}H NMR (DMSO-d_6) δ 1.0~2.5 (14H, br), 1.9~2.4 (2H, br), 2.4~3.7 (12H, br), 4.3~4.9 (1H, br), 5.00 (2H, s), 5.05 (4H, s), 5.2~6.4 (3H, br), 6.7~8.0 (5H, br), 7.33 (15H, s); Rf 0.4 (BuOH - AcOH -

H_2O, 4:1:1).

10-[*N*-(7-Guanidinoheptanoyl)-L-Asp]-1,5,10-triazadecane Trihydrochloride (**XI**, X=L-Asp, Y=$(CH_2)_6$) (Analogue **6**)

^1H NMR (DMSO-d_6) δ 0.9~1.8 (12H, br), 1.8~2.6 (6H, br), 2.7~3.5 (10H, br), 4.1~4.7 (1H, br), 6.9~8.7 (13H, br); IR ν_{max} (KBr) cm^{-1} 3320, 2935, 1640, 1550, 1470, 1390, 1310, 1170, 965; Rf 0.4 (PrOH - pyridine - H_2O - AcOH, 6:4:3:2); $[\alpha]_D^{27}$ −10.3° (*c* 1.5, H_2O); FD-MS *m/z* 430 (M+1).

10-[*N*-(7-Guanidinoheptanoyl)-L-Gln]-1,5-di-Z-1,5,10-triazadecane Hydrochloride (**X**, R_1=Z, X=L-Gln, Y=$(CH_2)_6$)

^1H NMR (DMSO-d_6) δ 0.9~2.4 (20H, br), 2.6~3.8 (10H, br), 3.9~4.3 (1H, br), 4.99 (2H, s), 5.04 (2H, s), 6.5~8.3 (10H, br), 7.34 (10H, s); Rf 0.1 ($CHCl_3$ - MeOH - AcOH, 8:2:0.5).

10-[*N*-(7-Guanidinoheptanoyl)-L-Gln]-1,5,10-triazadecane Trihydrochloride (**XI**, X=L-Gln, Y=$(CH_2)_6$) (Analogue **7**)

^1H NMR (DMSO-d_6) δ 0.8~1.8 (14H, br), 1.8~2.4 (6H, br), 2.6~3.8 (12H, br), 4.0~4.3 (1H, m), 6.5~9.2 (12H, br); IR ν_{max} (KBr) cm^{-1} 3400, 2940, 1655, 1540, 1455, 1160, 965; Rf 0.4 (PrOH - pyridine - H_2O - AcOH, 6:4:3:2); $[\alpha]_D^{23}$ −11.2° (*c* 1.1, H_2O); FD-MS *m/z* 443 (M+1).

10-[*N*-(7-Guanidinoheptanoyl)-L-Pro]-1,5-di-Z-1,5,10-triazadecane Hydrochloride (**X**, R_1=Z, X=L-Pro, Y=$(CH_2)_6$)

^1H NMR (DMSO-d_6) δ 0.9~2.4 (20H, br), 2.7~3.7 (12H, br), 4.0~4.4 (1H, br), 5.00 (2H, s), 5.04 (2H, s), 6.5~7.9 (7H, br), 7.33 (10H, s); Rf 0.3 (BuOH - AcOH - H_2O, 4:1:1).

10-[*N*-(7-Guanidinoheptanoyl)-L-Pro]-1,5,10-triazadecane Trihydrochloride (**XI**, X=L-Pro, Y=$(CH_2)_6$) (Analogue **8**)

^1H NMR (DMSO-d_6) δ 1.0~2.4 (20H, br), 2.5~3.9 (12H, br), 4.0~4.5 (1H, br), 6.7~9.3 (11H, br); IR ν_{max} (KBr) cm^{-1} 3400, 2940, 1640, 1450, 1160, 965; Rf 0.3 (PrOH - pyridine - H_2O - AcOH, 6:4:3:2); $[\alpha]_D^{27}$ −42.3° (*c* 1.3, H_2O); FD-MS *m/z* 412 (M+1).

10-[*N*-(7-Guanidinoheptanoyl)-N^{im}-Z-L-His]-1,5-di-Z-1,5,10-triazadecane Hydrochloride (**X**, R_1=Z, X=N^{im}-Z-L-His, Y=$(CH_2)_6$)

^1H NMR (DMSO-d_6) δ 0.9~2.0 (14H, br), 2.0~3.5 (14H, br), 4.0~4.5 (1H, br), 4.99 (2H, s), 5.03 (2H, s), 5.10 (2H, s), 6.7~8.0 (12, br), 7.31 (15H, s); Rf 0.4 (BuOH - AcOH - H_2O, 4:1:1).

10-[*N*-(7-Guanidinoheptanoyl)-L-His]-1,5,10-triazadecane Trihydrochloride (**XI**, X=L-His, Y=$(CH_2)_6$) (Analogue **9**)

^1H NMR (DMSO-d_6) δ 0.9~1.8 (12H, br), 1.8~2.3 (4H, br), 2.7~3.5 (12H, br), 4.3~4.9 (1H, br), 6.3~9.0 (17H, br); IR ν_{max} (KBr) cm^{-1} 3370, 2940, 1650, 1640, 1540, 1460, 1370, 1165, 1080; Rf 0.3 (PrOH - pyridine - H_2O - AcOH, 6:4:3:2); $[\alpha]_D^{27}$ −2.8° (*c* 1.2, H_2O); FD-MS *m/z* 452 (M+1).

