SYNTHESIS AND ANTITUMOR ACTIVITY OF SPERGUALIN ANALOGUES

II. CHEMICAL MODIFICATION OF THE SPERMIDINE MOIETY

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Chemical modifications of the spermidine moiety of an antitumor antibiotic, spergualin (**Ia**), and the structure-activity relationship are described. Replacement of spermidine with other polyamines decreased the antitumor activity against mouse leukemia L1210. Analogues containing an oxidized spermidine moiety that probably formed during oxidation with amine oxidase were inactive. Spermidine is indispensable for the antitumor activity. A facile method for the synthesis of glyoxyloyl polyamine, a key intermediate of spergualin-related compounds, is also reported.

Spergualin (**Ia**) is an antitumor antibiotic discovered by UMEZAWA and his co-workers, with the structure of (−)-(15*S*)-1-amino-19-guanidino-11,15-dihydroxy-4,9,12-triazanonadecane-10,13-dione.[1] We have reported a general method for the synthesis of spergualin analogues (**I**) by the acid-catalyzed condensation of ω-guanidino alkanamides (**III**) with glyoxyloylspermidine (**IIa**).[2] Structure-activity studies of analogues modified at the (3*S*)-7-guanidino-3-hydroxy fatty acid moiety showed that the carbon-chain length of the moiety was important for the activity.

KUNIMOTO *et al.*[3] have conjectured that spergualin is oxidized by amine oxidase and that the product of the enzymatic oxidation may inhibit the growth of leukemia cells. A search for such a product(s) might elucidate the mode of action of spergualin, and a study of the spermidine moiety should give information about the active form of spergualin.

In the second part of our analogue studies, we modified the spermidine moiety. Here, we report the synthesis and antitumor activity of polyamine analogues of spergualin.

Chemistry

The analogues in this report were synthesized with one of two approaches; acid-catalyzed condensation of ω-guanidino alkanamides (the amide component **III**) with glyoxyloyl polyamines (the aldehyde component **II**), or amide bond formation as usual in peptide synthesis. The first approach gives analogues containing α-hydroxyglycine (Type I), and the second gives analogues containing a common amino acid (Type II). The reactivity of the functional groups in the components was taken into account to select the approach. Type I analogues are, in general, more unstable but more potent

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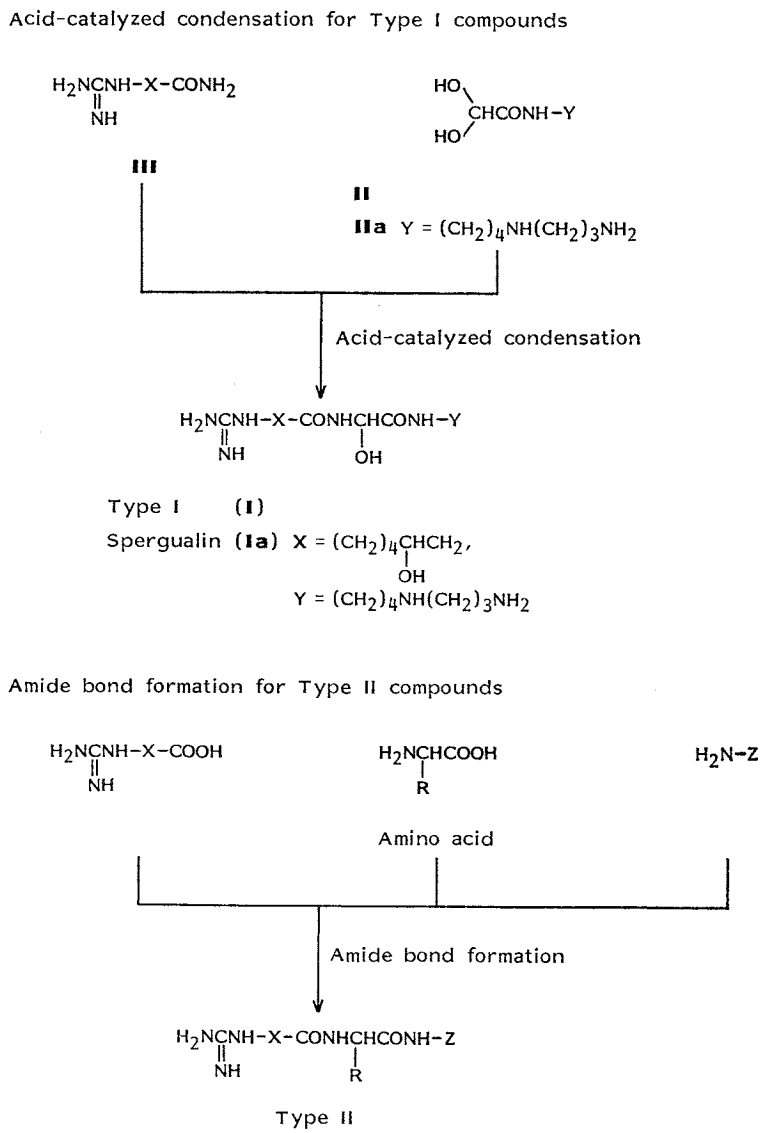
Fig. 1. General approach for the synthesis of spergualin analogues.



than Type II analogues.

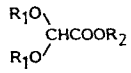
Preparation of Type I Analogues

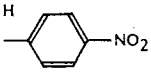
In the synthesis of Type I analogues, displacement of glyoxyloylspermidine (**IIa**) with other glyoxyloyl polyamines (**II**) affords a number of analogues. Condensation achieved in the presence of a carboxylic acid such as glutaric acid or citric acid and water at 60°C for 6~20 hours gives spergualin analogues in 30~40% yield; it is not necessary to protect functional groups such as amino, alkene, ester, guanidino, or hydroxyl groups.[2]

Two methods were used for the synthesis of glyoxyloyl polyamine. The first method (Method A) was reported by UMEZAWA *et al.*[4] in the synthesis of spergualin (**Ia**). Glyoxylic acid hydrate (**IV**) was used as the precursor of the glyoxyloyl moiety, and its aldehyde group was protected as diethyl

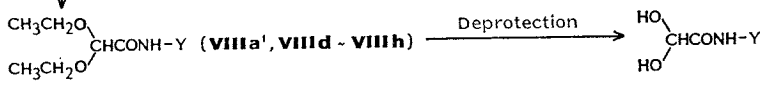
Scheme 1. Synthesis of glyoxyloyl polyamines.

