SYNTHESIS AND ANTITUMOR ACTIVITY OF SPERGUALIN ANALOGUES

I. CHEMICAL MODIFICATION OF 7-GUANIDINO-3-HYDROXYACYL MOIETY

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Many analogues and derivatives of an antitumor antibiotic, spergualin, were synthesized, and the relationships between the structure and the activity against mouse L-1210 tumor were studied. Both modification of the 15-hydroxyl group and alteration of chain-length of the ω-guanidinoacyl moiety affected the activity. 15-Deoxyspergualin (**18**, 1-amino-19-guanidino-11-hydroxy-4,9,12-triazanonadecane-10,13-dione) and its analogue **25** (1-amino-21-guanidino-11-hydroxy-4,9,12-triazauneicosane-10,13-dione) had strong activity, superior to that of spergualin.

Spergualin (**Ia**) is an antitumor antibiotic that was discovered in culture filtrates of *Bacillus laterosporus* BMG162-aF2, and has been determined to be (−)-(15*S*)-1-amino-19-guanidino-11,15-dihydroxy-4,9,12-triazanonadecane-10,13-dione.[1,2] Its total synthesis has also been accomplished; acid-catalyzed condensation of 11-amino-1,1-dihydroxy-3,8-diazaundecan-2-one [a hydrate of glyoxylylspermidine] (**II**) with (*S*)-7-guanidino-3-hydroxyheptanamide (**IIIa**) followed by separation from the 11-epimeric mixture gives spergualin.[3]

We applied this condensation reaction to analogue synthesis. Our first effort was directed to the modification of the 7-guanidino-3-hydroxyacyl moiety because of its versatile functional property. In addition, we synthesized the 11-*O*-alkyl-derivatives, because they were expected to be stable. We then examined their antitumor activity against mouse leukemia L-1210.

In this paper, we report on the preparation of the spergualin analogues and derivatives described above, and present the results of the structure-activity studies.

Chemistry

Acid-catalyzed Condensation Reaction

A number of analogues were synthesized by acid-catalyzed condensation of ω-guanidinoalkanamides (the amide component **III**) with glyoxylylspermidine (the aldehyde component **II**), as shown in Scheme 1. Primary amides can be added to aldehydes in the presence of acids or bases to give *N*-acylated aminoalcohols,[4] which correspond to the *N*-acylated-1-hydroxyglycine moiety of spergualin. During the study of chemical synthesis of spergualin it was necessary to examine various conditions of the condensation reaction including catalysts and solvents, because reactive functional groups such as guanidino or amino groups were present in both components. These two reactive groups were pro-

Scheme 1.

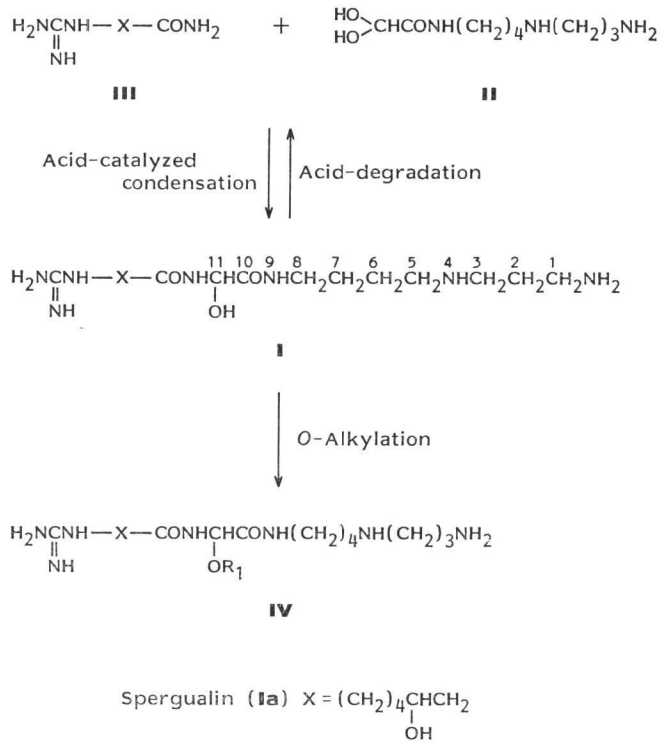


Table 1. Effect of acids on condensation of 7-guanidinoheptanamide (**IX**) with glyoxylylspermidine (**II**) to yield 15-deoxyspergualin (**18**).

Acid-catalyst	Equiv	Yield (%) of **18**	Acid-catalyst	Equiv	Yield (%) of **18**
None		32	Lactic acid	2	40
Acetic acid	1	34	L-Tartaric acid	1	37
	2	38	Citric acid	1/3	42
Propionic acid	1	19		2/3	41
Malonic acid	1	0		1	40
Succinic acid	1	42	*p*-Toluenesulfonic acid	1	10
Glutaric acid	1	43	HCl	1	10
Adipic acid	1	36	H_2SO_4	1	12
Lactic acid	1/2	40	H_3PO_4	1	25
	1	41	H_3BO_3	1	28

A mixture of 7-guanidinoheptanamide·HCl (**IX**, 0.17 mmol), glyoxylylspermidine·2HCl (**II**, 0.17 mmol) and respective acid in H_2O (5 mg) was heated at 60°C for 15 hours.

The yield of condensed product (**18**) was estimated by HPLC using Nucleosil $5C_{18}$ column, 9% acetonitrile - 0.005 M Na pentansulfonate - 0.01 M Na_2HPO_4 (pH 3) as the developing solvent and Na *o*-aminobenzoate as the internal standard.

