SYNTHESIS AND ANTITUMOR ACTIVITY OF SPERGUALIN ANALOGUES

III. NOVEL METHOD FOR SYNTHESIS OF OPTICALLY ACTIVE 15-DEOXYSPERGUALIN AND 15-DEOXY-11-*O*-METHYLSPERGUALIN

YOSHIHISA UMEDA, MAKOTO MORIGUCHI, KATSUSHIGE IKAI, HIROYUKI KURODA, TERUYA NAKAMURA, AKIO FUJII,[a] TOMIO TAKEUCHI[b] and HAMAO UMEZAWA[†]

Central Research Laboratory, Takara Shuzo Co., Ltd.,
3-4-1 Seta, Otsu-shi, Shiga-ken 520-21, Japan
[a]Research Laboratories, Pharmaceuticals Group, Nippon Kayaku Co., Ltd.,
3-31-12 Shimo, Kita-ku, Tokyo 115, Japan
[b]Institute of Microbial Chemistry,
3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

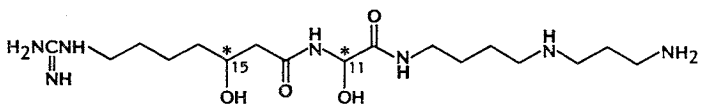
(Received for publication April 10, 1987)

Optically active 15-deoxyspergualin (**II**) and 15-deoxy-11-*O*-methylspergualin (**IIa**) were synthesized, and their antitumor activities were examined. The (−)-enantiomers of both **II** and **IIa** were active against mouse leukemia L1210, while the (+)-enantiomers were almost inactive. The optical resolution of the key intermediate, (±)-*N*-(7-guanidinoheptanoyl)-α-alkoxyglycine (**VI**) was achieved by use of an exopeptidase, serine (acid) carboxypeptidase [EC 3.4.16.1] and (±)-*N*-(7-guanidinoheptanoyl)-α-alkoxyglycyl-L-amino acid (**VIII**) as the substrate. Considering the enzymatic susceptibility of the substrate (**VIII**), we deduced that the absolute configuration of the carbon at 11 (C-11) of the bioactive (−)-enantiomer, and so that of natural spergualin (**I**), is *S*. This is, to our knowledge, the first report of the use of carboxypeptidase for the resolution of *N*-acyl amino acid.

The carbons at 11 (C-11) and 15 (C-15) of natural spergualin (**I**) are asymmetric (Fig. 1). The configuration at C-15 is *S* with respect to L-lysine,[1] and the configuration here has some slight effect on the antitumor activity of the compound. The configuration at C-11 has remained unknown, because optically active α-hydroxyglycine, the constituent containing the asymmetric C-11, can be neither isolated nor synthesized. The fact that the activity of 11-epimeric mixture of spergualin is about half that of the natural spergualin[2] indicates the importance of the configuration at C-11 for antitumor activity. This report is on the stereochemistry at C-11 of spergualin analogues, especially 15-deoxyspergualin, which is being tested in clinical trials.

Total synthesis of optically active spergualin has been achieved by the acid-catalyzed condensation of glyoxyloylspermidine (**IV**) with (*S*)-7-guanidino-3-hydroxyheptanamide (**III**) followed by the separation of the 11-epimeric mixture by reversed-phase HPLC.[1] This method is based upon the

Fig. 1. Spergualin (**I**).



† Institute of Microbial Chemistry, now deceased.

formation of diastereomers with the participation of the chiral C-15, so it is not useful for the synthesis of optically active 15-deoxyspergualin.

IWASAWA *et al.* reported a method for the preparation of (−)-15-deoxyspergualin from natural spergualin with retention of the configuration of C-11.[3] Thus, the configuration at C-11 of spergualin has been correlated with that of (−)-15-deoxyspergualin. However, this derivation neither afforded (+)-enantiomers nor was it used to synthesize other analogues with carbon skeletons different from that of spergualin.