Method A



IV $R_1 = R_2 = H$
V $R_1 = R_2 = CH_3CH_2$
VI $R_1 = CH_3CH_2$ $R_2 = H$
VII $R_1 = CH_3CH_2$ $R_2 =$ 

Coupling with polyamines

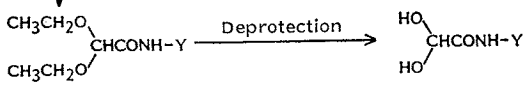


VIIIa' Y = $(CH_2)_4NH(CH_2)_2CN$
VIIIb' Y = $(CH_2)_3NH(CH_2)_3CN$
VIIIc' Y = $(CH_2)_5NH(CH_2)_2CN$
VIIId Y = $(CH_2)_4NH_2$
VIIIe Y = $(CH_2)_8NH_2$
VIIIf Y = $(CH_2)_3NH(CH_2)_3NH_2$
VIIIg Y = $(CH_2)_4NH(CH_2)_4NH_2$
VIIIh Y = $(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$

IIa', IId ~ IIh

VIIIa' ~ VIIIc'

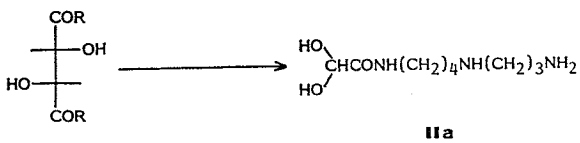
Reduction



VIIIa Y = $(CH_2)_4NH(CH_2)_3NH_2$
VIIIb Y = $(CH_2)_3NH(CH_2)_4NH_2$
VIIIc Y = $(CH_2)_5NH(CH_2)_3NH_2$

IIa ~ IIc

Method B



IIa

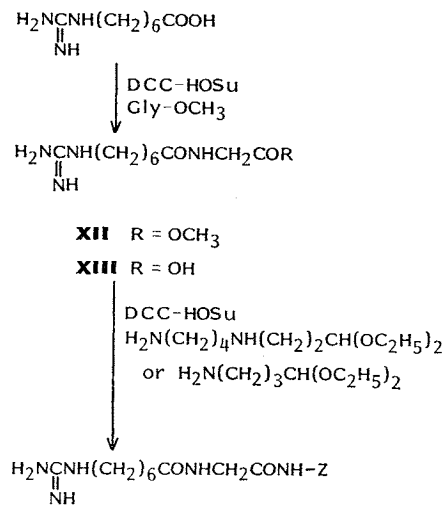
IX R = OH
X R = OC_2H_5
XIa' R = $NH(CH_2)_4NH(CH_2)_2CN$
XIa R = $NH(CH_2)_4NH(CH_2)_3NH_2$

acetal. The diethyl acetal **VI** was converted to its active ester (**VII**) with *p*-nitrophenol or *N*-hydroxysuccinimide and *N,N'*-dicyclohexylcarbodiimide (DCC), and coupled with polyamines to give *N*-acylated polyamines (**VIIId**~**VIIIh**). The group protecting the aldehyde of **VIIId**~**VIIIh** was removed by treatment in 0.1 N HCl at 100°C for 1 hour. For the synthesis of more complicated glyoxyloyl polyamines (**IIa**~**IIc**), *N'*-cyanoalkyl diaminoalkanes were used. *N*-Acylation of *N'*-cyanoalkyl diaminoalkanes with active ester **VII** gave **VIIIa'**~**VIIIc'**. The cyano group was reduced to an aminomethyl group by either catalytic hydrogenation with Raney nickel or $NaBH_4$ in the presence of $CoCl_2$.[5] Removal of the group protecting the aldehyde by acid hydrolysis gave the desired glyoxyloyl polyamines (**IIa**~**IIc**). This method is useful for the preparation of glyoxyloyl polyamines in laboratory scale, but it is less useful for large-scale preparation, because it involves aldehyde protection and deprotection procedures.

For the large-scale preparation of glyoxyloyl polyamines, we devised a facile method using tartaric acid (**IX**) as precursor (Method B). In Method B, oxidative cleavage of the adjacent diol in the final step affords the aldehyde group, and this eliminates the need for protection and deprotection of the aldehyde. For example, glyoxyloylspermidine (**IIa**) was synthesized as follows. First, diethyl tartrate (**X**) was aminolyzed with *N*-2-cyanoethylputrescine to give *N,N'-bis*[4-(2-cyanoethyl)aminobutyl]tartramide (**XIa'**). Catalytic hydrogenation of **XIa'** with Raney nickel in ammonia-saturated methanol or reduction with $NaBH_4$ in the presence of $CoCl_2$ gave *N,N'-bis*[4-(3-aminopropyl)aminobutyl]tartramide (**XIa**). Oxidative cleavage of **XIa** with sodium metaperiodate gave the desired glyoxyloylspermidine (**IIa**). This method does not involve any steps for aldehyde protection and deprotection, so it might be useful in the large-scale production of **IIa**.

Direct modifications of the amino group of spergaulin were carried out as follows. Acetylation of the primary amino group of spergualin was afforded with acetic anhydride in a mixed solvent of acetic acid and H_2O to give compound **13**. Reductive alkylation of amino group was achieved with sodium cyanoborohydride and the corresponding aldehyde. With acetaldehyde, only the terminal amino group was ethylated to give compound **14**.

Scheme 2. Synthesis of Type II compounds.

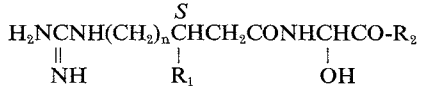


XIVi Z = $(CH_2)_4NH(CH_2)_2CH(OC_2H_5)_2$
XIVj Z = $(CH_2)_3CH(OC_2H_5)_2$
16 Z = $(CH_2)_4NH(CH_2)_2CHO$
17 Z = $(CH_2)_4NH(CH_2)_2CH_2OH$
18 Z = $(CH_2)_4NH(CH_2)_2COOH$
19 Z = $(CH_2)_3CHO$
20 Z = $(CH_2)_3CH_2OH$
21 Z = $(CH_2)_3COOH$

Preparation of Type II Analogues

In Type II analogues, the glyoxylic acid moiety is replaced with glycine moiety. Such replacement has little effect on antitumor activity.[6] The amide bonds were formed by methods used for peptide bond formation. Analogues containing aldehyde, alcohol or carboxylic acid groups in place of the terminal amino group (**16**~**21**) were synthesized as in Scheme 2. The aldehyde

Table 1. Antitumor activity of spergualin analogues (Type I) modified at the spermidine moiety.



Compound				Prolongation of life (T/C, %)								
				Dose (mg/kg/day)								
No.	n	R_1	R_2	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50
(−)-Ia	4	OH	$NH(CH_2)_4NH(CH_2)_3NH_2$		104	135	>207	>340	>401	>388	>372	>372
1	4	OH	$NH(CH_2)_4NH_2$					96	96	96	96	82
2	4	OH	$NH(CH_2)_8NH_2$				88	94	100	94	100	94
3	3	H	$NH(CH_2)_3NH(CH_2)_3NH_2$	97	97	97	97	104	97	97	104	97
4	4	H	$NH(CH_2)_3NH(CH_2)_3NH_2$	100	100	110	119	119	143	143	250	0
5	4	OH	$NH(CH_2)_3NH(CH_2)_3NH_2$				110	96	151	82	96	96
6	5	H	$NH(CH_2)_3NH(CH_2)_3NH_2$	97	97	97	97	174	118	104	97	0
7	6	H	$NH(CH_2)_3NH(CH_2)_3NH_2$	107	164	250	>321	>429	>429	>429	0	0
8	4	OH	$NH(CH_2)_3NH(CH_2)_4NH_2$				100	114	100	114	129	214
9	4	OH	$NH(CH_2)_4NH(CH_2)_4NH_2$				103	89	103	103	151	185
10	3	H	$NH(CH_2)_5NH(CH_2)_3NH_2$	100	100	100	100	100	107	100	107	100
11	4	H	$NH(CH_2)_5NH(CH_2)_3NH_2$	100	107	100	100	100	107	121	143	0
12	4	OH	$NH(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$				88	100	100	100	100	0
13	4	OH	$NH(CH_2)_4NH(CH_2)_3NHCOCH_3$				95	95	101	95	101	128
14	4	OH	$NH(CH_2)_4NH(CH_2)_3NHC_2H_5$				104	97	97	97	97	104
15	4	H	$NH(CH_2)_4NH(CH_2)_2CN$	100	100	100	100	100	100	100	100	100

Table 2. Antitumor activity of spergualin analogues (Type II).