10-[*N*-(7-Guanidinoheptanoyl)-N^g-NO_2-L-Arg]-1,5-di-Z-1,5,10-triazadecane Hydrochloride (**X**, R_1=Z, X=N^g-NO_2-L-Arg, Y=$(CH_2)_6$)

^1H NMR (DMSO-d_6) δ 1.0~1.9 (18H, br), 1.9~2.5 (2H, br), 2.7~3.8 (12H, br), 4.1~4.5 (1H, br), 5.01 (2H, s), 5.04 (2H, s), 6.0~8.4 (11H, br), 7.30 (10H, s); Rf 0.8 (PrOH - pyridine - H_2O - AcOH, 6:4:3:2).

10-[*N*-(7-Guanidinoheptanoyl)-L-Arg]-1,5,10-triazadecane Trihydrochloride (**XI**, X=L-Arg, Y=$(CH_2)_6$) (Analogue **10**)

^1H NMR (DMSO-d_6) δ 0.9~1.9 (16H, br), 1.9~2.3 (4H, br), 2.7~3.8 (12H, br), 4.0~4.5 (1H, br), 6.5~9.2 (17H, br); IR ν_{max} (KBr) cm^{-1} 3330, 2930, 1640, 1530, 1460, 1365, 1250, 1160, 1100; Rf 0.2 (PrOH - pyridine - H_2O - AcOH, 6:4:3:2); $[\alpha]_D^{23}$ −7.2° (*c* 1.2, H_2O); FD-MS *m/z* 471 (M+1).

10-[*N*-(7-Guanidinoheptanoyl)-OBzl-D-Ser]-1,5-di-Z-1,5,10-triazadecane Hydrochloride (**X**, R_1=Z, X=OBzl-D-Ser, Y=$(CH_2)_6$)

^1H NMR (DMSO-d_6) δ 1.0~2.0 (14H, br), 2.0~2.4 (2H, br), 2.7~3.5 (10H, br), 3.58 (2H, d, *J*=5

Hz), 4.2~4.8 (1H, br), 4.47 (2H, s), 5.01 (2H, s), 5.04 (2H, s), 6.7~8.3 (8H, br), 7.27 (5H, s), 7.30 (10H, s); Rf 0.2 ($CHCl_3$ - MeOH - AcOH, 9 : 1 : 0.5).

10-[*N*-(7-Guanidinoheptanoyl)-D-Ser]-1,5,10-triazadecane Trihydrochloride (**XI**, X=D-Ser, Y=$(CH_2)_6$) (Analogue **12**)

^{1}H NMR (DMSO-d_6) δ 0.8~1.9 (12H, br), 1.8~2.4 (4H, br), 2.6~3.4 (10H, br), 3.58 (2H, d, J=5 Hz), 4.18 (1H, m), 5.0~5.8 (1H, br), 6.7~9.4 (12H, br); IR ν_{max} (KBr) cm^{-1} 3350, 2940, 1640, 1535, 1460, 1360, 1160, 1060, 965; Rf 0.4 (PrOH - pyridine - H_2O - AcOH, 6 : 4 : 3 : 2); $[\alpha]_D^{27}$ +15.3° (*c* 1.0, H_2O), FD-MS *m/z* 402 (M+1).

10-[*N*-(7-Guanidinoheptanoyl)-OBzl-L-Thr]-1,5-di-Z-1,5,10-triazadecane Hydrochloride (**X**, R_1=Z, X=OBzl-L-Thr, Y=$(CH_2)_6$)

^{1}H NMR (DMSO-d_6) δ 0.6~2.4 (19H, br), 2.6~3.5 (10H, br), 3.5~4.3 (2H, br), 4.3~4.6 (2H, br), 4.6~5.2 (3H, br), 4.98 (2H, s), 5.02 (2H, s), 6.5~8.0 (5H, br), 7.2 (5H, s), 7.3 (10H, s); Rf 0.7 (BuOH - AcOH - H_2O, 4 : 1 : 1).

10-[*N*-(7-Guanidinoheptanoyl)-L-Thr]-1,5,10-triazadecane Trihydrochloride (**XI**, X=L-Thr, Y=$(CH_2)_6$) (Analogue **13**)

^{1}H NMR (DMSO-d_6) δ 1.05 (3H, d, J=6 Hz), 0.8~1.9 (12H, br), 1.9~2.4 (4H, br), 2.6~4.3 (15H, br), 6.5~9.5 (10H, br); IR ν_{max} (KBr) cm^{-1} 3330, 2940, 1650, 1530, 1465, 1380, 1160, 1110, 930; Rf 0.2 (PrOH - pyridine - H_2O - AcOH, 6 : 4 : 3 : 2); $[\alpha]_D^{22}$ −13.1° (*c* 1.1, H_2O); FD-MS *m/z* 416 (M+1).

10-[*N*-(7-Guanidinoheptanoyl)-DL-Hos]-1,5,10-triazadecane Trihydrochloride (**XI**, X=DL-Hos, Y=$(CH_2)_6$) (Analogue **14**)

1) 10-(*N*-Z-DL-Hos)-1,5-di-BOC-1,5,10-triazadecane (**VII**, R_1=BOC, R_2=Z, X=DL-Hos): To the solution of Z-DL-homoserine lactone 3.76 g (16 mmol) in 20 ml of THF was added 5 ml of DMF solution of 1,5-di-BOC-1,5,10-triazadecane **VI** (R_1=BOC, 2.07 g, 6.0 mmol), and the mixture was stirred for 3 days at room temperature. The reaction mixture was evaporated under reduced pressure and the resulting oily material was subjected to column chromatography on silica gel (Wako-Gel C-200, 400 g) using a mixed solvent of $CHCl_3$ and MeOH in a ratio of 20 : 1 as a eluant. Active fractions were collected and concentrated under reduced pressure to obtain 1.38 g of 10-(*N*-Z-DL-Hos)-1,5-di-BOC-1,5,10-triazadecane: ^{1}H NMR ($CDCl_3$) δ 1.0~2.3 (8H, br), 1.47 (18H, s), 2.8~3.5 (8H, br), 3.5~4.0 (3H, br), 4.1~4.6 (1H, m), 4.7~5.4 (1H, br), 5.09 (2H, s), 5.8~6.3 (1H, br), 6.6~7.1 (1H, br), 7.31 (5H, s); Rf 0.2 ($CHCl_3$ - MeOH, 20 : 1).