To explore the relationship between the configuration of C-11 and bioactivity, we synthesized both (−) and (+)-enantiomers of 15-deoxyspergualin, and examined the absolute configuration at C-11 of the bioactive enantiomer.

Chemistry

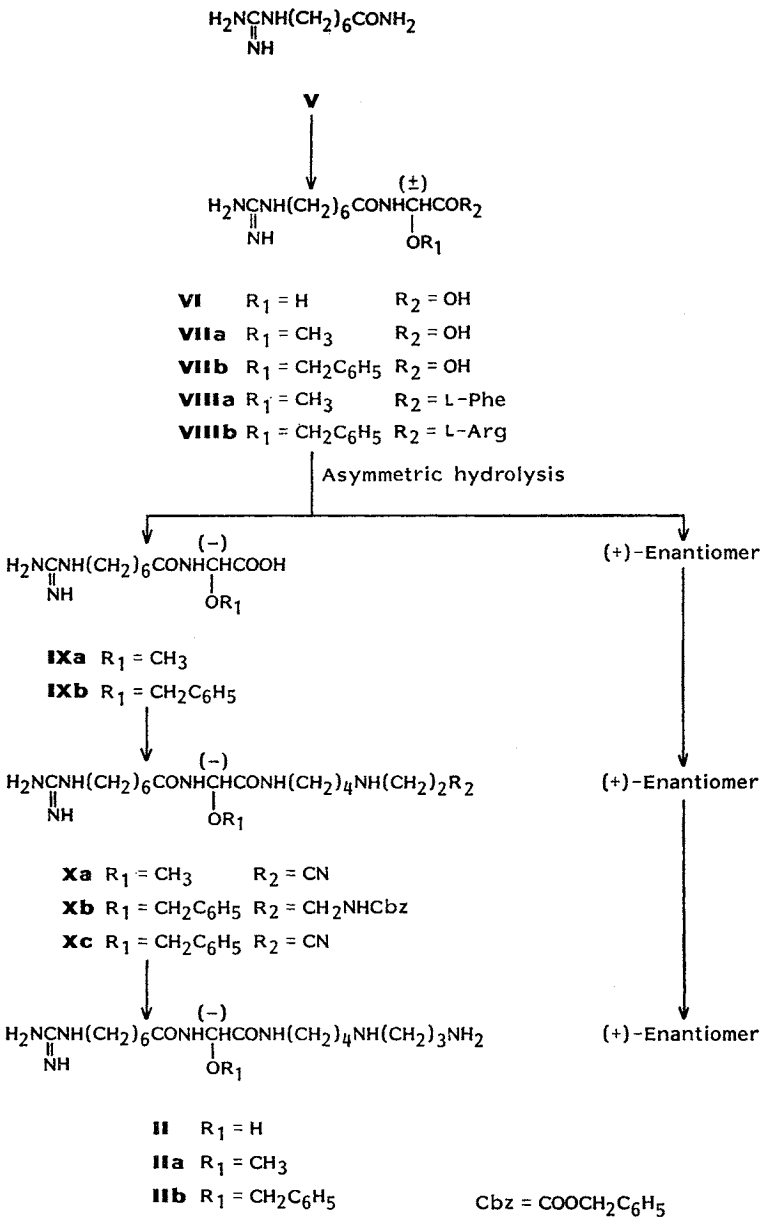
Synthesis of optically active 15-deoxyspergualin analogues are depicted in Scheme 1. The synthesis required the preparation of optically active and stable intermediates, *N*-acyl-*O*-alkoxyglycine (**IX**) in place of the unstable α-hydroxyglycine. Condensation of 7-guanidinoheptanamide (**V**) with glyoxylic acid gave a racemic adduct, *N*-(7-guanidinoheptanoyl)-α-hydroxyglycine (**VI**). The hydroxyl group of **VI** was protected by *O*-alkylation. Treatment of **VI** with alcohols in the presence of an acid catalyst gave *N*-(7-guanidinoheptanoyl)-α-alkoxyglycine (**VIIa**, R=CH_3; **VIIb**, R=$CH_2C_6H_5$), which was used as the key compound for the optical resolution. When benzyl alcohol was used for protection, the protective group can be removed by hydrogenation with retention of the configuration.