$H_2NCNH(CH_2)_6CONHCH_2CO\text{-}R_3$
‖
NH

Compound		Prolongation of life (T/C, %)							
		Dose (mg/kg/day)							
No.	R_3	0.39	0.78	1.56	3.13	6.25	12.5	25	50
16	$NH(CH_2)_4NH(CH_2)_2CHO$	96	103	105	96	99	112	24	
17	$NH(CH_2)_4NH(CH_2)_2CH_2OH$		96	100	96	100	100	96	100
18	$NH(CH_2)_4NH(CH_2)_2COOH$		96	103	100	100	103	96	100
19	$NH(CH_2)_3CHO$		103	103	96	96	96	103	96
20	$NH(CH_2)_3CH_2OH$		96	100	96	100	107	103	100
21	$NH(CH_2)_3COOH$		96	103	96	107	110	103	96

function was protected as diethyl acetal during the synthesis. Compounds **16** and **19** containing an aldehyde group were examined for antitumor activity, and were also used as the precursors for alcohols or carboxylic acids. Reduction of **16** and **19** with $NaBH_4$ gave the corresponding alcohols **17** and **20**. Oxidation of **16** and **19** with Jones reagent (CrO_3 - H_2SO_4) gave the corresponding carboxylic acids **18** and **21**.

Structure and Antitumor Activity Relationships

The structure and antitumor activity of spergualin analogues of Type I and Type II are shown in Tables 1 and 2, respectively.

The analogues in which the spermidine moiety was replaced by other polyamines had almost no antitumor activity, except for compound **7**. Our conclusions are as follows. First, the carbon-chain length of the polyamines is important for the activity. When the carbon chain and acylation position of acyl-$NH(CH_2)_mNH(CH_2)_nNH_2$ are expressed as (-m-n), polyamines with carbon chains of (-3-3), (-3-4), (-4-4) and (-5-3) were almost inactive, as in compounds **4**, **5**, **8**, **9** and **11**. In compound **7** (-3-3), the shortness of the carbon chain of the polyamine seems to have been compensated by the length of the 9-guanidinononanoyl moiety and the activity remained.

In addition to the carbon-chain length, coexistence of the primary and secondary amino groups in the molecule is also important; putrescine, 1,8-diaminooctane and spermine instead of spermidine as in compounds **1**, **2** and **12** were inactive.

Acylation or alkylation of the terminal amino group completely removed the antitumor activity (compounds **13** and **14**). The *N*-substituted compounds may not be substrates of amine oxidase, and our results seems to be consistent with the suggestion that amine oxidase in tumor cells activates spergualin.[3] However, analogues containing aldehyde, alcohol, or carboxylic acid functions (**16**~**21**) were also inactive.

From the results mentioned above and those reported earlier,[2] we concluded that functional groups such as guanidino or primary and secondary amino, and also two amide bonds, must be suitably arranged in the structure in order to fit the yet unknown target molecule.

Experimental

^1H NMR spectra were measured with a Hitachi R-24B (60 MHz) spectrometer or a Jeol FX-200 (200 MHz) spectrometer. Melting points were determined with a Yanagimoto melting point apparatus, and are reported uncorrected. None of the analogues of spergualin had a definite melting point.

The amide component (3*S*)-7-guanidino-3-hydroxyheptanamide was obtained by the acid hydrolysis of spergualin,[1] and the other ω-guanidino alkanamides were synthesized as described previously.[2]

(11*S*)-1-Amino-15-guanidino-7,11-dihydroxy-5,8-diazapentadecane-6,9-dione (**1**)

To a solution of 20 g (0.217 mol) of glyoxylic acid hydrate in EtOH (80 ml) was added 20 ml of 35% HCl, and the solution was refluxed for 4 hours. The solution was evaporated, and to the residue were added EtOH (80 ml) and HCl (20 ml). The solution was refluxed again for 4 hours. The solution was evaporated and the oily residue purified by distillation (bp 75~80°C/13 mmHg) to give 24.31 g (63.6%) of ethyl-(1,1-diethoxy)acetate (**V**): ^{1}H NMR ($CDCl_3$) δ 1.24 (6H, t, OCH_2CH_3), 1.30 (3H, t, $COOCH_2CH_3$), 3.16 (4H, q, OCH_2CH_3), 4.22 (2H, q, $COOCH_2CH_3$), 4.84 (1H, s, CH).

To a solution of 12.36 g (70.2 mmol) of **V** in EtOH (50 ml) was added 1 N NaOH (76 ml), and the solution was stirred and cooled in an ice bath for 1 hour. After being adjusted to pH 2 with 6 N HCl, the solution was extracted with EtOAc (120 ml). The EtOAc extract was dried over anhydrous Na_2SO_4 and evaporated to give 7.60 g (73.1%) of glyoxylic acid diethyl acetal (**VI**): ^{1}H NMR ($CDCl_3$) δ 1.25 (6H, t, OCH_2CH_3), 3.68 (4H, q, OCH_2CH_3), 4.91 (1H, s, CH), 9.0 (1H, s, COOH).

To a solution of 1.48 g (10 mmol) of glyoxylic acid diethyl acetal (**VI**) and 920 mg (10 mmol) of *p*-nitrophenol in EtOAc (30 ml) was added a solution of 2.48 g (12 mmol) of DCC in EtOAc (20 ml) and the mixture was stirred at room temp for 1 hour. The precipitate was filtered off and the filtrate was added dropwise to a solution of 1.06 g (12 mmol) of 1,4-diaminobutane in EtOAc (20 ml). The mixture was stirred at room temp for 2 hours and the yellow precipitate was filtered off. The filtrate was mixed with water (100 ml), adjusted to pH 2.5 with 1 N HCl and washed with EtOAc (100 ml). The aqueous layer was adjusted to pH 6.5 with 2 N NaOH, diluted with water, and chromatographed on a CM-Sephadex C-25 (Na-cycle) column (300 ml) with gradient elution with 2 liters each of H_2O and 0.5 M NaCl. The desired fractions (with a positive ninhydrin reaction) were collected and evaporated to dryness. The residue was extracted with MeOH and the MeOH extract desalted by chromatography on a Sephadex LH-20 column (200 ml) with MeOH as eluent. The fractions containing the purified material were combined and evaporated to dryness to give 1.4 g (55%) of 7-amino-1,1-diethoxy-3-azaheptan-2-one (**VIId**) (HCl salt) as an oil: ^{1}H NMR ($CDCl_3$) δ 1.22 (6H, t, CH_2CH_3), 1.4 (2H, s, NH_2), 1.5 (4H, m, CH_2), 2.72 (2H, t, CH_2NH_2), 3.27 (2H, q, $CONHCH_2$), 3.63 (4H, q, OCH_2CH_3), 4.75 (1H, s, CH), 6.97 (1H, br t, NH).