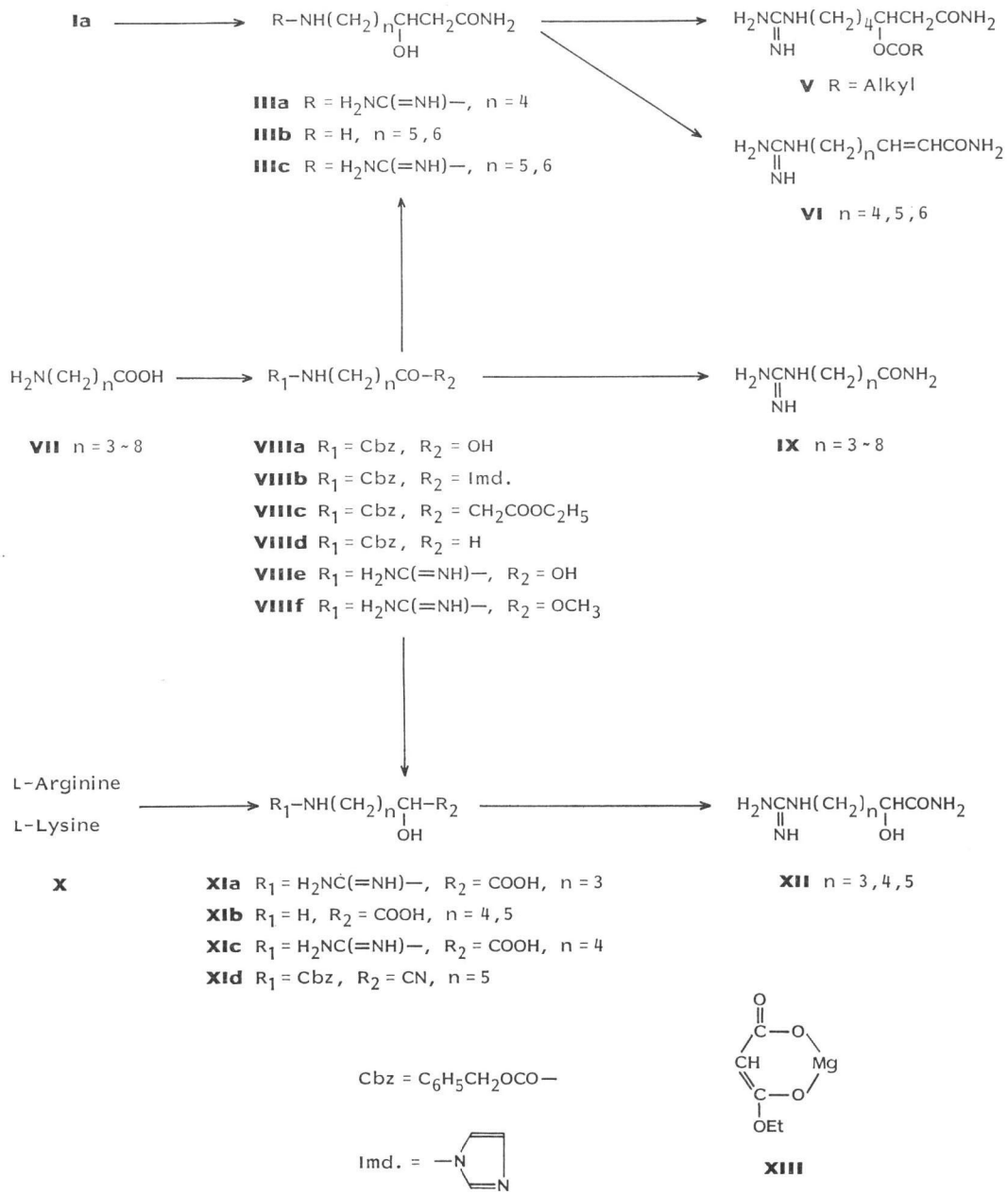
tected by protonation. Further we studied the effects of different catalysts. The results are summarized in Table 1.

Various carboxylic acids, especially dicarboxylic and tricarboxylic acids such as glutaric, succinic, and citric acid, were effective as catalysts. Mineral acids and *p*-toluenesulfonic acid were not useful.

Among solvents, water was the most suitable although the step of dehydration was necessary, and the amount of water affected the yield of the product. Using these results, we carried out the condensation reaction in the presence of glutaric acid (1~2.5 molar equiv) and water (10~100 molar equiv) at 60°C for 6~20 hours.

These relatively mild conditions can be generally used in analogue synthesis. In practice, we were successful in synthesizing analogues with various functional groups such as hydroxyl, acyloxy, or carbon-carbon double bond.

Scheme 2.



Preparation of ω-Guanidinoalkanamides

ω-Guanidinoalkanamides with various functional groups were prepared as shown in Scheme 2.

(*S*)-7-Guanidino-3-hydroxyheptanamide (**IIIa**), which was obtained by acid hydrolysis of spergualin (**Ia**), was used as a starting material. Acylation of the hydroxyl group of **IIIa** by acid anhydrides or acid chlorides with pyridine gave the corresponding (*S*)-3-acyloxy-7-guanidinoheptanamides (**V**). Racemic ω-guanidino-3-hydroxyalkanamides other than **IIIa** were synthesized from the corresponding *N*-protected ω-amino-3-keto-fatty acid esters (**VIIIc**). *N*-Protected ω-amino-fatty acids (**VIIIa**) were converted into reactive imidazolides (**VIIIb**) and were condensed with magnesium enolate of monoethyl malonate (**XIII**) to give 3-keto-esters (**VIIIc**), in which two-carbons units were increased.[5] Reduction of the carbonyl group with $NaBH_4$, ammonolysis of the ester group with ammonium hydroxide, and removal of the amino protective group gave ω-amino-3-hydroxyalkanamides (**IIIb**). Conversion of the regenerated amino group with *S*-methylisothiourea into a guanidino group gave the desired ω-guanidino-3-hydroxyalkanamides (**IIIc**).

ω-Guanidino-2-alkenamides (**VI**) were prepared by dehydration of **IIIa, c** with dicyclohexylcarbodiimide (DCC) and cupric chloride II.[6]

ω-Guanidinoalkanamides (**IX**) were synthesized from the corresponding ω-amino-fatty acids (**VII**). Treatment of ω-amino-fatty acids (**VII**) with *S*-methylisothiourea in alkaline solution gave ω-guanidino-fatty acids (**VIIIe**). Conversion of the carboxyl group into methyl ester (**VIIIf**) and ammonolysis with ammonium hydroxide gave ω-guanidinoalkanamides (**IX**).

ω-Guanidino-2-hydroxyalkanamides (**XII**) were synthesized by either of two methods from α-amino acids such as L-arginine or L-lysine (**X**), or from a cyanohydrin intermediate (**XId**). In the first method, conversion of α-amino group into α-hydroxyl group with nitrous acid and the successive transformation of carboxylic acid into amide, and ω-amino group into ω-guanidino group in the case of L-lysine, gave the desired compounds (**XII**). In the second method, racemic compound (**XII**) was synthesized *via* the cyanohydrin intermediate (**XId**); reduction of *N*-protected ω-amino-fatty acid imidazolide (**VIIIb**) with $LiAlH_4$ gave the corresponding aldehyde (**VIIId**); the aldehyde (**VIIId**) was converted to cyanohydrin (**XId**) with hydrogen cyanide. Removal of the *N*-protecting group of **XId** and hydration of cyano group with 35% hydrogen chloride gave ω-amino-2-hydroxyalkanamide (**XIb**). Conversion of the ω-amino group into a guanidino group gave the desired amide (**XII**).

All of these ω-guanidinoalkanamides were prepared as their hydrochlorides, and employed in the acid-catalyzed condensation with glyoxylylspermidine (**II**).

Preparation of Glyoxylylspermidine

Glyoxylylspermidine (**II**) was obtained by acid hydrolysis of natural spergualin (**Ia**), as shown in Scheme 1. The chemical synthesis of **II** has been accomplished by UMEZAWA *et al.*[3] Recently, we have devised a facile method that can be applied for preparation of **II** and its analogues. This method will be reported in another paper.

Preparation of 11-*O*-Alkyl-derivatives

Acid-catalyzed reaction of various alcohols with the condensation products described above or spergualin readily gave 11-*O*-alkylated derivatives. An advantage of 11-*O*-alkylation is that these derivatives are more stable than the original compound in aqueous solution.

All analogues and derivatives thus prepared were mixtures of epimers in 11-C. Effective methods for preparing optically active analogues and 11-*O*-alkylated derivatives will be reported elsewhere.

Structure and Antitumor Activity Relationships

Results concerning the antitumor activity of spergualin analogues and derivatives are shown in Tables 2 to 4.