2) 10-(DL-Hos)-1,5-di-BOC-1,5,10-triazadecane (**VIII**, R_1=BOC, X=DL-Hos): 10-(*N*-Z-DL-Hos)-1,5-di-BOC-1,5,10-triazadecane (1.35 g, 2.22 mmol) was dissolved in 20 ml of MeOH and was hydrogenated over palladium black for 8 hours. After a completion of the reaction, the filtrate was concentrated to give oily 10-(DL-Hos)-1,5-di-BOC-1,5,10-triazadecane: ^{1}H NMR ($CDCl_3$) δ 1.1~2.2 (10H, br), 1.47 (18H, s), 2.8~3.5 (9H, br), 3.5~4.0 (3H, m), 4.6~5.6 (1H, br), 7.4~7.9 (1H, br); Rf 0.1 ($CHCl_3$ - MeOH, 9 : 1).

3) 10-[*N*-(7-Guanidinoheptanoyl)-DL-Hos]-1,5-di-BOC-1,5,10-triazadecane Acetate (**X**, R_1=BOC, X=DL-Hos, Y=$(CH_2)_6$): 10-(DL-Hos)-1,5-di-BOC-1,5,10-triazadecane (0.88 g, 1.85 mmol), 0.30 g (2.96 mmol) of Et_3N and 1.19 g (3.71 mmol) of 7-guanidinoheptanoic acid *N*-hydroxysuccinimide ester hydrochloride were treated in a mixed solvent of 5 ml of THF and 8 ml of DMF at room temperature overnight. The reaction mixture was evaporated and the resulting oily material was dissolved in 50 ml of 2 % aqueous solution of H_3PO_4 and the solution was washed with EtOAc. Na_2CO_3 was added to the solution to adjust the pH to 10.5. Then the aqueous layer was extracted with two 50-ml portions of EtOAc and AcOH was added to the EtOAc solution until it became substantially neutral. Concentrating the mixture under reduced pressure gave 1.08 g of oily 10-[*N*-(7-guanidinoheptanoyl)-DL-Hos]-1,5-di-BOC-1,5,10-triazadecane acetate in a yield of 86.4%: ^{1}H NMR (DMSO-d_6) δ 0.9~2.0 (16H, br), 1.43 (18H, s), 1.84 (3H, s), 2.0~2.4 (2H, br), 2.7~3.7 (11H, br), 3.37 (2H, t), 4.1~4.6 (1H, br), 6.3~8.4 (8H, br); Rf 0.3 ($CHCl_3$ - MeOH - AcOH, 8 : 2 : 0.5).

4) 10-[*N*-(7-Gaunidinoheptanoyl)-DL-Hos]-1,5,10-triazadecane Trihydrochloride (**XI**, X=DL-Hos,

Y=$(CH_2)_6$) (Analogue **14**): 10-[*N*-(7-Guanidinoheptanoyl)-DL-Hos]-1,5-di-BOC-1,5,10-triazadecane acetate (1.04 g, 1.54 mmol) was treated with TFA at 0°C for 5 hours, then TFA was removed by evaporation under reduced pressure. To the residue was added 1 N HCl, and the mixture was concentrated to provide 0.97 g of oil. This oil was dissolved in 10 ml of H_2O and chromatographed on columns of CM-Sephadex C-25 and Sephadex LH-20, in a similar manner as described in the preparation of analogue **2** or **11**, to provide 0.38 g of purified oily material. This oil was lyophilized to give 0.37 g of **14** in a yield of 45%: ^{1}H NMR (DMSO-d_6) δ 0.6~2.0 (14H, br), 2.0~2.3 (4H, br), 2.6~4.0 (15H, br), 4.0~4.7 (1H, br), 6.0~9.5 (10H, br); IR ν_{max} (KBr) cm^{-1} 3380, 2940, 1650, 1530, 1470, 1380, 1165, 1055, 965; Rf 0.4 (PrOH - pyridine - H_2O - AcOH, 6:4:3:2); FD-MS *m/z* 416 (M+1).

10-[*N*-(7-Guanidinoheptanoyl)-β-Ala]-1,5-di-Z-1,5,10-triazadecane Hydrochloride (**X**, R_1=Z, X=β-Ala, Y=$(CH_2)_6$)

^{1}H NMR (DMSO-d_6) δ 1.0~2.1 (14H, br), 2.0~2.5 (4H, br), 2.6~3.1 (12H, br), 4.99 (2H, s), 5.03 (2H, s), 6.7~8.3 (8H, br), 7.3 (10H, s); Rf 0.8 ($CHCl_3$ - MeOH - 29% NH_3, 2:2:1).

10-[*N*-(7-Guanidinoheptanoyl)-β-Ala]-1,5,10-triazadecane Trihydrochloride (**XI**, X=β-Ala, Y=$(CH_2)_6$) (Analogue **15**)

^{1}H NMR (DMSO-d_6) δ 1.0~1.8 (12H, br), 1.9~2.5 (6H, br), 2.6~3.5 (12H, br), 7.0~9.3 (12H, br); IR ν_{max} (KBr) cm^{-1} 3350, 2935, 1635, 1545, 1460, 1365, 1165, 965; Rf 0.6 ($CHCl_3$ - MeOH - 29% NH_3, 2:2:1); FD-MS *m/z* 386 (M+1).