Many attempts to resolve **VII** failed, including salt formation with optically active bases or acids, diastereomeric ester formation with active alcohols, and selective crystallization. The resolution of **VII** was achieved by enzymatic hydrolysis of the *N*-acyl dipeptide (**VIIIa** and **VIIIb**), in which another L-amino acid was attached to **VII**.

Carboxypeptidase (CPase) P from *Penicillium janthinellum*[4] or CPase W from wheat bran,[5] which are classified as serine (acid) carboxypeptidases [EC 3.4.16.1], catalyze the asymmetric hydrolysis of the peptide bond of **VIII** to give the (−)-enantiomer of **IX**. Here, we designated the configuration of the enantiomers of **IX** as (−) or (+) in accordance with the optical rotation of the final compound derived from them. These enzymes eliminate the *C*-terminal amino acid while distinguishing the *S-S* configuration of the sequent amino acid from the *R-S* configuration.

The remaining *R-S* isomer of **VIII** was hydrolyzed by a metallo-CPase such as CPase A from bovine pancreas [EC 3.4.17.1] or CPase B from porcine pancreas [EC 3.4.17.2] to give the (+)-enantiomer of **IX**. We chose an appropriate CPase according to the terminal amino acid; CPase A was used for the cleavage of neutral amino acids, and CPase B for basic ones. Any L-amino acid would be suitable for the terminal amino acid, but L-Phe and L-Arg were particularly suitable, because the product **IX** was readily separated from the substrate **VIII** containing these amino acids by absorption chromatography or ion-exchange chromatography. Although the optical rotation of the compounds of the α-methoxyglycine series (**IXa-Xa-IIa**) was of the same sign, optical rotation of the α-benzyloxyglycine derivative (**IXb**) obtained by asymmetric hydrolysis with CPase W was dextro-rotatory and the sign was reversed by the introduction of a bulky group to the carboxyl function (**Xb** and **IIb**). Specific rotations of the related compounds are listed in Table 1. Thus, the enantiomer derived from CPase P or W hydrolysis gave a (−) series of spergualin analogues, and the other enantiomer gave a (+) series.

Scheme 1.



The assumption that the alkoxy group of **VIII** is sterically equivalent to the alkyl group of normal amino acids led us to deduce that the absolute configuration at C-11 of the (−) series is *S*.

Condensation of optically active 7-guanidino-α-alkoxyglycine (**IX**) with *N*-cyanoethylputrescine or partly *N*-protected spermidine gave **Xa** or **Xb**. Reduction of **Xa** with $NaBH_4$ in the presence of $CoCl_2$ gave optically active 15-deoxy-11-*O*-methylspergualin (**IIa**). Removal of the protective groups of **Xb** by catalytic hydrogenation gave optically active 15-deoxyspergualin (**II**).

The optical purity of each enantiomer of the final products, (**IIa** and **II**), was measured by reversed-phase liquid chromatography of their diastereomeric derivatives. The diastereomers were