In the course of analogue synthesis, we first modified the 15-hydroxyl group of spergualin by acylation.[7] The structures of 15-*O*-acylated spergualins and their antitumor activity are shown in Table 2. 15-*O*-Propionyl **4** and *n*-butyryl **6** derivatives had satisfactory activity. Acylation of the hydroxyl group with long-chain fatty acids decreased the activity (compounds **7**, **8**, **9**, **10**, **11** and **12**). 15-Benzoyl derivative **13** had little activity. These results suggested that if the hydrophilic portion becomes hydrophobic or a sterically bulky group is attached to the 15-hydroxyl group, the activity decreases. In addition, the 15-hydroxyl group may not be essential for the activity. The following

Table 2. Antitumor activity of 15-*O*-acylspergualins.

$$H_2NC(=NH)NH(CH_2)_4CH(OR_2)CH_2CONHCH(OR_1)CONH(CH_2)_4NH(CH_2)_3NH_2$$

Compound			Dose (mg/kg/day)							
No.	R_2	R_1	0.39	0.78	1.56	3.13	6.25	12.5	25	50
1	H: (−)**Ia**	H	104	135	>207	>340	>401	>388	>372	>372
2	H: (±)**Ia**	H		112	139	>269	>335	>400	>371	>359
3	$COCH_3$	H			110	123	>411	>411	>411	260
4	COC_2H_5	H			125	>375	>375	>375	>375	338
5	COC_2H_5	CH_3		100	114	129	200	>429	>295	>214
6	CO-*n*-C_3H_7	H			113	>280	>400	>400	>393	0
7	CO-*iso*-C_3H_7	H			113	167	>313	340	0	0
8	CO-*n*-C_4H_9	H			113	167	>280	>400	273	300
9	CO-*n*-C_5H_{11}	H			109	135	>276	>385	>336	250
10	CO-*n*-C_7H_{15}	H			128	192	199	>346	>327	>359
11	CO-*n*-C_9H_{19}	H			115	115	135	210	203	>318
12	CO-*n*-$C_{13}H_{27}$	H			97	111	125	139	>417	0
13	COC_6H_5	H			94	98	104	104	27	0

Results are shown as life prolongation (T/C %) in this Table and Tables 3 and 4.

Table 3. Antitumor activity of 15-deoxyspergualin analogues and their 11-*O*-alkyl-derivatives.

$$H_2NC(=NH)NH(CH_2)_nCONHCH(OR_3)CONH(CH_2)_4NH(CH_2)_3NH_2$$

Compound			Dose (mg/kg/day)									
No.	n	R_3	0.1	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50
14	3	H					94	94	100	100	88	100
15	4	H					100	100	100	100	100	100
16	5	H					95	101	95	95	95	101
17	5	CH_3					100	100	100	100	107	0
18	6	H	121	190	>372	>401	>397	>397	>342	>374	>349	0
19	6	CH_3		113	169	>346	>363	>411	>366	>387	6	0
20	6	C_2H_5		100	114	171	157	229	>429	>371	0	
21	6	C_2H_4OH		100	100	107	114	136	236	>329	>329	0
22	6	$CH_2C_6H_5$		100	107	107	114	129	171	264	0	0
23	7	H					104	118	125	181	306	0
24	7	CH_3					100	100	107	121	0	
25	8	H	>236	>290	>350	>416	>354	>379	>371	>386	0	0
26	8	CH_3	100	111	136	169	293	>393	>379	>314	0	

Table 4. Antitumor activity of miscellaneous analogues of spergualin.

X-NHCHCONH(CH$_2$)$_4$NH(CH$_2$)$_3$NH$_2$
|
OH

Compound		Dose (mg/kg/day)								
No.	X	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50
27	$H_2NC(=NH)NH(CH_2)_3CH(OH)CO$				100	100	100	100	107	100
28	$H_2NC(=NH)NH(CH_2)_4CH(OH)CO$				100	100	107	100	100	100
29	$H_2NC(=NH)NH(CH_2)_5CH(OH)CO$				100	100	100	100	100	100
30	$H_2NC(=NH)NH(CH_2)_4CH(OH)CH_2CO$				118	125	226	>313	>417	>417
31	$H_2NC(=NH)NH(CH_2)_5CH(OH)CH_2CO$				97	97	97	97	104	97
32	$H_2NC(=NH)NH(CH_2)_6CH(OH)CH_2CO$		100	100	114	129	164	200	229	0
33	$H_2NC(=NH)NH(CH_2)_4CH=CHCO$			129	229	>307	>350	>357	229	0
34	$H_2NC(=NH)NH(CH_2)_5CH=CHCO$			100	100	100	100	100	0	
35	$H_2NC(=NH)NH(CH_2)_6CH=CHCO$	136	157	200	>429	>429	>350	0		
36	$H_2N(CH_2)_4CH(OH)CH_2CO$				110	103	96	103	103	96

showed that this group was, in fact, unnecessary.

We prepared analogues in which the 7-guanidino-3-hydroxyheptanamide (**IIIa**) of spergualin was displaced by ω-guanidinoalkanamide comprising 4 to 9 carbon atoms. The structures and their antitumor activities are given in Table 3. Compound **18** with 7-guanidinoheptanamide and **25** with 9-guanidinononanamide had much improved activity. This indicates that the 15-hydroxyl group is not essential for the antitumor activity, and in fact, by removing the group, derivatives with improved activity were obtained. Concerning the carbon-chain length of the amide components, compounds **14**, **15**, **16**, **18**, **23** and **25** showed an interesting pattern. Those compounds in which the carbon-chain length of the ω-guanidino-acyl residue was 6 or shorter were inactive. Compound **23**, containing 8-guanidinooctanamide, was also inactive. The chain length of the ω-guanidino-acyl residue was important for activity, and this was also true in other analogues having a hydroxyl or double-bond in the ω-guanidinoalkan amide moiety, as for compounds **27**, **28**, **31** and **34** (Table 4).

It was also interesting that 14-hydroxyl analogue **29** was inactive, although its carbon-chain length was sufficient for activity.

The ω-guanidino group was essential for activity, as shown by the results for compound **36**.

11-*O*-Alkylated compounds **5**, **19**, **20**, **21**, **22** and **26** had less activity than the corresponding 11-hydroxyl compounds.

In conclusion, among the spergualin analogues and derivatives so far tested, 15-deoxyspergualin **18** and its homolog **25** had strong activity, superior to that of spergualin.

Experimental

MP were determined using a Yanagimoto melting point apparatus, and are uncorrected. None of the analogues or derivatives of spergualin in this report had a definite mp. IR spectra were taken on a Hitachi EPI-G2 or 273-30 spectrophotometer. NMR spectra were determined with a Hitachi R-24B (60 MHz) spectrometer. High performance liquid chromatography (HPLC) was done using a system consisting of an Altex 100A pump, Shimadzu CTO-2A column oven, Soma S-310A UV detector, and Shimadzu C-R1B Chromatopac recorder.

(A) Preparation of 15-*O*-Acylspergualins (Compounds **3**~**13**)

(15*S*)-15-Acetoxy-1-amino-19-guanidino-11-hydroxy-4,9,12-triazanonadecane-10,13-dione (15-*O*-Acetylspergualin **3**)

To 813 mg (3.41 mmol) of (*S*)-7-guanidino-3-hydroxyheptanamide (**IIIa**) (HCl salt) were added 7 ml of pyridine and 7 ml of acetic anhydride. The mixture was stirred at room temp overnight. The reaction mixture was poured into 100 ml of H_2O, concentrated under reduced pressure, and chromatographed on a CM-Sephadex C-25 (Na^+) column (450 ml) with linear gradient elution using H_2O (1.5 liters) and 1 M NaCl solution (1.5 liters). The fractions containing the desired product were collected and evaporated to dryness, and the residue was extracted with MeOH. The MeOH extract was passed through a Sephadex LH-20 column (1.5 liters), and then eluted with MeOH. The fractions positive for the Sakaguchi reaction were combined and evaporated to dryness, giving 753 mg (78.8%) of (*S*)-7-guanidino-3-acetoxyheptanamide (HCl salt) as a syrup: IR (KBr) 3330, 3160, 2930, 2850, 1720, 1660, 1425, 1370, 1255, 1175, 1025 cm^{-1}; ^1H NMR (CD_3OD) 1.4~1.9 (6H, m, CH_2), 2.00 (3H, s, $COCH_3$), 2.49 (2H, d, CH_2CO), 3.18 (2H, t, NCH_2), 5.19 (1H, m, 3-CH).