10-[*N*-(7-Guanidinoheptanoyl)-γ-Aba)-1,5-di-Z-1,5,10-triazadecane Hydrochloride (**X**, R_1=Z, X=γ-Aba, Y=$(CH_2)_6$)

^{1}H NMR (DMSO-d_6) δ 0.9~2.0 (16H, br), 2.0~2.5 (4H, br), 2.7~3.7 (12H, br), 4.99 (2H, s), 5.03 (2H, s), 6.5~8.5 (8H, br), 7.3 (10H, s); Rf 0.6 (BuOH - AcOH - H_2O, 4:1:1).

10-[*N*-(7-Guanidinoheptanoyl)-γ-Aba]-1,5,10-triazadecane Trihydrochloride (**XI**, X=γ-Aba, Y=$(CH_2)_6$) (Analogue **16**)

^{1}H NMR (DMSO-d_6) δ 0.9~1.8 (14H, br), 1.8~2.4 (6H, br), 2.6~3.4 (12H, br), 6.7~9.2 (12H, br); IR ν_{max} (KBr) cm^{-1} 3350, 2940, 1640, 1550, 1460, 1360; Rf 0.4 (PrOH - pyridine - H_2O - AcOH, 6:4:3:2); FD-MS *m/z* 400 (M+1).

10-[*N*-(7-Guanidinoheptanoyl)-HABA]-1,5-di-BOC-1,5,10-triazadecane Hydrochloride (**X**, R_1=BOC, X=HABA, Y=$(CH_2)_6$)

^{1}H NMR (CD_3OD) δ 0.9~2.5 (18H, m), 2.7~3.6 (12H, m), 3.8~4.2 (H, m), 4.2~8.9 (8H, br), 4.8~5.3 (4H, d, *J*=2 Hz), 7.3 (5H, s); IR ν_{max} (KBr) cm^{-1} 3330, 2930, 1645, 1535, 1460, 1425, 1360, 1260, 1215, 1150; Rf 0.45 ($CHCl_3$ - MeOH - 29% NH_3, 12:5:1).

10-[*N*-(7-Guanidinoheptanoyl)-HABA]-1,5,10-triazadecane Trihydrochloride (**XI**, X=HABA, Y=$(CH_2)_6$) (Analogue **17**)

^{1}H NMR (D_2O, external TMS) δ 1.4~3.1 (18H, m), 3.3~4.1 (12H, m), 4.5~4.9 (H, m); IR ν_{max} (KBr) cm^{-1} 3320, 2930, 1635, 1545, 1455, 1115; Rf 0.3 (PrOH - pyridine - H_2O - AcOH, 6:4:3:2).

10-(*N*-(9-Guanidinononanoyl)-OBzl-L-Ser]-1,5-di-Z-1,5,10-triazadecane Hydrochloride (**X**, R_1=Z, X=OBzl-L-Ser, Y=$(CH_2)_8$)

^{1}H NMR (DMSO-d_6) δ 1.0~2.0 (18H, br), 2.0~2.4 (2H, br), 2.7~3.5 (10H, br), 3.58 (2H, d, *J*=6 Hz), 4.2~4.8 (1H, br), 4.47 (2H, s), 5.01 (2H, s), 5.05 (2H, s), 6.9~8.3 (8H, br), 7.29 (5H, s), 7.33 (10H, s); Rf 0.2 ($CHCl_3$ - MeOH - AcOH, 9:1:0.3).

10-[*N*-(9-Guanidinononanoyl)-L-Ser]-1,5,10-triazadecane Trihydrochloride (**XI**, X=L-Ser, Y=$(CH_2)_8$) (Analogue **18**)

^{1}H NMR (DMSO-d_6) δ 1.0~1.8 (16H, br), 1.8~2.4 (4H, br), 2.6~3.5 (11H, br), 3.55 (2H, d, *J*=5 Hz), 4.0~4.3 (1H, br), 7.0~9.6 (12H, br); IR ν_{max} (KBr) cm^{-1} 3350, 2940, 1655, 1540, 1470, 1160, 1060; Rf 0.6 (PrOH - pyridine - H_2O - AcOH, 6:4:3:2); $[\alpha]_D^{25}$ −5.0° (*c* 1.1, H_2O); FD-MS *m/z* 430 (M+1).

10-[*N*-4-(4-Guanidinophenyl)butyryl-Gly]-1,5,10-triazadecane Trihydrochloride (**XI**, X=Gly, Y=4-(4-Guanidinophenyl)butyryl) (Analogue **19**)

4-(4-Guanidinophenyl)butyric Acid: The solution of 4-(4-aminophenyl)butyric acid 1.60 g (8.93 mmol), 1-amidino-3,5-dimethylpyrazole nitrate 2.70 g (13.4 mmol) and *N*,*N*-diisopropylethylamine 2.19 g (17 mmol) in THF (40 ml) was refluxed overnight. Then the precipitated crystals were filtered and washed with acetone, MeOH and THF, dissolved in dil HCl and this solution was then evaporated to dryness. The resulting residue was washed with ether and acetone to provide 1.54 g of 4-(4-guanidinophenyl)butyric acid in a yield of 67%: MP 157~160°C; ^{1}H NMR (D_2O+DCl, external TMS) δ 2.1~2.6 (2H, m), 2.6~3.3 (4H, m), 7.5~7.9 (4H, m); IR ν_{max} (KBr) cm^{-1} 3370, 3170, 2330, 1730, 1680, 1660, 1620, 1600, 1575, 1510, 1240, 1220.