One gof **VIId** (HCl salt) was dissolved in 0.1 N HCl (28 ml), and refluxed for 1.5 hours. The reaction mixture was adjusted to pH 6.5 with 2 N NaOH and chromatographed on a CM-Sephadex C-25 (Na-cycle) column by gradient elution with 1 liter each of H_2O and 1 N NaCl. The fractions containing the desired product were combined and desalted by a procedure similar to that described above to give 540 mg (69.2%) of 7-amino-1,1-dihydroxy-3-azaheptan-2-one (**IId**) (HCl salt): ^{1}H NMR (CD_3OD) δ 1.4~1.9 (4H, m, CH_2), 2.8~3.6 (4H, m, NCH_2), 4.80 (1H, s, CH).

Acid-catalyzed condensation of glyoxyloyl polyamines (**IId~IIh**) with ω-guanidino alkanamide and purification of the product were carried out by the following procedure. A mixture of 601 mg (2.52 mmol) of (3*S*)-7-guanidino-3-hydroxyheptanamide (HCl), 500 mg (2.52 mmol) of **IId**, 333 mg (2.52 mmol) of glutaric acid, and 410 mg of H_2O was kept at 60°C for 8 hours. The reaction mixture was diluted with H_2O and chromatographed on a CM-Sephadex C-25 (Na-cycle) column by gradient elution with 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 extract chromatographed on a Sephadex LH-20 column with MeOH as the eluent. The fractions containing the purified product were combined and evaporated to dryness to give 370 mg (35.0%) of compound **1** (2HCl salt): ^{1}H NMR (CD_3OD) δ 1.4~2.1 (10H, m, CH_2), 2.40 (2H, d, CH_2), 2.8~3.3 (6H, m, NCH_2), 4.0 (1H, m, CH), 5.55 (1H, s, CH).

(15*S*)-1-Amino-19-guanidino-11,15-dihydroxy-9,12-diazanonadecane-10,13-dione (**2**)

Starting with 2.96 g (20 mmol) of **VI** and 5.77 g (40 mmol) of 1,8-diaminooctane, a procedure similar to that used for compound **1** afforded 3.22 g (52%) of 11-amino-1,1-diethoxy-3-azaoctan-2-one (**VIIIe**) (HCl salt) as crystals: ^{1}H NMR (CD_3OD) δ 1.23 (6H, t, OCH_2CH_3), 1.2~1.7 (12H, m, CH_2), 2.92 (2H, t, CH_2NH_2), 3.22 (2H, t, $CONHCH_2$), 3.62 (4H, q, OCH_2CH_3), 4.80 (1H, s, CH).

Acetal **VIIIe** (3.02 g, 9.72 mmol) was acid-hydrolyzed by heating under reflux for 1.5 hours in 0.1 N HCl (45 ml), and purified by chromatography on a CM-Sephadex C-25 (Na-cycle) column (500 ml) and then on a Sephadex LH-20 column (500 ml) to give 1.92 g (81.2%) of 11-amino-1,1-dihydroxy-3-azaoctan-2-one (**IIe**) (HCl salt): ^{1}H NMR (CD_3OD) δ 1.2~1.8 (12H, m, CH_2), 2.8~3.3 (4H, m, NCH_2), 5.10 (1H, s, CH).

Acid-catalyzed condensation of 205 mg (0.805 mmol) of **IIe** with 192 mg (0.805 mmol) of (3*S*)-7-guanidino-3-hydroxyheptanamide in the presence of 106 mg (0.805 mmol) of glutaric acid and 163 mg of H_2O followed by purification gave 115 mg (30%) of **2**: ^{1}H NMR (CD_3OD) δ 1.4~2.2 (18H, m, CH_2), 2.34 (2H, d, CH_2), 2.9~3.5 (6H, m, NCH_2), 4.00 (1H, m, CH), 5.50 (1H, s, CH).

1-Amino-17-guanidino-10-hydroxy-4,8,11-triazaheptadecane-9,12-dione (3)

With 2.96 g (20 mmol) of **VI**, and 5.24 g (40 mmol) of *N*-(3-aminopropyl)-1,3-diaminopropane, a procedure similar to that applied for compound **1** afforded 2.73 g (41%) of 10-amino-1,1-diethoxy-3,7-diazadecan-2-one (**VIIIf**) as oil: ^{1}H NMR ($CDCl_3$) δ 1.23 (6H, t, CH_2CH_3), 1.87 (4H, quintet, CH_2), 2.5~3.2 (6H, m, NCH_2), 3.2~3.45 (2H, m, CH_2NH_2), 3.63 (4H, q, OCH_2), 4.79 (1H, s, CH), 5.17 (3H, br s, NH, NH_2).

Acid hydrolysis of the acetal (1 g) gave 397 mg (48%) of 10-amino-1,1-dihydroxy-3,7-diazadecan-2-one (**IIf**): ^{1}H NMR (CD_3OD) δ 1.6~2.5 (4H, m, CH_2), 2.9~3.3 (8H, m, NCH_2), 4.90 (1H, s, CH).

Acid-catalyzed condensation of 120 mg (0.432 mmol) of **IIf** with 90 mg (0.432 mmol) of 6-guanidinohexanamide (HCl) gave 60.6 mg (29%) of **3**: ^{1}H NMR (CD_3OD) δ 1.2~2.5 (12H, m, CH_2), 2.9~3.4 (10H, m, NCH_2), 5.49 (1H, s, CH).

1-Amino-18-guanidino-10-hydroxy-4,8,11-triazaoctadecane-9,12-dione (4)

Acid-catalyzed condensation of 134 mg (0.48 mmol) of **IIf** with 107 mg (6.48 mmol) of 7-guanidinoheptanamide (HCl) gave 85.2 mg (37%) of **4**: ^{1}H NMR (CD_3OD) δ 1.2~2.5 (14H, m, CH_2), 2.9~3.3 (10H, m, NCH_2), 5.50 (1H, s, CH).

(14*S*)-1-Amino-18-guanidino-10,14-dihydroxy-4,8,11-triazaoctadecane-9,12-dione (5)

Acid-catalyzed condensation of 147 mg (0.528 mmol) of **IIf** with 126 mg (0.528 mmol) of (3*S*)-7-guanidino-3-hydroxyheptanamide (HCl) gave 81.3 mg (31%) of **5**: ^{1}H NMR (CD_3OD) δ 1.3~2.3 (10H, m, CH_2), 2.42 (2H, d, CH_2), 2.9~3.6 (10H, m, NCH_2), 4.01 (1H, m, CH_2), 5.50 (1H, d, CH).

1-Amino-19-guanidino-10-hydroxy-4,8,11-triazanonadecane-9,12-dione (6)

Acid-catalyzed condensation of 102 mg (0.37 mmol) of **IIf** with 72 mg (0.30 mmol) of 8-guanidinooctaneamide (HCl) gave 46.6 mg (31%) of **6**: ^{1}H NMR (CD_3OD) δ 1.1~2.5 (16H, m, CH_2), 2.9~3.3 (10H, m, NCH_2), 5.50 (1H, s, CH).