A mixture of 354 mg (1.26 mmol) of (*S*)-7-guanidino-3-acetoxyheptanamide (HCl salt), 429 mg (1.47 mmol) of 11-amino-1,1-dihydroxy-3,8-diazaundecane-2-one **IIa** (2HCl salt) and 333 mg (2.52 mmol) of glutaric acid was dissolved in H_2O (0.5 ml) and heated overnight at 60°C. The reaction mixture was diluted with H_2O and chromatographed on CM-Sephadex C-25 (Na^+) with gradient elution using H_2O and 1 M NaCl solution. Fractions containing the desired product were collected and evaporated to dryness. The residue was extracted with MeOH and the MeOH extract chromatographed on Sephadex LH-20 with elution by MeOH; the active fractions were combined and evaporated to dryness, giving 146 mg (21.6%) of compound **3** (3HCl salt): $[\alpha]_D$ +2.4° (*c* 1, H_2O); IR (KBr) 3400, 2930, 1720, 1655, 1525, 1460, 1370, 1250, 1160, 1075, 1025 cm^{-1}; ^1H NMR (CD_3OD) 1.4~1.9 (10H, m, CH_2), 2.03 (3H, s, $COCH_3$), 2.18 (2H, m, CH_2), 2.59 (2H, d, CH_2CO), 2.9~3.4 (10H, NCH_2), 5.22 (1H, m, 15-CH), 5.51 (1H, s, s, 11-CH).

A procedure similar to that used for compound **3** was followed in the preparation of the 15-*O*-acylspergualins. To purify the intermediate and final products, chromatography on Diaion HP-20 was used as well as CM-Sephadex C-25 (Na^+) and Sephadex LH-20. For acylation of **IIIa**, propionic anhydride, isobutyric anhydride, *n*-valeric anhydride, *n*-hexanoic anhydride, *n*-octanoic anhydride, *n*-decanoic anhydride, *n*-tetradecanoic anhydride, or benzyl chloride was used with pyridine and *N*,*N*-dimethylformamide. Spectral data of the final products numbered from **4** to **13** were as follows.

(15*S*)-1-Amino-19-guanidino-11-hydroxy-15-propionyloxy-4,9,12-triazanonadecane-10,13-dione (15-*O*-Propionylspergualin **4**)

$[\alpha]_D$ +2.4° (*c* 1, H_2O); IR (KBr) 3320, 2925, 1720, 1655, 1520, 1450, 1370, 1270, 1190, 1075 cm^{-1}; ^1H NMR (CD_3OD) 1.10 (3H, t, CH_3), 1.4~1.9 (10H, m, CH_2), 2.21 (2H, m, 2-CH_2), 2.32 (2H, q, $COCH_2$), 2.54 (2H, d, 14-CH_2), 2.9~3.4 (10H, NCH_2), 5.21 (1H, m, 15-CH), 5.45 (1H, s, s, 11-CH).

(15*S*)-1-Amino-15-butyryloxy-19-guanidino-11-hydroxy-4,9,12-triazanonadecane-10,13-dione (15-*O*-Butyrylspergualin **6**)

$[\alpha]_D$ +3.1° (*c* 1, H_2O); IR (KBr) 3350, 3170, 2950, 2930, 2860, 1720, 1660, 1525, 1450, 1380, 1260,

1180, 1080 cm^{-1}; ^1H NMR (CD_3OD) 0.94 (3H, t, CH_3), 1.4~1.9 (12H, m, CH_2), 2.14 (2H, m, 2-CH_2), 2.29 (2H, t, $COCH_2$), 2.54 (2H, d, 14-CH_2CO), 2.9~3.4 (10H, NCH_2), 5.24 (1H, m, 15-CH), 5.48 (1H, s, s, 11-CH).

(15*S*)-1-Amino-19-guanidino-11-hydroxy-15-isobutylyloxy-4,9,12-triazanonadecane-10,13-dione (15-*O*-Isobutyrylspergualin **7**)

$[\alpha]_D$ +3.1° (*c* 1, H_2O); IR (KBr) 3320, 3150, 2955, 2930, 2860, 1725, 1655, 1520, 1460, 1380, 1265, 1190, 1155, 1070, 990 cm^{-1}; ^1H NMR (CD_3OD) 1.13 (6H, d, CH_3), 1.4~1.9 (10H, m, CH_2), 2.15 (2H, m, 2-CH_2), 2.56 and 2.59 (2H, d, d, 14-CH_2CO), 2.6 (1H, m, CHCO), 2.9~3.4 (10H, NCH_2), 5.25 (1H, m, 15-CH), 5.48 (1H, s, s, 11-CH).

(15*S*)-1-Amino-19-guanidino-11-hydroxy-15-pentanoyloxy-4,9,12-triazanonadecane-10,13-dione (15-*O*-Pentanoylspergualin **8**)

$[\alpha]_D$ +1.3° (*c* 1, H_2O); IR (KBr) 3320, 3160, 2950, 2930, 2860, 1725, 1660, 1525, 1460, 1375, 1260, 1170, 1085 cm^{-1}; ^1H NMR (CD_3OD) 0.91 (3H, t, CH_3), 1.2~1.9 (14H, m, CH_2), 2.23 (2H, m, 2-CH_2), 2.30 (2H, t, CH_2CO), 2.55 and 2.58 (2H, d, d, 14-CH_2), 2.9~3.4 (10H, NCH_2), 5.20 (1H, m, 15-CH), 5.25 and 5.47 (1H, d, d, 11-CH).

(15*S*)-1-Amino-19-guanidino-15-hexanoyl-11-hydroxy-4,9,12-triazanonadecane-10,13-dione (15-*O*-Hexanoylspergualin **9**)

$[\alpha]_D$ +3.6° (*c* 1, H_2O); IR (KBr) 3395, 3160, 2940, 2920, 2850, 1720, 1655, 1520, 1455, 1375, 1245, 1165, 1090 cm^{-1}; ^1H NMR (CD_3OD) 0.91 (3H, t, CH_3), 1.1~2.0 (16H, m, CH_2), 2.26 (2H, m, 2-CH_2), 2.33 (2H, t, CH_2), 2.58 and 2.63 (2H, d, d, 14-CH_2), 2.9~3.4 (10H, NCH_2), 5.20 (1H, m, 15-CH), 5.30 and 5.51 (1H, d, d, 11-CH).

(15*S*)-1-Amino-19-guanidino-11-hydroxy-15-octanoyloxy-4,9,12-triazanonadecane-10,13-dione (15-*O*-Octanoylspergualin **10**)

$[\alpha]_D$ +2.5° (*c* 1, H_2O); IR (KBr) 3390, 3170, 2950, 2920, 2850, 1720, 1655, 1520, 1460, 1370, 1260, 1165, 1100 cm^{-1}; ^1H NMR (CD_3OD) 0.89 (3H, t, CH_3), 1.1~2.0 (10H, m, CH_2), 2.22 (2H, m, 2-CH_2), 2.32 (2H, t, CH_2CO), 2.66 (2H, d, 14-CH_2), 2.9~3.4 (10H, NCH_2), 5.23 (1H, m, 15-CH), 5.49 (1H, s, s, 11-CH).