10-[*N*-4-(4-Guanidinophenyl)butyryl-Gly]-1,5-di-Z-1,5,10-triazadecane (**X**, R_1=Z, X=Gly, Y=4-(4-Guanidinophenyl)butyryl): 4-(4-Guanidinophenyl)butyric acid hydrochloride 1.56 g (6.05 mmol) was dissolved in DMF (20 ml). To the solution was added HOSu 0.84 g (7.26 mmol) and DCCD 1.50 g (7.26 mmol) under ice cooling and the solution was reacted overnight. The precipitates were filtered off and the filtrate was added to the solution of 10-glycyl-1,5-di-Z-1,5,10-triazadecane 2.59 g (5.5 mmol) and Et_3N 0.61 g (6.05 mmol) in DMF (30 ml). The mixture was stirred overnight at room temperature then evaporated to give an oily material. This was dissolved in EtOAc (300 ml) and EtOH (60 ml), and the solution was washed with 5% H_3PO_4, 5% Na_2CO_3 and satd NaCl solution, successively. The solution was dried over anhydrous Na_2SO_4 and the filtrate was concentrated under reduced pressure to give 3.3 g of pale yellow oil in a yield of 89.1%: ^{1}H NMR ($CDCl_3$) δ 1.1~2.8 (12H, br), 2.8~4.1 (10H, br), 5.04 (4H, s), 4.8~8.1 (11H, br), 7.3 (10H, s); Rf 0.59 ($CHCl_3$ - MeOH - 17% NH_3, 6:3.5:1).

10-[*N*-4-(4-Guanidinophenyl)butyryl-Gly]-1,5,10-triazadecane Trihydrochloride (**19**): 10-[*N*-4-(4-Guanidinophenyl)butyryl-Gly]-1,5-di-Z-1,5,10-triazadecane 3.3 g (4.90 mmol) was hydrogenated over palladium black in 40 ml of AcOH for 10 hours at 50°C. The filtrates was evaporated to yield 2.10 g of oily material. This was dissolved in 10 ml of H_2O and the solution was passed through a column packed with 220 ml of CM-Sephadex C-25 (Na^+). The adsorbent was eluted in a mode of gradient between 1,100 ml of distilled H_2O and 1,100 ml of 1.0 M NaCl solution. The fractions containing the title compound were collected and evaporated to dryness. To this residue was added MeOH to remove insoluble materials by filtration. The filtrate was purified in the same manner as described for analogue **2**: ^{1}H NMR (DMSO-d_6) δ 1.1~2.5 (12H, br), 2.5~3.3 (8H, br), 3.5~3.9 (2H, br d), 6.9~7.7 (8H, m), 7.7~8.3 (3H, br), 8.3~10.0 (5H, br); IR ν_{max} (KBr) cm^{-1} 3290, 2940, 2320, 1640, 1540, 1455; Rf 0.26 (PrOH - pyridine - H_2O - AcOH, 6:4:3:2).

In the same manner, following analogues were synthesized.

10-[*N*-4-(4-Guanidinophenyl)butyryl-L-Ser]-1,5,10-triazadecane Trihydrochloride (**XI**, X=L-Ser, Y=4-(4-Guanidinophenyl)butyryl) (Analogue **20**)

10-[*N*-4-(4-Guanidinophenyl)butyryl-OBzl-L-Ser]-1,5-di-Z-1,5,10-triazadecane Hydrochloride (**X**, R_1=Z, X=OBzl-L-Ser, Y=4-(4-Guanidinophenyl)butyryl): ^{1}H NMR (DMSO-d_6) δ 1.1~2.8 (12H, br), 2.8~3.8 (10H, br), 4.2~4.8 (3H, br s), 5.02 (2H, s), 5.06 (2H, s), 7.3 (15H, s), 7.7~10.1 (12H, br); Rf 0.16 ($CHCl_3$ - MeOH - 17% NH_3, 6:1.5:0.25).

10-[*N*-4-(4-Guanidinophenyl)butyryl-L-Ser]-1,5,10-triazadecane Trihydrochloride (**20**): ^{1}H NMR (DMSO-d_6) δ 1.1~2.5 (12H, br), 2.5~3.4 (8H, br), 3.4~3.8 (3H, br d), 4.0~4.5 (1H, br), 6.8~7.7 (8H, m), 7.7~8.8 (5H, br), 8.8~9.7 (2H, br), 10.13 (1H, br s); IR ν_{max} (KBr) cm^{-1} 3290, 2940, 2320, 1635, 1510, 1450; Rf 0.34 (PrOH - pyridine - H_2O - AcOH, 6:4:3:2); $[\alpha]_D^{19.5}$ −13.8° (*c* 1.17, H_2O).

10-[*N*-4-(3-Guanidinopropyl)benzoyl-Gly]-1,5,10-triazadecane Trihydrochloride (**XI**, X=Gly, Y=4-(3-Guanidinopropyl)benzoyl) (Analogue **21**)

4-(3-Guanidinopropyl)benzoic Acid Hydrochloride: The solution of methyl 4-(3-aminopropyl)-benzoate 4.00 g (20.7 mmol), 1-amidino-3,5-dimethylpyrazole nitrate 6.25 g (31.0 mmol) and *N*,*N*-diisopropylethylamine 5.08 g (39.3 mmol) in THF (150 ml) was refluxed overnight.

Then it was concentrated to provide an oily material. Subsequently, this oil was hydrolyzed in

6 N HCl (100 ml) for 4 hours under reflux. The resulting solution was washed with EtOAc (50 ml×4) and the pH of the solution was brought to 6.0 by addition of 20% aqueous NH_3. The deposited crystal was filtered: ^{1}H NMR (DMSO-d_6+DCl, external TMS)) δ 2.0~2.7 (2H, m), 3.1~3.5 (2H, m), 3.5~4.0 (2H, m), 7.9 (2H, d, J=8 Hz), 8.4 (2H, d, J=8 Hz), 8.4 (2H, d, J=8 Hz).