1-Amino-20-guanidino-10-hydroxy-4,8,11-triazaeicosane-9,12-dione (7)

Acid-catalyzed condensation of 134 mg (0.481 mmol) of **IIf** with 121 mg (0.481 mmol) of 9-guanidinononanamide (HCl) gave 76 mg (31%) of **7**: ^{1}H NMR (CD_3OD) δ 1.2~2.5 (18H, m, CH_2), 2.9~3.3 (10H, m, NCH_2), 5.50 (1H, s, CH).

(15*S*)-1-Amino-19-guanidino-11,15-dihydroxy-5,9,12-triazanonadecane-10,13-dione (8)

To 14.8 g (0.1 mol) of 4-bromobutyronitrile cooled in a water bath was added dropwise 37.07 g (0.5 mol) of 1,3-diaminopropane, and the mixture was stirred at room temp for 1 hour. The mixture was dissolved in 2 N NaOH and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with a K_2CO_3 solution, dried over anhydrous Na_2SO_4 and evaporated. The residue was purified with distillation (bp 103~106°C/3 mmHg) to give 5.92 g of *N*-(3-aminopropyl)aminobutyronitrile.

To a solution of 4.37 g (29.5 mmol) of **VI** and 3.73 g (32.5 mmol) of *N*-hydroxysuccinimide in dioxane (20 ml) was added a solution of 6.71 g (32.5 mmol) of DCC in dioxane (40 ml), and the mixture was stirred at room temp for 4 hours. The precipitate was filtered off and the filtrate added dropwise to a solution of 5.0 g (35.4 mmol) of *N*-(3-aminopropyl)aminobutyronitrile in dioxane (20 ml). The mixture was stirred overnight, then diluted with 10 ml of water and evaporated. The residue was dissolved in water (100 ml) and adjusted to pH 7.0. The aqueous layer was washed with EtOAc and chromatographed on Diaion SP-207 with elution by aq MeOH to give 4.62 g (58%)

of 10-cyano-1,1-diethoxy-3,7-diazadecan-2-one (**VIIIb'**): ^{1}H NMR ($CDCl_3$) δ 1.26 (6H, t, CH_2CH_3), 1.9~2.4 (4H, m, CH_2), 2.71 (2H, t, CH_2CN), 5.05 (1H, s, CH).

To a solution of 4.47 g (16.47 mmol) of **VIIIb'** and 7.84 g (32.9 mmol) of $CoCl_2 \cdot 6H_2O$ in MeOH (200 ml) was added 6.23 g (165 mmol) of $NaBH_4$ with stirring in an ice bath. The mixture was stirred at room temp for 1 hour, poured into 200 ml of H_2O, and adjusted to pH 6.5 with 2 N HCl. The black precipitate was filtered off, the filtrate was diluted with H_2O and chromatographed on CM-Sephadex C-25 and Sephadex LH-20 to give 2.10 g (37%) of 11-amino-1,1-diethoxy-3,7-diazaundecan-2-one (**VIIIb**): ^{1}H NMR (CD_3OD) δ 1.22 (6H, t, CH_2CH_3), 1.7~2.2 (6H, m, CH_2), 2.8~3.2 (6H, m, NCH_2), 3.2~3.4 (2H, m, $CONHCH_2$), 3.65 (4H, q, OCH_2), 4.82 (1H, s, CH).

Acid hydrolysis of 1.83 g (5.27 mmol) of acetal (**VIIIb**) followed by purification with CM-Sephadex C-25 and Sephadex LH-20 gave 1.16 g (75%) of 11-amino-1,1-dihydroxy-3,7-diazaundecan-2-one (**IIb**): ^{1}H NMR (CD_3OD) δ 1.6~2.4 (6H, m, CH_2), 2.9~3.3 (8H, m, NCH_2), 4.85 (1H, s, CH).

Acid-catalyzed condensation of 210 mg (0.717 mmol) of **IIb** with 171 mg (0.717 mmol) of (3*S*)-7-guanidino-3-hydroxyheptanamide gave 118 mg (32%) of **8**: ^{1}H NMR (CD_3OD) δ 1.2~2.2 (12H, m, CH_2), 2.40 (2H, d, CH_2), 2.8~3.4 (10H, m, NCH_2), 4.0 (1H, m, CH), 5.55 (1H, s, CH).

(16*S*)-1-Amino-20-guanidino-12,16-dihydroxy-5,10,13-triazaeicosane-11,14-dione (**9**)

To a solution of 1.22 g (5.57 mmol) of 7-amino-1,1-diethoxy-3-azaheptan-2-one (**VIIId**) in absolute EtOH was added 1.19 g (4.18 mmol) of *N*-(4-bromobutyl)phthalimide and the mixture was refluxed for 10 hours. The reaction mixture was evaporated to dryness, the residue dissolved in 1 N HCl and washed with EtOAc (50 ml). The aqueous layer was adjusted to pH 6 with 1 N NaOH and purified with a CM-Sephadex C-25 column (350 ml) and then with a Sephadex LH-20 column (150 ml) to give 516 mg (34%) of **VIIIg**: ^{1}H NMR (CD_3OD) δ 1.24 (6H, t, CH_2CH_3), 1.5~2.1 (8H, m, CH_2), 2.9~3.3 (8H, m, NCH_2), 3.65 (4H, q, OCH_2), 4.85 (1H, s, CH).

Acid hydrolysis of 480 mg (1.32 mmol) of acetal (**VIIIg**) gave 238 mg (59%) of 12-amino-1,1-dihydroxy-3,8-diazadodecan-2-one (**IIg**): ^{1}H NMR (CD_3OD) δ 1.4~1.8 (8H, m, CH_2), 2.7~3.2 (8H, m, NCH_2), 4.92 (1H, s, CH).

Acid-catalyzed condensation of 238 mg (0.78 mmol) of **IIg** with 186 mg (0.78 mmol) of (3*S*)-7-guanidino-3-hydroxyheptanamide gave 144 mg (40.6%) of **9**: ^{1}H NMR (CD_3OD) δ 1.5~2.0 (14H, m, CH_2), 2.46 (2H, d, CH_2), 2.95~3.50 (10H, m, NCH_2), 4.05 (1H, m, CH), 5.62 (1H, m, CH).

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

To 7.34 g (72 mmol) of cadaverine was added dropwise 3.82 g (72 mmol) of acrylonitrile. The mixture was stirred at room temp for 1 hour and then heated at 60°C for 3 hours. Distillation (bp 100°C/0.15 mmHg) yielded 4.61 g (41%) of *N*-2-cyanoethylcadaverine.

Coupling of 4.19 g (27 mmol) of *N*-2-cyanoethylcadaverine with 2.67 g (18 mmol) of **VI** followed by a purification procedure similar to that used for compound **VIIIb'** gave 3.47 g (64.4%) of 11-cyano-1,1-diethoxy-3,9-diazaundecan-2-one (**VIIIc'**): ^{1}H NMR ($CDCl_3$) δ 1.24 (6H, t, CH_2CH_3), 1.4~1.9 (6H, m, CH_2), 2.5~2.8 (2H, t, CH_2CN), 2.8~3.4 (8H, m, NCH_2), 3.72 (4H, q, OCH_2), 4.80 (1H, s, CH).