(15*S*)-1-Amino-15-decanoyloxy-19-guanidino-11-hydroxy-4,9,12-triazanonadecane-10,13-dione (15-*O*-Decanoylspergualin **11**)

$[\alpha]_D$ +3.3° (*c* 1, H_2O); IR (KBr) 3340, 3160, 2950, 2920, 2850, 1730, 1650, 1525, 1460, 1370, 1245, 1160, 1105, 1070 cm^{-1}; ^1H NMR (CD_3OD): 0.89 (3H, t, CH_3), 1.1~2.0 (24H, m, CH_2), 2.26 (2H, m, 2-CH), 2.31 (2H, t, CH_2), 2.59 (1H, m, 15-CH), 2.9~3.4 (10H, NCH_2), 5.24 (1H, m, 15-CH), 5.50 (1H, s, s, 11-CH).

(15*S*)-1-Amino-19-guanidino-11-hydroxy-15-tetradecanoyloxy-4,9,12-triazanonadecane-10,13-dione (15-*O*-Tetradecanoylspergualin **12**)

$[\alpha]_D$ +4° (*c* 1, H_2O); IR (KBr) 3400, 2930, 2850, 1725, 1650, 1525, 1460, 1170, 1110 cm^{-1}; ^1H NMR (CD_3OD) 0.89 (3H, t, CH_3), 1.2~2.1 (32H, m, CH_2), 2.1~2.4 (4H, m, CH_2), 2.56 (2H, d, 14-CH_2), 2.9~3.4 (10H, NCH_2), 5.25 (1H, m, 15-CH), 5.50 (1H, d, 11-CH).

(15*S*)-1-Amino-15-benzoyloxy-19-guanidino-11-hydroxy-4,9,12-triazanonadecane-10,13-dione (15-*O*-Benzyloxyspergualin **13**)

$[\alpha]_D$ −5° (*c* 1, H_2O); IR (KBr) 3370, 2930, 1700 (sh), 1655, 1520, 1440, 1310, 1275, 1110, 1065, 1020, 710 cm^{-1}; ^1H NMR (CD_3OD) 1.4~1.9 (10H, m, CH_2), 2.16 (2H, m, 2-CH_2), 2.72 (2H, d, 14-CH_2), 2.9~3.4 (10H, NCH_2), 5.50 (1H, m, 15-CH), 5.52 (1H, s, s, 11-CH), 7.3~8.2 (5H, C_6H_5).

(B) Preparation of 15-Deoxyspergualin Analogues (Compounds **14**, **15**, **16**, **18**, **23** and **25**)

1-Amino-19-guanidino-11-hydroxy-4,9,12-triazanonadecane-10,13-dione (15-Deoxyspergualin **18**)

A solution of 8.4 g (58 mmol) of 7-aminoheptanoic acid and 10.07 g (74 mmol) of *S*-methylisothiourea hemisulfate in 43.4 ml of 3 N NaOH was stirred at 55°C overnight. The resulting solid was

collected by filtration and washed with cold water and acetone to give 7-guanidinoheptanoic acid as crude crystals. The crystals were dissolved in 36 ml of 2 N HCl with heat and left at room temp overnight, giving 11.0 g (84%) of 7-guanidinoheptanoic acid (HCl salt) as white crystals: mp 166~167.5°C; IR (KBr) 3400, 3250, 3170, 3100, 2930, 2850, 1720, 1675, 1635, 1605, 1470, 1410, 1395, 1225, 1170, 800, 790 cm^{-1}; ^1H NMR (D_2O) 1.2~1.8 (8H, m, CH_2), 2.39 (2H, t, CH_2CO), 3.20 (2H, t, NCH_2).

To a solution of 10 g (44.6 mmol) of 7-guanidinoheptanoic acid (HCl salt) in 56 ml of MeOH was added 0.5 ml of Dowex 50W (H^+), and the mixture was refluxed for 3.5 hours. After cooling, the catalyst was removed by filtration and the solution was evaporated to dryness, giving methyl 7-guanidinoheptanoate (HCl salt) as a syrup. The methyl ester underwent ammonolysis without further purification when 35 ml of ammonium hydroxide was added. After being stirred overnight at room temp, the solution was evaporated and dissolved in H_2O (10 ml). The solid, which was 7-guanidinoheptanoic acid, was removed by filtration and the remaining solution was passed through a column (160 ml) of CM-Sephadex C-25 (Na^+). After being washed with 600 ml of H_2O, the column was eluted with 0.5 M NaCl solution. Fractions positive for the Sakaguchi reaction were combined, evaporated, desalted with EtOH extraction, and chromatographed on a Sephadex LH-20 column as described in Section (A), giving 7.52 g (76%) of 7-guanidinoheptanamide (HCl salt): mp 127~130°C; IR (KBr) 3350, 3150, 2920, 1655, 1630, 1590, 1455, 1430, 1400, 1220, 1165, 1130, 1065 cm^{-1}; ^1H NMR (CD_3OD) 1.2~1.9 (8H, m, CH_2), 2.23 (2H, t, CH_2CO), 3.20 (2H, t, NCH_2)

A mixture of 7.50 g (33.7 mmol) of 7-guanidinoheptanamide (HCl salt), 8.97 g (30.7 mmol) of **IIa**, 4.05 g (30.7 mmol) of glutaric acid, and 2.0 g of H_2O was kept at 60°C for 8 hours. The reaction mixture was chromatographed and purified as described for compound **3** in Section (A), giving 6.08 g (39.3%) of **18**: IR (KBr) 3300, 3250, 3160, 2925, 2850, 1645, 1520, 1460, 1360, 1260, 1160, 1115, 1070, 1000, 580 cm^{-1}; ^1H NMR (D_2O) 1.2~2.4 (16H, m, CH_2), 2.9~3.4 (10H, m, NCH_2), 5.40 (1H, s, 11-CH).

Spectral data of compounds **14**, **15**, **16**, **23** and **25** were as follows.

1-Amino-16-guanidino-11-hydroxy-4,9,12-triazahexadecane-10,13-dione (**14**)

IR (KBr) 3320, 2950, 1655, 1525, 1460, 1365, 1260, 1160, 1115, 1070 cm^{-1}; ^1H NMR (CD_3OD) 1.4~2.4 (8H, m, CH_2), 2.40 (2H, m, 2-CH_2), 2.9~3.4 (10H, NCH_2), 5.55 (1H, s, 11-CH).

1-Amino-17-guanidino-11-hydroxy-4,9,12-triazaheptadecane-10,13-dione (**15**)

IR (KBr) 3400, 2950, 1660, 1530, 1465, 1170, 1120, 1080, 1020 cm^{-1}; ^1H NMR (CD_3OD) 1.4~2.4 (10H, m, CH_2), 2.4 (2H, m, 2-CH_2), 2.9~3.4 (10H, NCH_2), 5.35 (1H, s, 11-CH).