10-[*N*-4-(3-Guanidinopropyl)benzoyl-Gly]-1,5-di-Z-1,5,10-triazadecane Hydrochloride (X, R_1=Z, X=Gly, Y=4-(3-Guanidinopropyl)benzoyl): ^{1}H NMR (DMSO-d_6) δ 1.1~2.1 (8H, br), 2.1~3.5 (12H, br), 3.7~4.0 (2H, br), 5.01 (2H, s), 5.05 (2H, s), 6.8~8.9 (11H, br), 7.30 (10H, s); Rf 0.34 ($CHCl_3$ - MeOH - 17% NH_3, 6 : 1.5 : 0.25).

10-[*N*-4-(3-Guanidinopropyl)benzoyl-Gly]-1,5,10-triazadecane Trihydrochloride (**21**): ^{1}H NMR (DMSO-d_6) δ 1.1~2.4 (8H, br), 2.5~3.4 (12H, br), 3.7~4.0 (2H, br d), 7.1~7.5 (4H, br), 7.33 (2H, d, J=8 Hz), 7.7~8.5 (8H, br), 7.90 (2H, d, J=8 Hz), 8.5~8.9 (1H, br), 8.9~9.6 (2H, br); IR ν_{max} (KBr) cm^{-1} 3270, 2950, 2930, 1640, 1540, 1500, 1460, 1300; Rf 0.33 (PrOH - pyridine - H_2O - AcOH, 6 : 4 : 3 : 2).

10-[*N*-4-(3-Guanidinopropyl)benzoyl-L-Ser)-1,5,10-triazadecane Trihydrochloride (XI, X=L-Ser, Y=4-(3-Guanidinopropyl)benzoyl) (Analogue **22**)

10-[*N*-4-(3-Guanidinopropyl)benzoyl)-OBzl-L-Ser]-1,5-di-Z-1,5,10-triazadecane Hydrochloride (X, R_1=Z, X=OBzl-L-Ser, Y=4-(3-guanidinopropyl)benzoyl): ^{1}H NMR ($CDCl_3$) δ 1.3~2.1 (8H, br), 2.5~4.1 (12H, br), 4.5 (2H, s), 5.02 (2H, s), 5.05 (2H, s), 6.9~8.9 (11H, br), 7.3 (15H, s); Rf 0.32 ($CHCl_3$ - MeOH - 17% NH_3, 6 : 1.5 : 0.25).

10-[*N*-4-(3-Guanidinopropyl)benzoyl-L-Ser]-1,5,10-triazadecane Trihydrochloride (**22**): ^{1}H NMR (DMSO-d_6) δ 1.2~2.4 (8H, br), 2.6~3.4 (12H, br), 3.4~4.2 (3H, br d), 4.2~4.7 (1H, br), 7.1~7.5 (4H, br), 7.33 (2H, d, J=8 Hz), 7.7~8.8 (6H, br), 7.90 (2H, d, J=8 Hz), 8.8~9.7 (2H, br); IR ν_{max} (KBr) cm^{-1} 3300, 3150, 2950, 1640, 1535, 1500, 1460, 1290, 1060; Rf 0.31 (PrOH - pyridine - H_2O - AcOH, 6 : 4 : 3 : 2); $[\alpha]_D^{19.5}$ +24.4° (c 0.97, H_2O).

10-[*N*-3-(4-Guanidinomethylphenyl)propionyl-L-Ser]-1,5,10-triazadecane Trihydrochloride (XI, X=L-Ser, Y=3-(4-Guanidinomethylphenyl)propionyl) (Analogue **23**)

Methyl 3-(4-Aminomethylphenyl)propionate: Methyl 3-(4-cyanophenyl)propionate 4.30 g (22.97 mmol) in MeOH (350 ml) saturated with ammonia was hydrogenated over Raney-Ni (3 g) for 2 hours at 60 atom. The filtrate was concentrated under reduced pressure to give 4.02 g of title compound. Yield 90.5%: ^{1}H NMR ($CDCl_3$) δ 2.4~3.2 (6H, m), 3.63 (3H, s), 3.8~4.7 (2H, br), 7.16 (4H, s); Rf 0.16 ($CHCl_3$ - MeOH, 10 : 1).

3-(4-Guanidinomethylphenyl)propionic Acid Hydrochloride: MP >300°C; ^{1}H NMR (D_2O+DCl, external TMS) δ 3.0~3.6 (4H, m), 4.84 (2H, s), 7.7 (4H, s); IR ν_{max} (KBr) cm^{-1} 3350, 3060, 2330, 1675, 1610, 1550, 1460, 1405, 1150; Rf 0.60 ($CHCl_3$ - MeOH - 17% NH_3, 4 : 4 : 2).

10-[*N*-3-(4-Guanidinomethylphenyl)propionyl-OBzl-L-Ser]-1,5-di-Z-1,5,10-triazadecane (X, R_1=Z, X=OBzl-L-Ser, Y=3-(4-Guanidinomethylphenyl)propionyl): ^{1}H NMR ($CDCl_3$) δ 1.0~2.0 (6H, br), 2.0~3.9 (14H, br), 4.0~4.8 (5H, br d), 5.0 (2H, s), 5.50 (2H, s), 5.1~8.3 (11H, br), 7.2 (15H, s); Rf 0.27 ($CHCl_3$ - MeOH - 17% NH_3, 6 : 1.5 : 0.25).

10-[*N*-3-(4-Guanidinomethylphenyl)propionyl-L-Ser]-1,5,10-triazadecane Trihydrochloride (**23**): ^{1}H NMR (DMSO-d_6) δ 1.1~2.4 (6H, br), 2.4~3.4 (12H, br), 3.4~3.9 (3H, br d), 4.0~4.6 (3H, br d), 6.8~7.7 (4H, br), 7.2 (4H, s), 7.7~8.7 (6H, br), 8.7~9.7 (2H, br); IR ν_{max} (KBr) cm^{-1} 3310, 2940, 2320, 1640, 1535, 1450; Rf 0.25 (PrOH - pyridine - H_2O - AcOH, 6 : 4 : 3 : 2); $[\alpha]_D^{19.5}$ −24.8° (c 1.0, H_2O).