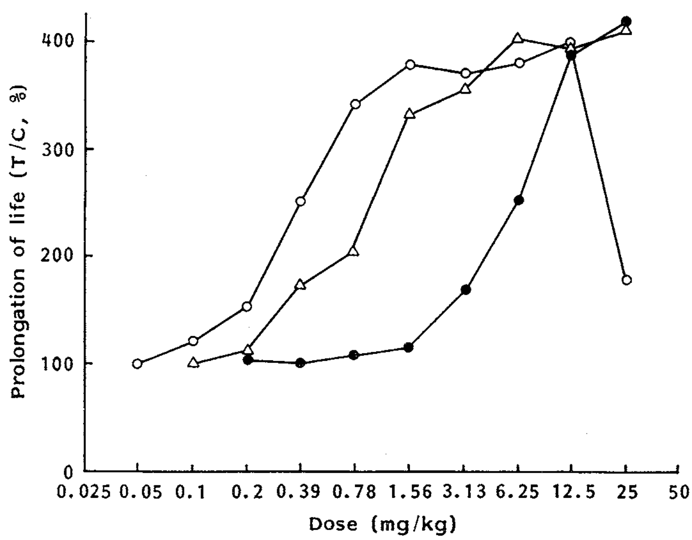
Table 1. Specific rotation of *N*-(7-guanidinoheptanoyl)-α-alkoxyglycine derivatives (IX, X and II).

$$H_2NCNH(CH_2)_6CONHCHCOR_2$$
(with =NH on the guanidino C and OR_1 on the α-carbon)

Compound	R_1	R_2	$[\alpha]_D$ (c 1, H_2O)	
			(−) Series	(+) Series
IXa	CH_3	OH	−20.2°	+20.5°
Xa	CH_3	$NH(CH_2)_4NH(CH_2)_2CN$	−33.9°	+38.5°
IIa	CH_3	$NH(CH_2)_4NH(CH_2)_3NH_2$	−29.4°	+29.5°
IXb	$CH_2C_6H_5$	OH	+15.2°*	−14.2°*
Xb	$CH_2C_6H_5$	$NH(CH_2)_4NH(CH_2)_3NHCbz$	−16.3°	+16.5°
Xc	$CH_2C_6H_5$	$NH(CH_2)_4NH(CH_2)_2CN$	−20.8°*	+20.0°*
IIb	$CH_2C_6H_5$	$NH(CH_2)_4NH(CH_2)_3NH_2$	−22.1°	+21.0°
II	H	$NH(CH_2)_4NH(CH_2)_3NH_2$	−14.3°	+14.7°

* In MeOH.
Cbz: Benzyloxycarbonyl.

Fig. 2. Antitumor activity of optically active 15-deoxy-11-*O*-methylspergualin compared with the racemic compound.
○ (−)-IIa, △ (±)-IIa, ● (+)-IIa.

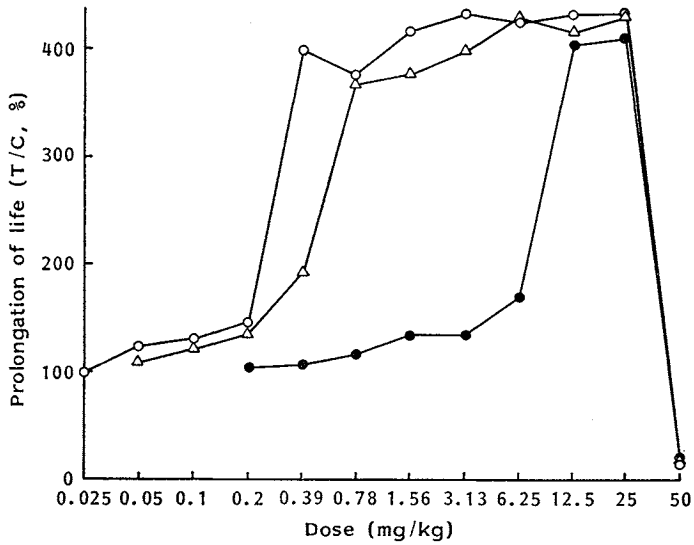


formed with the help of the chiral reagent, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (GITC), developed by NIMURA *et al.* for the resolution of amino acid enantiomers.[5] Analysis showed that the optical purity of each enantiomer of (**II** and **IIa**) was high (≥98%).

Bioactivity

The antitumor activity of optically active 15-deoxyspergualin (**II**) and 15-deoxy-11-*O*-methylspergualin (**IIa**) was tested and compared with the corresponding racemic compounds (Figs. 2 and 3). Six female Slc-CDF$_1$ mice (6 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 mean survival time at 30 days after the tumor inoculation.