1-Amino-18-guanidino-11-hydroxy-4,9,12-triazaoctadecane-10,13-dione (**16**)

IR (KBr) 3270, 2950, 1660, 1530, 1460, 1370, 1240, 1165, 1120, 1080 cm^{-1}; ^1H NMR (CD_3OD) 1.4~2.0 (10H, m, CH_2), 2.0~2.5 (4H, m, CH_2), 2.9~3.4 (10H, NCH_2), 5.52 (1H, s, 11-CH).

1-Amino-20-guanidino-11-hydroxy-4,9,12-triazaeicosane-10,13-dione (**23**)

IR (KBr) 3330, 2925, 1655, 1520, 1460, 1360, 1160, 1120, 1080 cm^{-1}; ^1H NMR (CD_3OD) 1.2~2.0 (12H, m, CH_2), 2.0~2.4 (4H, m, CH_2), 2.9~3.4 (10H, NCH_2), 5.52 (1H, s, 11-CH).

1-Amino-21-guanidino-11-hydroxy-4,9,12-triazauneicosane-10,13-dione (**25**)

IR (KBr) 3370, 2925, 1655, 1520, 1460, 1155, 1115, 1080 cm^{-1}; ^1H NMR (CD_3OD) 1.2~2.0 (16H, m, CH_2), 2.0~2.4 (4H, m, CH_2), 2.9~3.4 (10H, NCH_2), 5.50 (1H, s, 11-CH).

(C) Preparation of 11-*O*-Alkylspergualin Derivative (Compounds **5**, **17**, **19**, **20**, **21**, **22**, **24** and **26**)

1-Amino-19-guanidino-11-methoxy-4,9,12-triazanonadecane-10,13-dione (**19**)

A mixture of 27.5 g (55.3 mmol) of **18**, 150 ml of MeOH, and 2.5 ml of 36% HCl was stirred at room temp overnight. The reaction mixture was evaporated to dryness and dissolved in H_2O (200 ml). The solution was adjusted to pH 5 with 0.5 N NaOH and chromatographed on CM-Sephadex C-25 (Na^+) and Sephadex LH-20 as described for compound **3** in Section (A), giving 22.0 g (78%) of **19**. IR (KBr) 3420, 2950, 1650, 1520, 1460, 1360, 1190, 1160, 1090 cm^{-1}; ^1H NMR

(CD_3OD) 1.2~2.0 (12H, m, CH_2), 2.0~2.5 (4H, m, CH_2), 2.9~3.4 (10H, NCH_2), 3.37 (3H, s, OCH_3), 5.26 (1H, s, 11-CH).

By a procedure similar to that described above for compound **19**, compounds **5**, **17**, **20**, **21**, **22**, **24** and **26** were prepared by replacing MeOH with the corresponding alcohol. Their spectral data were as follows.

1-Amino-19-guanidino-11-methoxy-15-propionyloxy-4,9,12-triazanonadecane-10,13-dione (**5**)

$[\alpha]_D$ +2.2° (*c* 1, H_2O); IR (KBr) 3400, 3180, 2940, 1720, 1660, 1530, 1460, 1200, 1085 cm^{-1}; ^1H NMR (CD_3OD) 1.10 (3H, t, CH_3), 1.4~2.0 (10H, m, CH_2), 2.2 (2H, m, 2-CH_2), 2.34 (2H, q, $COCH_2$), 2.63 (2H, d, 14-CH_2), 2.9~3.4 (10H, NCH_2), 3.38 (3H, s, OCH_3), 5.20 (1H, m, 15-CH), 5.29, 5.30 (1H, s, s, 11-CH).

1-Amino-18-guanidino-11-methoxy-4,9,12-triazaoctadecane-10,13-dione (**17**)

IR (KBr) 3420, 2940, 1650, 1520, 1460, 1355, 1190, 1160, 1090 cm^{-1}; ^1H NMR (CD_3OD) 1.4~2.0 (10H, m, CH_2), 2.0~2.5 (4H, m, CH_2), 2.9~3.4 (10H, NCH_2), 3.38 (3H, s, OCH_3), 5.29 (1H, s, 11-CH).

1-Amino-19-guanidino-11-ethoxy-4,9,12-triazanonadecane-10,13-dione (**20**)

IR (KBr) 3400, 2930, 1655, 1520, 1460, 1360, 1160, 1085 cm^{-1}; ^1H NMR (CD_3OD) 1.23 (3H, t, CH_3), 1.3~2.0 (12H, m, CH_2), 2.0~2.5 (4H, m, CH_2), 2.9~3.4 (10H, NCH_2), 3.64 (2H, q, OCH_2), 5.42 (1H, s, 11-CH).

1-Amino-19-guanidino-11-(2′-hydroxy)ethoxy-4,9,12-triazanonadecane-10,13-dione (**21**)

IR (KBr) 3370, 2930, 1655, 1520, 1455, 1355, 1165, 1110, 1060 cm^{-1}; ^1H NMR (CD_3OD) 1.2~2.0 (12H, m, CH_2), 2.0~2.5 (4H, m, CH_2), 2.9~3.4 (10H, NCH_2), 3.70 (4H, t, t, OCH_2), 5.45 (1H, s, 11-CH).

1-Amino-19-guanidino-11-benzyloxy-4,9,12-triazanonadecane-10,13-dione (**22**)

IR (KBr) 3340, 2930, 1655, 1520, 1450, 1160, 1065, 1020, 740, 695 cm^{-1}; ^1H NMR (CD_3OD) 1.2~2.0 (12H, m, CH_2), 2.0~2.5 (4H, m, CH_2), 2.9~3.4 (10H, NCH_2), 4.64 (2H, s, OCH_2), 5.51 (1H, s, 11-CH), 7.32 (5H, s, C_6H_5).

1-Amino-20-guanidino-11-methoxy-4,9,12-triazaeicosane-10,13-dione (**24**)

IR (KBr) 3400, 2925, 1650, 1520, 1455, 1355, 1250, 1190, 1160, 1090 cm^{-1}; ^1H NMR (CD_3OD) 1.2~2.0 (14H, m, CH_2), 2.0~2.5 (4H, m, CH_2), 2.9~3.4 (10H, NCH_2), 3.37 (3H, s, OCH_3), 5.28 (1H, s, 11-CH).

1-Amino-21-guanidino-11-methoxy-4,9,12-triazauneicosane-10,13-dione (**26**)

IR (KBr) 3400, 2930, 1655, 1520, 1460, 1360, 1190, 1160, 1090 cm^{-1}; ^1H NMR (CD_3OD) 1.2~2.0 (16H, m, CH_2), 2.0~2.5 (4H, m, CH_2), 2.9~3.4 (10H, NCH_2), 3.37 (3H, s, OCH_3), 5.29 (1H, s, 11-CH).

(D) Preparation of 15-Deoxy-14-hydroxyspergualin Analogues (Compounds **27**, **28** and **29**)

(14*S*)-1-Amino-18-guanidino-11,14-dihydroxy-4,9,12-triazaoctadecane-10,13-dione (**28**)

To a solution of 2 g (11.0 mmol) of L-lysine in H_2O (10 ml) were added 1.90 g (6.0 mmol) of $Ba(OH)_2$ and 1.68 g (6.0 mmol) of *S*-methylisothiourea hemisulfate. The mixture was stirred at 80°C for 2 hours and brought to pH 6.5 with 2 N H_2SO_4. The resulting precipitate was removed by filtration and the filtrate was purified by chromatography on CM-Sephadex C-25 (Na^+) and Dowex 50 W (H^+), giving 1.48 g (72%) of (*S*)-6-guanidino-2-aminohexanoic acid: mp 220~222°C; $[\alpha]_D$ +13.6° (*c* 1, H_2O).