10-[*N*-3-(3-Guanidinomethylphenyl)propionyl-L-Ser]-1,5,10-triazadecane Triyhdrochloride (XI, X=L-Ser, Y=3-(3-Guanidinomethylphenyl)propionyl) (Analogue **24**)

3-(3-Guanidinomethylphenyl)propanoic Acid Hydrochloride: Methyl 3-(3-aminomethylphenyl)-propionate 4.40 g (22.77 mmol) was treated with 1-amidino-3,5-dimethylpyrazole nitrate in the same manner as described above. Yield 41.7%: MP 273~276°C; ^{1}H NMR (D_2O+DCl, external TMS) δ 2.8~3.5 (4H, m), 4.8 (2H, s), 7.3~7.9 (4H, m); IR ν_{max} (KBr) cm^{-1} 3340, 3100, 2330, 1645, 1535, 1400, 1330; Rf 0.5 ($CHCl_3$ - MeOH - 17% NH_3, 4 : 4 : 2).

10-[*N*-3-(3-Guanidinomethylphenyl)propionyl-OBzl-L-Ser)-1,5-di-Z-1,5,10-triazadecane Hydrochloride (X, R_1=Z, X=OBzl-L-Ser, Y=3-(3-Guanidinomethylphenyl)propionyl): ^{1}H NMR ($CDCl_3$)

δ 1.1~2.1 (6H, br), 2.1~3.9 (14H, br), 3.9~4.7 (5H, br d), 5.0 (2H, s), 5.05 (2H, s), 6.3~8.5 (11H, br), 7.2 (15H, s); Rf 0.42 ($CHCl_3$ - MeOH - 17% NH_3, 6 : 1.5 : 0.25).

10-[*N*-3-(3-Guanidinomethylphenyl)propionyl-L-Ser]-1,5,10-triazadecane Trihydrochloride (**24**): ^1H NMR (DMSO-d_6) δ 1.1~2.3 (6H, br), 2.3~3.4 (12H, br), 3.4~3.8 (3H, br d), 4.0~4.6 (3H, br d), 6.8~7.8 (8H, m), 7.8~8.8 (6H, br), 8.8~9.7 (2H, br); IR ν_{max} (KBr) cm^{-1} 3240, 2320, 1630, 1530, 1450; Rf 0.30 (PrOH - pyridine - H_2O - AcOH, 6 : 4 : 3 : 2); $[\alpha]_D^{19.5}$ −22.0° (*c* 1.0, H_2O).

10-[*N*-5-(4-Guanidinophenyl)pentanoyl-L-Ser]-1,5,10-triazadecane Trihydrochloride (**XI**, X=L-Ser, Y=5-(4-Guanidinophenyl)pentanoyl) (Analogue **25**)

5-(4-Guanidinophenyl)pentanoic Acid Hydrochloride: ^1H NMR (D_2O+DCl, external TMS) δ 1.7~2.4 (4H, m), 2.5~3.4 (4H, m), 7.5~8.0 (4H, m); Rf 0.50 ($CHCl_3$ - MeOH - 17% NH_3, 4 : 4 : 2).

10-[*N*-5-(4-Guanidinophenyl)pentanoyl-OBzl-L-Ser]-1,5-di-Z-1,5,10-triazadecane (X, R_1=Z, X=OBzl-L-Ser, Y=5-(4-guanidinophenyl)pentanoyl): ^1H NMR ($CDCl_3$) δ 0.9~2.8 (14H, br), 2.8~3.9 (10H, br), 4.47 (3H, br s), 5.03 (2H, s), 5.06 (2H, s), 5.0~7.9 (11H, br), 7.3 (15H, s); Rf 0.38 ($CHCl_3$ - MeOH - 17% NH_3, 6 : 1.5 : 0.25).

10-[*N*-(5-(4-Guanidinophenyl)pentanoyl)-L-Ser]-1,5,10-triazadecane Trihydrochloride (**25**): ^1H NMR (DMSO-d_6) δ 1.1~2.5 (14H, br), 2.5~3.3 (8H, br), 3.4~3.7 (3H, br d), 4.0~4.4 (1H, br), 6.8~7.7 (8H, m), 7.7~8.7 (5H, br), 8.7~9.7 (2H, br), 10.05 (1H, br s); IR ν_{max} (KBr) cm^{-1} 3300, 2940, 2330, 1640, 1510, 1450; Rf 0.31 (PrOH - pyridine - H_2O - AcOH, 6 : 4 : 3 : 2); $[\alpha]_D^{19.5}$ −13.9° (*c* 1.07, H_2O).

10-[*N*-6-(4-Guanidinophenyl)hexanoyl-L-Ser]-1,5,10-triazadecane Trihydrochloride (**XI**, X=L-Ser, Y=6-(4-Guanidinophenyl)hexanoyl) (Analogue **26**)

10-[*N*-6-(4-Guanidinophenyl)hexanoyl-OBzl-L-Ser]-1,5-di-Z-1,5,10-triazadecane Hydrochloride (X, R_1=Z, X=OBzl-L-Ser, Y=6-(4-Guanidinophenyl)hexanoyl): ^1H NMR ($CDCl_3$) δ 0.80~1.95 (14H, br), 1.95~2.30 (2H, br), 2.30~2.85 (2H, br), 2.85~3.45 (8H br), 3.45~4.00 (2H, br m), 4.30~4.80 (1H, br), 4.67 (2H, s), 5.05 (4H, s), 5.60~7.50 (8H, br), 7.05 (4H, br s), 7.25 (15H, s), 7.28 (10H, s); IR ν_{max} (Neat) cm^{-1} 3320, 2940, 1670, 1650, 1540, 1515, 1475, 1450, 1425, 1255, 1210, 740, 700; Rf 0.25 ($CHCl_3$ - MeOH - 17% NH_3, 6 : 1.5 : 0.25).