Reduction of 3.47 g (11.6 mmol) of **VIIIc'** with 2.32 g (9.76 mmol) of $CoCl_2 \cdot 6H_2O$ and 2.31 g (61 mmol) of $NaBH_4$ in MeOH (50 ml) followed by a purification procedure similar to that used for **VIIIb** gave 2.41 g (57.8%) of 12-amino-1,1-diethoxy-3,9-diazadodecan-2-one (**VIIIc**): ^{1}H NMR (CD_3OD) δ 1.21 (6H, t, CH_2CH_3), 1.2~1.9 (6H, m, CH_2), 1.9~2.4 (2H, m, CH_2), 2.8~3.4 (8H, m, NCH_2), 3.58 (4H, q, OCH_2), 4.77 (1H, s, CH).

Acid hydrolysis of 2.37 g (6.5 mmol) of acetal (**VIIIc**) gave 1.54 g (77%) of 12-amino-1,1-dihydroxy-3,9-diazadodecan-2-one (**IIc**): ^{1}H NMR (CD_3OD) δ 1.5~2.4 (8H, m, CH_2), 2.8~3.3 (8H, m, NCH_2), 4.85 (1H, s, CH).

Acid-catalyzed condensation 500 mg (1.63 mmol) of **IIc** with 284 mg (1.36 mmol) of 6-guanidinohexanamide (HCl) gave 283 mg (42%) of **10**: ^{1}H NMR (CD_3OD) δ 1.1~2.1 (16H, m, CH_2), 2.9~3.4 (10H, m, NCH_2), 5.53 (1H, s, CH).

1-Amino-20-guanidino-12-hydroxy-4,10,13-triazaeicosane-11,14-dione (**11**)

Acid-catalyzed condensation of 500 mg (1.63 mmol) of **IIc** with 303 mg (1.36 mmol) of 7-guani-

dinoheptanamide gave 289 mg (42%) of **11**: ^{1}H NMR (CD_3OD) δ 1.1~2.6 (18H, m, CH_2), 2.9~3.4 (10H, m, NCH_2), 5.53 (1H, s, CH).

(19*S*)-1-Amino-23-guanidino-15,19-dihydroxy-4,9,13,16-tetraazatrieicosane-14,17-dione (**12**)

With 1.98 g (10 mmol) of **VI** and 2.43 g (12 mmol) of spermine, a procedure similar to that for compound **1** gave 898 mg (20.3%) of 15-amino-1,1-diethoxy-3,7,12-triazapentadecan-2-one (**VIIIh**): ^{1}H NMR (CD_3OD) δ 1.23 (6H, t, CH_2CH_3), 1.7~2.3 (8H, m, CH_2), 2.9~3.3 (12H, m, NCH_2), 3.64 (4H, q, OCH_2), 4.80 (1H, s, CH).

Acid hydrolysis of 707 mg (1.60 mg) of acetal (**VIIIh**) gave 339 mg (55%) of **IIh**: ^{1}H NMR (CD_3OD) δ 1.5~1.9 (8H, m, CH_2), 2.4~3.3 (12H, m, NCH_2), 4.85 (1H, s, CH).

Acid-catalyzed condensation of 300 mg (0.778 mmol) of **IIh** with 209 mg (0.875 mmol) of (3*S*)-7-guanidino-3-hydroxyheptanamide (HCl) gave 198 mg (37%) of **12**: ^{1}H NMR (CD_3OD) δ 1.3~2.3 (14H, m, CH_2), 2.42 (2H, d, CH_2), 2.9~3.4 (14H, m, NCH_2), 4.00 (1H, m, CH), 5.52 (1H, s, CH).

(−)-(15*S*)-1-Acetoamino-19-guanidino-11,15-dihydroxy-4,9,12-triazanonadecane-10,13-dione (**13**)

To a solution of 660 mg (1.29 mmol) of spergualin in H_2O (20 ml) was added 7 ml of acetic anhydride, and the solution was stirred at room temp overnight. The reaction mixture was diluted with H_2O and purified with CM-Sephadex C-25 and Sephadex LH-20 to give 129 mg (22.4%) of **13**: ^{1}H NMR (CD_3OD) δ 1.4~1.9 (10H, m, CH_2), 1.98 (3H, s, $NCOCH_3$), 2.41 (2H, d, CH_2), 2.9~3.4 (10H, m, NCH_2), 5.49 (1H, s, CH).

(−)-(15*S*)-1-Ethylamino-19-guanidino-11,15-dihydroxy-4,9,12-triazanonadecane-10,13-dione (**14**)

To a solution of 500 mg (0.976 mmol) of spergualin and 560 mg (12.7 mmol) of acetaldehyde in H_2O (10 ml) was added 370 mg (5.9 mmol) of $NaBH_3CN$ while the solution was kept at pH 6.5 to 7.5. The reaction mixture was stirred for 1 hour at room temp and then chromatographed on CM-Sephadex C-25 and on Sephadex LH-20 to give 514 mg (97.6%) of **14**: ^{1}H NMR (CD_3OD) δ 1.35 (3H, t, CH_3), 1.4~1.9 (10H, m, CH_2), 2.45 (2H, d, CH_2), 2.9~3.4 (10H, m, NCH_2), 3.3 (2H, q, NCH_2), 4.05 (1H, m, CH), 5.51 (1H, s, CH).

1-Cyano-18-guanidino-10-hydroxy-3,8,11-triazaoctadecane-9,12-dione (**15**)

To a solution of 2.96 g (20 mmol) of **VI** and 2.78 g (20 mmol) of *p*-nitrophenol in EtOAc (60 ml) was added a solution of 4.95 g (24 mmol) of DCC in EtOAc (60 ml), and the mixture was stirred at room temp for 1 hour. The precipitate was filtered off and the filtrate was added dropwise to a solution of 5.65 g (40 mmol) of cyanoethylputrescine in EtOAc (120 ml). The mixture was stirred for 2 hours and evaporated. The residue was dissolved in $CHCl_3$ and washed with 2 N NaOH. The $CHCl_3$ layer was evaporated. The residue was dissolved in H_2O, adjusted to pH 6.5 with 2 N HCl, and washed with EtOAc. The aqueous layer was purified with CM-Sephadex C-25 and Sephadex LH-20 to give 3.65 g (59%) of **VIIIa'**: ^{1}H NMR ($CDCl_3$) δ 1.23 (6H, t, CH_2CH_3), 1.3~1.8 (4H, m, CH_2), 2.51 (2H, t, CH_2CN), 2.67 (2H, t, NCH_2), 2.91 (2H, t, NCH_2), 3.28 (2H, q, $CONHCH_2$), 3.63 (4H, q, OCH_2), 4.74 (1H, s, CH).

Acid hydrolysis of 3.32 g (10.8 mmol) of acetal (**VIIIa'**) gave 1.33 g of **IIa'**: ^{1}H NMR (CD_3OD) δ 1.4~1.9 (4H, m, CH_2), 2.9~3.2 (2H, t, CH_2CN), 3.2~3.6 (6H, m, NCH_2), 5.05 (1H, s, CH).