To a solution of 970 mg (5.2 mmol) of (*S*)-6-guanidino-2-aminohexanoic acid in 35% acetic acid (68 ml) was added a solution of 1.28 g (18.6 mmol) of sodium nitrite in H_2O (3 ml). The mixture was stirred at room temp for 4 hours and the resulting mixture was purified by chromatography on Dowex 50 W (H^+), giving 674 mg (69%) of (*S*)-6-guanidino-2-hydroxyhexanoic acid: mp 242~246°C; $[\alpha]_D$ −0.2° (*c* 1, 1 N HCl).

Methylation and amidation of (*S*)-6-guanidino-2-hydroxyhexanoic acid as in the preparation of

III in Section (B) gave 543 mg (78%) of (*S*)-6-guanidino-2-hydroxyhexanamide (HCl salt) as a colorless crystal: mp 116~118°C; $[\alpha]_D$ −18.6° (*c* 1, H_2O); IR (KBr) 3325, 3170, 2925, 1655, 1450, 1390, 1110, 1080 cm^{-1}; ^1H NMR (CD_3OD) 1.4~1.8 (6H, m, CH_2), 3.17 (2H, NCH_2), 3.94 (1H, t, CHCO).

A mixture of 500 mg (2.2 mmol) of (*S*)-6-guanidino-2-hydroxyhexanamide (HCl salt), 773 mg (2.6 mmol) of **IIa**, 291 mg (2.2 mmol) of glutaric acid, and 380 mg of H_2O was kept at 60°C overnight. The reaction mixture was purified as for **3** in Section (A), giving 262 mg (24%) of **28**: $[\alpha]_D$ −10.6° (*c* 1, H_2O); IR (KBr) 3375, 2930, 1650, 1520, 1455, 1110, 1070 cm^{-1}; ^1H NMR (CD_3OD) 1.5~2.0 (10H, m, CH_2), 2.15 (2H, m, 2-CH_2), 2.9~3.4 (10H, NCH_2), 4.08 (1H, t, 14-CH), 5.50 (1H, s, 11-CH).

(14*S*)-1-Amino-17-guanidino-11,14-dihydroxy-4,9,12-triazahexadecane-10,13-dione (**27**)

$[\alpha]_D$ −10.5° (*c* 1, H_2O); IR (KBr) 3360, 3180, 2950, 1660, 1525, 1515, 1460, 1115, 1080 cm^{-1}; ^1H NMR (CD_3OD) 1.5~2.0 (8H, m, CH_2), 2.12 (2H, 2-CH_2), 2.9~3.5 (10H, NCH_2), 4.10 (1H, t, 14-CH), 5.51 (1H, s, 11-CH).

1-Amino-19-guanidino-11,14-dihydroxy-4,9,12-triazanonadecane-10,13-dione (**29**)

A solution of 2.56 g (10 mmol) of 6-benzyloxycarbonylaminohexanoic acid and 1.78 g (11 mmol) of 1,1-carbonyldiimidazole in tetrahydrofuran (THF, 70 ml) was stirred for 20 minutes at room temp. To the solution, while being cooled at −15°C, was added 759 mg (20 mmol) of lithium aluminum hydride with THF (30 ml) for 30 minutes at the same temp, and the solution was acidified with 2 N HCl (5 ml) and evaporated *in vacuo*. The resulting residue was extracted with $CHCl_3$ (120 ml), and the $CHCl_3$ layer was washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was chromatographed on silica gel (70 g) with elution by $CHCl_3$, giving 1.51 g (59%) of 6-benzyloxycarbonylaminohexanal: ^1H NMR (neat) 1.2~1.9 (6H, m, CH_2), 2.42 (2H, t, 2-CH_2), 3.18 (2H, q, 6-CH_2), 4.85 (1H, NH), 5.10 (2H, s, CH_2), 7.32 (5H, s, C_6H_5), 9.75 (1H, t, CHO).

A mixture of 1.35 g (5.42 mmol) of 6-benzyloxycarbonylaminohexanal, 664 mg (13.55 mmol) of sodium cyanide and 36% HCl (1.19 mmol) was stirred for 15 minutes. To this mixture were slowly added H_2O (6 ml) and ether (6 ml), over a period of 1 hour, and it was stirred for another 1 hour. The reaction mixture was extracted with ether (10 ml×3) and washed with brine (15 ml×2). The ether portion was evaporated *in vacuo*, giving 1.34 g (89.6%) of the desired cyanohydrin, 6-benzyloxycarbonylamino-1-hydroxyhexanecarbonitrile: ^1H NMR (CD_3OD) 1.2~1.9 (8H, m, CH_2), 3.12 (2H, m, NCH_2), 4.44 (1H, t, CH), 5.07 (2H, s, CH_2), 7.30 (5H, s, C_6H_5).

The cyanohydrin (1.18 mg, 4.27 mol) in MeOH (20 ml) and 1 N HCl (5 ml) was hydrogenated with 10% palladium on charcoal under a stream of hydrogen at atmospheric pressure for 2.5 hours at room temp. The catalyst was removed by filtration and the filtrate was evaporated. The residue was dissolved in H_2O (50 ml), and the solution was washed with EtOAc (50 ml). The aqueous layer was evaporated to dryness, giving 794 mg of crude 6-amino-1-hydroxyhexanecarbonitrile. The residue obtained above was dissolved in 36% HCl (5 ml) and stirred overnight. The reaction mixture was diluted with H_2O (30 ml), brought to pH 6.5 with 0.5 N NaOH, and purified by chromatography on CM-Sephadex C-25 and Sephadex LH-20 as described in Section (A), giving 277.8 mg of 7-amino-2-hydroxyheptanamide (HCl salt): ^1H NMR (D_2O) 1.3~2.0 (8H, m, CH_2), 2.98 (2H, t, NCH_2), 4.04 (1H, t, 2-CH).

Conversion of the generated amino group into a guanidino group as for **18** in Section (B), gave 199.5 mg (59.3%) of 7-guanidino-2-hydroxyheptanamide (HCl salt): ^1H NMR (D_2O) 1.3~2.0 (8H, m, CH_2), 3.2 (2H, t, NCH_2), 4.03 (1H, t, 2-CH).

Acid-catalyzed condensation of 200 mg (0.84 mmol) of 7-guanidino-2-hydroxyheptanamide with 295 mg (1.01 mmol) of **II**, as described in Sections (A) and (B), gave 79 mg (18%) of **29**: IR (KBr) 3360, 2950, 1660, 1525, 1460, 1110 cm^{-1}; ^1H NMR (CD_3OD) 1.2~2.4 (14H, m, CH_2), 2.9~3.4 (10H, NCH_2), 4.03 (1H, m, 14-CH), 5.50 (1H, s, 11-CH).