10-[*N*-6-(4-Guanidinophenyl)hexanoyl-L-Ser]-1,5,10-triazadecane Trihydrochloride (**26**): MP 159.5~161.5°C; ^1H NMR (DMSO-d_6) δ 1.00~1.90 (12H, br), 1.90~2.38 (4H, br), 2.57~3.22 (8H, br), 3.22~3.85 (3H, br), 3.85~4.55 (1H, br), 4.55~5.38 (1H, br), 6.95~7.40 (4H, m), 7.40~7.72 (4H, br), 7.72~8.15 (2H, br), 8.15~9.32 (4H, br), 9.32~10.53 (1H, br); IR ν_{max} (KBr) cm^{-1} 3280, 2930, 2775, 1640, 1555, 1510, 1450, 1295, 1245, 1060; Rf 0.43 (PrOH - pyridine - H_2O - AcOH, 6 : 4 : 3 : 2); $[\alpha]_D^{20.5}$ −13.7° (*c* 1.28. H_2O).

10-[*N*-3-(2-Chloro-4-guanidinomethylphenyl)propionyl)-L-Ser]-1,5,10-triazadecane Trihydrochloride (**XI**, X=L-Ser, Y=3-(2-Chloro-4-guanidinomethylphenyl)propionyl) (Analogue **27**)

Methyl 3-(2-Chloro-4-acetylaminomethylphenyl)propionate: Methyl 3-(4-acetylaminomethylphenyl)-propionate 5.0 g (21.25 mmol) was added to a sulfuryl chloride (85 ml) solution of anhydrous aluminum chloride 14.2 g (106 mmol) at −25°C. Thereafter, the temperature of the mixture was gradually elevated to 5°C, then the reaction mixture was subjected to react for 24 hours. It was poured onto crushed ice. The resulting solid material was extracted with $CHCl_3$ (1,000 ml). It was washed with 5% Na_2CO_3, 5% H_3PO_4 and water and dried over anhydrous $MgSO_4$. The filtrates was dried up and residual oily product was subjected to a column chromatography of silica gel (300 g). Eluting with a mixture of toluene and EtOAc (1 : 1), the fractions with desired product was collected and evaporated to dryness to provide 1.93 g of the title compound in a yield of 33.7%: MP 91~93°C; ^1H NMR (400 MHz, $CDCl_3$) δ 2.03 (3H, s), 2.63 (2H, t, J=7.7 Hz), 3.04 (2H, t, J=7.7 Hz), 3.67 (3H, s), 4.37 (2H, d, J=5.9 Hz), 5.85 (1H, br s), 7.10 (1H, d, J=7.8 Hz), 7.20 (1H, d, J=7.8 Hz), 7.26 (1H, s).

3-(2-Chloro-4-aminomethylphenyl)propionic Acid Hydrochloride: Methyl 3-(2-chloro-4-acetylaminomethylphenyl)propionate 1.10 g (4.08 mmol) was hydrolyzed in 2 N HCl and worked up in a usual manner to yield 1.05 g of the title compound. Yield quantitative: MP 191~194°C; ^1H NMR (D_2O, external TMS) δ 2.9~3.7 (4H, m), 4.60 (2H, s), 7.6~8.1 (3H, m); Rf 0.20 ($CHCl_3$ - MeOH - 17% NH_3, 6 : 3 : 0.5).

3-(2-Chloro-4-guanidinomethylphenyl)propionic Acid Hydrochloride: 3-(2-Chloro-4-aminomethylphenyl)propionic acid hydrochloride 1.00 g (4.0 mmol), 1-amidino-3,5-dimethylpyrazole nitrate 1.03 g (5.12 mmol) was treated as the same manner as described above to afford 0.75 g of the title compound in a 73.3% yield: MP 260~264°C; ^{1}H NMR (D_2O+DCl, external TMS) δ 2.8~3.7 (4H, m), 4.82 (2H, s), 7.5~7.9 (3H, m); Rf 0.06 ($CHCl_3$ - MeOH - 17% NH_3, 6:3:0.5).

10-[*N*-3-(2-Chloro-4-guanidinomethylphenyl)propionyl-OBzl-L-Ser]-1,5-di-Z-1,5,10-triazadecane Hydrochloride (X, R_1=Z, X=OBzl-L-Ser, Y=3-(2-Chloro-4-guanidinomethylphenyl)propionyl): Yield 83.8%; ^{1}H NMR (DMSO-d_6) δ 1.0~2.0 (6H, br), 2.2~3.5 (12H, br), 3.5~3.8 (2H, br d), 4.3~4.8 (5H, br m), 5.02 (2H, s), 5.05 (2H, s), 6.8~8.8 (11H, br), 7.10 (5H, s), 7.30 (10H, s); Rf 0.21 ($CHCl_3$ - MeOH - 17% NH_3, 6:1.5:0.25).

10-[*N*-(3-(2-Chloro-4-guanidinomethylphenyl)propionyl)-L-Ser]-1,5,10-triazadecane Trihydrochloride (**27**): Yield 49.0%; ^{1}H NMR (DMSO-d_6) δ 1.2~2.3 (6H, br), 2.5~3.3 (12H, br), 3.3~3.7 (3H, br d), 4.1~4.5 (3H, br d), 7.1~7.7 (7H, m), 7.7~8.6 (6H, br), 8.6~9.5 (2H, br); IR ν_{max} (KBr) cm^{-1} 3300, 3150, 3050, 2950, 1645, 1540, 1450, 1050; Rf 0.35 (PrOH - pyridine - H_2O - AcOH, 6:4:3:2); $[\alpha]_D^{19}$ −26.1° (*c* 1.1, H_2O).

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