Fig. 3. Antitumor activity of optically active 15-deoxyspergualin compared with the racemic compound.
○ (−)-II, △ (±)-II, ● (+)-II.



The activity of the racemic compound was about 50% that of the (−)-enantiomer, but the activity of the (+)-enantiomer was about 3% to 6% of the (−)-enantiomer (Figs. 2 and 3). The activity of the (+)-enantiomer observed may arise from (−)-enantiomer contaminating the sample. The results showed that the antitumor activity of spergualin and its analogues depended upon the (−)-enantiomer, and that the (+)-enantiomer is virtually inactive. In contrast, all compounds were acutely toxic at 50 mg/kg/day irrespective of their optical activity.

Experimental

^{1}H NMR spectra were recorded on a Jeol FX-200 (200 MHz) spectrometer. Melting points were measured with a Yanagimoto melting point apparatus, and are uncorrected. Optical rotations were measured with a Jasco DIP-181 digital polarimeter. HPLC was carried out with a system consisting of an Altex 100A pump, a Shimadzu CTO-2A column oven, a Soma S-310A UV detector, and a Shimadzu C-R1B Chromatopac recorder.

CPase P was provided by Takara Shuzo Co., Ltd., CPase A, CPase B and CPase W were purchased from Sigma Chemical Co., Boehringer Mannheim Yamanouchi Co., and Seikagaku Kogyo Co., Ltd., respectively.

(I) Synthesis of Optically Active 15-Deoxy-11-*O*-methylspergualin (IIa)

(±)-*N*-(7-Guanidinoheptanoyl)-α-hydroxyglycine (VI)

To a solution of 22.3 g (100 mmol) of 7-guanidinoheptanamide (HCl salt) in 150 ml of DMF was added 10.2 g (110 mmol) of glyoxylic acid, and the mixture was heated at 70°C for 4 hours. After being cooled, the reaction mixture was evaporated and the residue was dissolved in 100 ml of H_2O and chromatographed on a CM-Sephadex C-25 (Na^+ form, 1,100-ml) column with elution by H_2O. Fractions containing the desired product were combined and evaporated to give 20.9 g (80%) of (±)-*N*-(7-guanidinoheptanoyl)-α-hydroxyglycine (**VI**): MP 165~166°C (dec).

(±)-*N*-(7-Guanidinoheptanoyl)-α-methoxyglycine (VIIa)

To a cooled solution of 9.36 g (36.0 mmol) of **VI** in MeOH (300 ml) was added 5 ml of concentrated H_2SO_4, and the mixture was stirred at room temp for 3 hours. The reaction mixture was concentrated to 100 ml and poured into 200 ml of H_2O. The pH of the resulting solution was

adjusted to 10 with 2 N NaOH and stirred for 1 hour. Then, the pH was adjusted to 6.5 with 2 N HCl and evaporated. The residue was purified with a CM-Sephadex C-25 (Na^+ form) column to give 7.90 g (80%) of (±)-*N*-(7-guanidinoheptanoyl)-α-methoxyglycine (**VIIa**): MP 201~202°C (dec); ^{1}H NMR (CD_3OD) δ 1.2~1.9 (8H, m, CH_2), 2.30 (2H, t, CH_2), 3.17 (2H, m, NCH_2), 3.32 (3H, s, OCH_3), 5.23 (1H, s, CH).

The solid, 7.80 g of **VIIa**, was dissolved in 1 N HCl (28.4 ml) and evaporated to give a white powder of (±)-*N*-(7-guanidinoheptanoyl)-α-methoxyglycine HCl salt (**VIIa**).