(E) Preparation of 15-Epimeric-spergualin Analogues (Compounds **30**, **31** and **32**)

1-Amino-20-guanidino-11,15-dihydroxy-4,9,12-triazaeicosane-10,13-dione (**31**)

A solution of 2.65 g (10 mmol) of 6-benzyloxycarbonylaminohexanoic acid and 1.62 g (10 mmol)

of 1,1-carbonyldiimidazole in anhydrous THF (25 ml) was stirred for 15 minutes at room temp. To the reaction mixture was added a suspension of 6.18 g (40 mmol) of magnesium enolate of monoethyl malonate (prepared from 5.28 g of monoethyl malonate and 972 mg of magnesium) in 50 ml of THF. The mixture was stirred for 2 hours at room temp. After 50 ml of 1 N HCl was added and stirred for 10 minutes, the reaction mixture was extracted with $CHCl_3$ (50 ml×3). The $CHCl_3$ layer was washed successively with 1 N HCl, saturated $NaHCO_3$, and saturated brine, dried over anhydrous Na_2SO_4 and freed from the solvent by distillation. The residue was chromatographed on a column of silica gel (100 g) eluted with $CHCl_3$, giving 2.35 g (70%) of ethyl-8-benzyloxycarbonylamino-3-ketooctanoate: ^{1}H NMR (CD_3Cl) 1.27 (3H, t, CH_3), 1.1~1.9 (6H, m, CH_2), 2.52 (2H, t, CH_2CO), 3.17 (2H, t, NCH_2), 3.40 (2H, q, OCH_2), 4.18 (2H, s, $COCH_2CO$), 5.05 (1H, NH), 5.09 (2H, s, $CH_2C_6H_5$), 7.32 (5H, s, C_6H_5).

To a solution of 2.01 g (6 mmol) of ethyl 8-benzyloxycarbonylamino-3-ketooctanoate in EtOH (20 ml) was added 227 mg (6 mmol) of $NaBH_4$ with stirring at room temp. The mixture was stirred for 30 minutes, then mixed with several drops of acetic acid, poured into 100 ml of H_2O, and extracted with $CHCl_3$ (50 ml×3). The $CHCl_3$ layer was washed successively with 1 N HCl, saturated $NaHCO_3$, and saturated saline, then dried over anhydrous Na_2SO_4 and freed from the solvent by distillation, giving 2.00 g (99%) of ethyl 8-benzyloxycarbonylamino-3-hydroxyoctanoate: mp 47~50°C.

Into 40 ml of MeOH saturated with gaseous ammonia was dissolved 1.69 g (5 mmol) of ethyl 8-benzyloxycarbonylamino-3-hydroxyoctanoate. The solution was stirred for 3 days at room temp, and the reaction mixture was evaporated to dryness. The residue was crystallized from EtOH, giving 1.18 g (72.5%) of 8-benzyloxycarbonylamino-3-hydroxyoctanamide as crystals, mp 100~101°C.

Removal of the *N*-protective group by hydrogenation and conversion of the regenerated amino group into guanidino group gave 687 mg (85%) of 8-guanidino-3-hydroxyoctanamide (HCl salt).

Acid-catalyzed condensation of 150 mg (0.59 mmol) of 8-guanidino-3-hydroxyoctanamide with 208 mg (0.71 mmol) of **II**, gave 121 mg (38.6%) of **31**; IR (KBr) 3450, 2925, 1650, 1525, 1460, 1160, 1110, 1075 cm^{-1}; ^{1}H NMR (CD_3OD) 1.4~2.3 (14H, m, CH_2), 2.38 (2H, d, 14-CH_2), 2.9~3.4 (10H, NCH_2), 4.0 (1H, m, 15-CH), 5.52 (1H, s, 11-CH).

1-Amino-21-guanidino-11,15-dihydroxy-4,9,12-triazauneicosane-10,13-dione (**32**)

IR (KBr) 3400, 2950, 1655, 1520, 1460, 1165, 1110, 1075 cm^{-1}; ^{1}H NMR (CD_3OD) 1.2~2.4 (16H, m, CH_2), 2.41 (2H, d, 14-CH_2), 2.9~3.4 (10H, NCH_2), 4.0 (1H, m, 15-CH), 5.58 (1H, s, 11-CH).

(F) Preparation of 15-Deoxy-14,15-didehydrospergualin Analogues (Compounds **33**, **34** and **35**)

1-Amino-19-guanidino-11-hydroxy-4,9,12-triaza-14-nonadecene-10,13-dione (**33**)

To a solution of 955 mg (4 mmol) of **IIIa** (HCl salt) in DMF (20 ml) were added 2.48 g (12 mmol) of DCC and 40 mg of cupric chloride II. The mixture was stirred at room temp for 2 days. The resulting precipitate was filtered off and the filtrate evaporated *in vacuo*. The residue was dissolved in H_2O (10 ml) and the solution was washed twice with EtOAc (10 ml). The aqueous layer was chromatographed on CM-Sephadex C-25 (Na^+) and Sephadex LH-20 as described above, giving 950 mg (89.5%) of 7-guanidino-2-heptenamide (HCl salt) as colorless crystals: mp 162~168°C; IR (KBr) 3370, 3150, 1660, 1625, 1610, 1590, 1415, 1395, 1370 cm^{-1}; ^{1}H NMR (CD_3OD) 1.4~1.8 (4H, m, CH_2), 2.27 (2H, m, 4-CH_4), 3.20 (2H, t, NCH_2), 5.98 (1H, m, 3-CH), 6.80 (1H, d, 2-CH).

A mixture of 235 mg (1.1 mmol) of 7-guanidino-2-heptenamide (HCl salt), 372 mg (1.3 mmol) of **IIa** (2HCl salt), 140 mg (1.1 mmol) of glutaric acid, and 200 mg of H_2O was kept at 60°C overnight. The desired product was purified with CM-Sephadex and Sephadex LH-20 chromatography as described above, giving 245 mg (46.5%) of **33** (3HCl salt): IR (KBr) 3350, 2930, 1660, 1520, 1460, 1355, 1160, 1110, 1075 cm^{-1}; ^{1}H NMR (CD_3OD) 1.4~2.5 (14H, m, CH_2), 2.9~3.4 (10H, NCH_2), 5.56 (1H, s, 11-CH), 6.01 (1H, m, 15-CH), 6.81 (1H, d, 14-CH).

Spectra data of compounds **34** and **35** were as follows.

1-Amino-20-guanidino-11-hydroxy-4,9,12-triaza-14-eicosene-10,13-dione (**34**)

IR (KBr) 3400, 2925, 1600, 1530, 1460, 1360, 1165, 1115, 1080 cm^{-1}; ^{1}H NMR (CD_3OD) 1.4~2.5 (14H, m, CH_2), 2.9~3.4 (10H, NCH_2), 5.60 (1H, s, 11-CH), 6.02 (1H, m, 15-CH), 6.85 (1H, d,

14-CH).

1-Amino-21-guanidino-11-hydroxy-4,9,12-triaza-14-uneicosene-10,13-dione (**35**)

IR (KBr) 3400, 2940, 2850, 1660, 1530, 1460, 1360, 1225, 1115, 1080 cm^{-1}; ^1H NMR (CD_3OD) 1.2~2.5 (16H, m, CH_2), 2.9~3.4 (10H, NCH_2), 5.65 (1H, s, CH), 6.04 (1H, m, 15-CH), 6.88 (1H, m, 14-CH).

(G) Antitumor Effects on Mouse Leukemia L-1210

Two to ten male BDF_1 mice (5 weeks of age) were inoculated intraperitoneally with 10^5 L-1210 cells, and immediately thereafter each mouse was given a physiological saline solution of the sample ip once a day for 6 consecutive days to determine the life prolongation (T/C, %) on 30 days according to the equation:

$$\text{Life prolongation} = \frac{\text{Mean survival period of treated group}}{\text{Mean survival period of control group}} \times 100$$

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