Acid-catalyzed condensation of 1.23 g (4.9 mmol) of **IIa'** with 913 mg (4.1 mmol) of 7-guanidinoheptanamide gave 1.14 g (51%) of **15**: ^{1}H NMR (CD_3OD) δ 1.2~2.1 (12H, m, CH_2), 2.30 (2H, t, CH_2), 2.9~3.5 (10H, m, CH_2), 5.52 (1H, s, CH).

19-Guanidino-4,9,12-triazanonadecane-1,10,13-trione (**16**)

To a solution of 10 g (44.7 mmol) of 7-guanidinoheptanoic acid (HCl salt) and 6.17 g (53.6 mmol) of *N*-hydroxysuccinimide in *N*,*N*-dimethylformamide (DMF, 40 ml) was added a solution of 11.1 g (53.6 mmol) of DCC in DMF (30 ml), and the solution was stirred for 3 hours at room temp. The precipitate was filtered off and the filtrate was added to a solution of 6.73 g (53.6 mmol) of glycine methyl ester hydrochloride and triethylamine (7.54 ml) in DMF (10 ml). Stirring was continued at room temp overnight, the precipitate was filtered off, and the filtrate evaporated under reduced pressure. Water (150 ml) was added and pH of the solution was adjusted to 6.5. The aqueous layer

was purified with chromatography on Diaion HP-20 (H_2O and then 20% aq MeOH), CM-Sephadex C-25, and Sephadex LH-20 (MeOH) to give 13.64 g (46%) of 7-guanidinoheptanoylglycine methyl ester (**XII**, HCl salt): ^{1}H NMR (CD_3OD) δ 1.36 (4H, m, CH_2), 1.6 (4H, m, CH_2), 2.32 (2H, t, CH_2CO), 3.16 (2H, t, NCH_2), 3.75 (3H, s, OCH_3), 3.98 (2H, s, $NHCH_2CO$).

To an ice-cooled solution of 13.6 g (46 mmol) of **XII** in MeOH (60 ml) was added 54 ml of 1 N NaOH and the mixture was stirred for 1 hour. After adjustment of the pH to 7.0 with 1 N HCl the mixture was evaporated under reduced pressure. The residue was dissolved in water, the pH adjusted to 1.8 and evaporated to give crude 7-guanidinoheptanoylglycine (**XIII**, HCl salt).

To a solution of 6.26 g (22.3 mmol) of **XIII** and 3.08 g (26.8 mmol) of *N*-hydroxysuccinimide in DMF (30 ml) was added a solution of 5.52 g (26.8 mmol) of DCC in DMF (20 ml). The mixture was filtered and the filtrate added to a solution of 5.85 g (26.8 mmol) of *N*-(3,3-diethoxy)propyl-1,4-diaminobutane (prepared from 3,3-diethoxypropyl chloride and 1,4-diaminobutane) in DMF (10 ml) and stirred at room temp overnight. The reaction mixture was added to water (200 ml), adjusted to pH 6, and filtered. The filtrate was chromatographed first on a CM-Sephadex C-25 column and then on a Sephadex LH-20 column to give 5.98 g (53%) of acetal **XIVi**: MP 99~100°C; ^{1}H NMR (CD_3OD) δ 1.21 (6H, t, CH_3), 1.4 (4H, m, CH_2), 1.5~1.8 (8H, m, CH_2), 2.0 (2H, m, $CH_2CH\langle^{O}_{O}$), 2.3 (2H, t, CH_2CO), 3.0~3.3 (8H, m, NCH_2), 3.5~3.8 (4H, m, OCH_2), 3.8 (2H, s, $NHCH_2CO$), 4.66 (1H, t, $CH\langle^{O}_{O}$).

Acetal **XIVi** (500 mg, 0.99 mmol) was dissolved in 0.1 N HCl (5 ml) and warmed at 50°C for 30 minutes. The mixture was freeze-dried to give 470 mg (quantitative yield) of 19-guanidino-4,9,12-triazanonadecane-1,10,13-trione (hydrate, **16**): ^{1}H NMR (Me_2SO-d_6 and D_2O) δ 1.28 (4H, m, CH_2), 1.5 (8H, m, CH_2), 1.8 (2H, m, $CH_2CH\langle^{OH}_{OH}$), 2.19 (2H, t, CH_2CONH), 2.9 (4H, m, NCH_2), 3.1 (4H, m, NCH_2), 3.63 (2H, s, $NHCH_2CO$), 4.98 (t, $CH\langle^{OH}_{OH}$) and 9.6 (s, CHO).

19-Guanidino-1-hydroxy-4,9,12-triazanonadecane-10,13-dione (**17**)

To a solution of 300 mg (0.68 mmol) of aldehyde **16** in water (7 ml) was added 128 mg (3.4 mmol) of $NaBH_4$ with stirring, which was continued for 0.5 hour at room temp. The solution was adjusted to pH 6 with 1 N HCl and chromatographed on CM-Sephadex C-25 and Sephadex LH-20 to give 172 mg (57%) of alcohol **17**: ^{1}H NMR (D_2O) δ 1.35 (4H, m, CH_2), 1.60 (8H, m, CH_2), 1.91 (2H, t, CH_2CH_2OH), 2.32 (2H, t, CH_2CO), 3.01~3.24 (8H, m, NCH_2), 3.68 (2H, t, CH_2OH), 3.82 (2H, s, $NHCH_2CO$).

19-Guanidino-10,13-dioxo-4,9,12-triazanonadecanoic acid (**18**)

A solution of 300 mg (0.60 mmol) of acetal **XIVi** in 0.1 N acetic acid (5 ml) was stirred at 50°C for 30 minutes. To the solution was added a few drops of Jones reagent, and the solution was stirred at room temp overnight. After isopropanol (2 ml) was added, the solution was neutralized with $NaHCO_3$ and filtered. The filtrate was chromatographed on a column (100 ml) of CM-Sephadex C-25 and on a column (70 ml) of Sephadex LH-20 to give 183 mg (59%) of acid **18**: ^{1}H NMR (D_2O) δ 1.35 (4H, m, CH_2), 1.6 (8H, m, CH_2), 2.35 (2H, t, CH_2CO), 2.59 (2H, t, CH_2CO), 3.04~3.30 (8H, m, CH_2NH), 3.84 (2H, s, $NHCH_2CO$).

A similar synthetic procedure afforded the homologous compounds **19**, **20** and **21**. ^{1}H NMR spectra were as follows.

15-Guanidino-5,8-diazapentadecane-1,6,9-trione (**19**)

^{1}H NMR (D_2O - CD_3COOD) δ 1.34 (4H, m, CH_2), 1.56 (6H, m, CH_2), 1.99 (2H, m, $CH_2CH\langle^{OH}_{OH}$), 2.30 (2H, t, CH_2CO), 3.15 (4H, m, CH_2NH), 3.80, 3.99, 4.15 (2H, ddd, $NHCH_2CO$), 5.58 (m, $CH\langle^{OH}_{OH}$).