(±)-*N*-(7-Guanidinoheptanoyl)-α-methoxyglycyl-L-phenylalanine (**VIIIa**)

To a solution of 6.58 g (2.21 mmol) of **VIIa** (HCl salt) and 4.88 g (42.4 mmol) of *N*-hydroxysuccinimide in DMF (70 ml) were added 4.87 g (21.2 mmol) of L-phenylalanine ethyl ester (HCl salt) and 2.98 ml of triethylamine. To the solution was added dropwise a solution of 8.75 g (42.4 mmol) of *N*,*N*′-dicyclohexylcarbodiimide (DCC) in DMF (30 ml) and the mixture stirred at room temp for 4 hours. The precipitate was filtered off and the filtrate was evaporated. The residue was dissolved in 300 ml of H_2O and chromatographed on a CM-Sephadex C-25 (Na^+ form, 1 liter) column by elution with H_2O and a 0.2 M solution of NaCl. Fractions containing the desired product were combined and evaporated. The residue was dissolved in H_2O and chromatographed on a Diaion HP-20 column (1 liter) by successive elution with 1.5 liters of H_2O, 2 liters of 25% aq MeOH and 4 liters of 50% aq MeOH. Fractions containing the desired product were combined and evaporated to give 5.31 g (51.6%) of (±)-*N*-(7-guanidinoheptanoyl)-α-methoxyglycyl-L-phenylalanine ethyl ester (HCl salt): ^{1}H NMR (CD_3OD) δ 1.17 (3H, t, CH_3), 1.1~1.9 (8H, m, CH_2), 2.27 (2H, t, CH_2), 2.9~3.4 (5H), 3.31 (3H, s, OCH_3), 4.11 (2H, m, CH_2), 5.30 (1H, s, CH), 7.20 (5H, s, C_6H_5).

To a cooled solution of 5.21 g (10.7 mmol) of (±)-*N*-(7-guanidinoheptanoyl)-α-methoxyglycyl-L-phenylalanine ethyl ester (HCl salt) in MeOH (35 ml) was added 12.8 ml 1 N NaOH and the mixture stirred at room temp for 1.5 hours. The reaction mixture was adjusted to pH 6.5 and evaporated to give the calculated amount of (±)-*N*-(7-guanidinoheptanoyl)-α-methoxyglycyl-L-phenylalanine (**VIIIa**).

(−)-*N*-(7-Guanidinoheptanoyl)-α-methoxyglycine ((−)-**IXa**)

The residue of **VIIIa** was dissolved in 100 ml of 0.05 M AcONa - HCl buffer (pH 5.0). To the solution was added 10 mg of CPase P and the mixture was shaken at 30°C for 8 hours. The reaction mixture was evaporated and the residue was chromatographed on a Sephadex LH-20 column (4 liters) by elution with H_2O. Fractions containing the desired product were combined and concentrated to 30 ml, adjusted to pH 2.1, and chromatographed on CM-Sephadex C-25 (Na^+ form) to give 1.16 g (32.6%) of (−)-*N*-(7-guanidinoheptanoyl)-α-methoxyglycine ((−)-**IXa**): MP 197~198°C (dec); $[\alpha]_D$ −20.2° (*c* 1, H_2O); ^{1}H NMR (CD_3OD) δ 1.2~1.9 (8H, m, CH_2), 2.30 (2H, t, CH_2), 3.17 (2H, m, NCH_2), 3.32 (3H, s, OCH_3), 5.23 (1H, s, CH).

One g (3.65 mmol) of (−)-**IXa** was dissolved in 1 N HCl (3.65 ml), and then the solution was evaporated to dryness to give 1.13 g of (−)-*N*-(7-guanidinoheptanoyl)-α-methoxyglycine HCl salt ((−)-**IXa**).