15-Guanidino-1-hydroxy-5,8-diazapentadecane-6,9-dione (**20**)

^{1}H NMR (D_2O) δ 1.35 (4H, m, CH_2), 1.55 (8H, m, CH_2), 2.32 (2H, t, CH_2CO), 3.14~3.22 (4H, m, CH_2NH), 3.59 (2H, s, CH_2OH), 3.84 (2H, s, $NHCH_2CO$).

15-Guanidino-6,9-dioxo-5,8-diazapentadecanoic acid (**21**)

^{1}H NMR (D_2O) δ 1.30 (4H, m, CH_2), 1.58 (4H, m, CH_2), 1.70 (2H, t, CH_2), 2.06 (2H, t, CH_2CONH), 2.30 (2H, t, CH_2COOH), 3.14 (4H, m, CH_2NH), 3.80 (2H, s, $NHCH_2CO$).

Glyoxyloylspermidine (**IIa**)

A mixture of 23.31 g (0.165 mol) of *N*-2-cyanoethylputrescine and 15.45 g (0.075 mol) of diethyl L-tartarate (**X**) was heated at 80°C for 2 hours. After the reaction mixture was cooled, the solidified product was washed with acetone to give 29.64 g (99%) of *N,N'-bis*[4-(2-cyanoethyl)aminobutyl]tartramide (**XIa'**) as pale yellow crystals.

Compound **XIa'** (5 g) was purified with chromatography on a Diaion HP-20 column (1.5 liters) eluted with 10 liters of water, 5 liters of 5% aq MeOH, and 5 liters of 20% aq MeOH to give 3.1 g (62%) of **XIa'** as white crystals: ^{1}H NMR (Me_2SO-d_6) δ 1.3~1.7 (8H, m, CH_2), 2.4~2.9 (16H, m, NCH_2), 3.3~3.7 (4H, m, NH, OH), 4.20 (2H, m, CH), 7.60 (2H, m, CONH).

To a solution of 19.8 g (0.05 mol) of **XIa'** and 28.55 g (0.12 mol) of $CoCl_2 \cdot 6H_2O$ in MeOH was added 22.71 g (0.6 mol) of $NaBH_4$ with stirring and cooling on ice. The mixture was stirred at room temp for 1.5 hours and then poured into 220 ml of H_2O. The solution was adjusted to pH 6.5 with 6 N HCl. The black precipitate was filtered off, and the filtrate was evaporated. The residue was dissolved in water (400 ml) and purified with CM-Sephadex C-25 and Sephadex LH-20 to give 16.5 g (60%) of *N,N'-bis*[4-(3-aminopropyl)aminobutyl]tartramide (**XIa**) (4HCl salt): ^{1}H NMR (Me_2SO-d_6) δ 1.5~2.4 (12H, m, CH_2), 2.9~3.4 (16H, m, NCH_2), 4.45 (2H, m, CH).

To a solution of 24.8 g (0.045 mol) of **XIa** (4HCl salt) in H_2O (360 ml) was added dropwise a solution of 16.04 g (0.075 mol) of sodium metaperiodate in H_2O (200 ml) and the mixture was stirred at room temp for 2 hours. Ethylene glycol was added to the reaction mixture to consume excess periodate and the yellowish-green precipitate was filtered off. The filtrate was adjusted to pH 5.0 with 2 N NaOH and purified with CM-Sephadex C-25 and Sephadex LH-20 to give 8.23 g (31%) of glyoxyloylspermidine (**IIa**) (2HCl salt): ^{1}H NMR (CD_3OD) δ 1.4~2.4 (6H, m, CH_2), 2.8~3.4 (8H, m, NCH_2), 4.89 (1H, s, CH).

Large-scale Production of **IIa**

A mixture of 450 g (3.19 mol) of *N*-2-cyanoethylputrescine and 300 g (1.45 mol) of diethyl L-tartarate (**X**) was heated at 80°C for 3 hours. The reaction mixture was dissolved in 4 liters of MeOH and saturated with ammonia. To the solution was added 180 g of Raney nickel, and the mixture hydrogenated under the pressure of 12 kg/cm^2 at 40°C for 24 hours. The catalyst was filtered off, and the filtrate was evaporated. The residue was dissolved in 3 liters of H_2O and adjusted to pH 5.0 with 6 N HCl. To this solution was added a solution of 397 g (1.66 mol) of sodium metaperiodate in 5 liters of H_2O and the mixture was stirred at room temp for 3 hours. Ethylene glycol (10 ml) was added to consume excess periodate. The solution was adjusted to pH 5.0 with 6 N NaOH, and purified with CM-Sephadex C-25 and Sephadex LH-20 to give 400.4 g (44%) of glyoxyloylspermidine (**IIa**) (2HCl salt).

Antitumor Effects on Mouse Leukemia L1210

Two to ten male Slc-BDF_1 mice (5 weeks old) were inoculated intraperitoneally with 10^5 cells of L1210 (IMC), a subline of L1210, and the test compound was injected intraperitoneally once a day for 6 days starting the day of the tumor inoculation.

The antitumor activity was expressed by the T/C (%) value based on the mean survival period at 30 days after the tumor inoculation according to the equation:

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

References

1) UMEZAWA, H.; S. KONDO, H. IINUMA, S. KUNIMOTO, Y. IKEDA, H. IWASAWA, D. IKEDA & T. TAKEUCHI: Structure of an antitumor antibiotic, spergualin. J. Antibiotics 34: 1622~1624, 1981

2) UMEDA, Y.; M. MORIGUCHI, H. KURODA, T. NAKAMURA, H. IINUMA, T. TAKEUCHI & H. UMEZAWA: Synthesis and antitumor activity of spergualin analogues. I. Chemical modification of 7-guanidino-3-hydroxyacyl moiety. J. Antibiotics 38: 886~898, 1985. The common name "glyoxylylspermidine" in the previous reports should be corrected as "glyoxyloylspermidine". According to the IUPAC nomenclature, it is "11-dihydroxy-10-oxo-4,9-diazaundecane-1-amine dihydrochloride".
3) KUNIMOTO, S.; K. MIURA, H. IINUMA, T. TAKEUCHI & H. UMEZAWA: Cytotoxicity of spergualin and amine oxidase activity in medium. J. Antibiotics 38: 899~903, 1985
4) KONDO, S.; H. IWASAWA, D. IKEDA, Y. UMEDA, Y. IKEDA, H. IINUMA & H. UMEZAWA: The total synthesis of spergualin, an antitumor antibiotic. J. Antibiotics 34: 1625~1627, 1981
5) SATOH, T.; S. SUZUKI, Y. SUZUKI, Y. MIYAJI & Z. IMAI: Reduction of organic compounds with sodium borohydride-transition metal salt systems. (1). Reduction of organic nitrile, nitro and amide compounds to primary amines. Tetrahedron Lett. 1969: 4555~4558, 1969
6) UMEZAWA, H.; T. TAKEUCHI, R. NISHIZAWA, K. TAKAHASHI, T. NAKAMURA & Y. UMEDA (Zaidan Hojin Biseibutsu Kagaku Kenkyukai): Spergualin-related compounds and process for the preparation thereof. Jpn. Kokai 42356 ('84), Mar. 8, 1984