(+)-*N*-(7-Guanidinoheptanoyl)-α-methoxyglycine ((+)-**IXa**)

The solid recovered, 1.66 g (3.46 mmol) of (+)-*N*-(7-guanidinoheptanoyl)-α-methoxyglycyl-L-phenylalanine was dissolved in 0.05 M $NaHPO_4$ buffer (pH 7.5, 70 ml). To the solution was added 34 mg of CPase A and the mixture was stirred at room temp for 9 hours. The reaction mixture was evaporated and the residue was purified with CM-Sephadex C-25 (Na^+ form) to give 915 mg (79.5%) of (+)-*N*-(7-guanidinoheptanoyl)-α-methoxyglycine ((+)-**IXa**): MP 196~198°C; $[\alpha]_D$ +20.5° (*c* 1, H_2O); ^{1}H NMR (CD_3OD) δ 1.2~1.9 (8H, m, CH_2), 2.30 (2H, t, CH_2), 3.17 (2H, m, NCH_2), 3.32 (3H, s, OCH_3), 5.23 (1H, s, CH).

The solid obtained, 900 mg (3.29 mmol) of (+)-**IXa**, was dissolved in 1 N HCl (3.29 ml) and then evaporated to give 1.00 g of (+)-*N*-(7-guanidinoheptanoyl)-α-methoxyglycine HCl salt ((+)-**IXa**).

(−)-8-[*N*-(7-Guanidinoheptanoyl)-α-methoxyglycyl]-1-cyano-3,8-diazaoctane ((−)-**Xa**)

To a solution of 709 mg (2.28 mmol) of (−)-*N*-(7-guanidinoheptanoyl)-α-methoxyglycine (HCl

salt) in DMF (10 ml) were added a solution of 810 mg (4.56 mmol) of *N*-2-cyanoethyl-1,4-diaminobutane (mono HCl salt) in DMF (5 ml) and 524 mg (4.56 mmol) of *N*-hydroxysuccinimide. A solution of 940 mg of DCC in DMF (5 ml) was added to the mixture and the solution stirred at room temp overnight. The resulting precipitate was filtered off and the filtrate was diluted with water and washed with EtOAc. The aqueous layer was purified with a CM-Sephadex C-25 (Na^+ form, 400-ml) column and a Sephadex LH-20 (500-ml) column to give 599 mg (56%) of (−)-8-[*N*-(7-guanidinoheptanoyl)-α-methoxyglycyl]-1-cyano-3,8-diazaoctane ((−)-**Xa**, HCl salt): $[\alpha]_D$ −33.9° (*c* 1, H_2O); ^1H NMR (CD_3OD) δ 1.3~1.9 (12H, m, CH_2), 2.32 (2H, t, CH_2), 2.98 (2H, t, CH_2), 2.9~3.4 (8H, m, NCH_2), 3.37 (3H, s, OCH_3), 5.28 (1H, s, CH).

(−)-10-[*N*-(7-Guanidinoheptanoyl)-α-methoxyglycyl]-1,5,10-triazadecane ((−)-**IIa**)

To a solution of 584 mg (1.24 mmol) of ((−)-**Xa**) and 355 mg (1.49 mmol) of $CoCl_2$ in MeOH (13 ml) was added 284 mg (7.44 mmol) of $NaBH_4$ with stirring and cooling in an ice bath. The mixture was stirred for one more hour at room temp, and purified with a CM-Sephadex C-25 (Na^+ form) column and a Sephadex LH-20 column to give 405 mg (64%) of (−)-10-[*N*-(7-guanidinoheptanoyl)-α-methoxyglycyl]-1,5,10-triazadecane ((−)-**IIa**): $[\alpha]_D$ −29.4° (*c* 1, H_2O); IR (KBr) cm^{-1} 3420, 2950, 1650, 1520, 1460, 1360, 1190, 1160, 1090; ^1H NMR (CD_3OD) δ 1.2~2.0 (12H, m, CH_2), 2.0~2.5 (4H, m, CH_2), 2.9~3.4 (10H, m, NCH_2), 3.37 (3H, s, OCH_3), 5.26 (1H, s, CH).

A procedure similar to that for the (−)-enantiomer afforded the (+)-enantiomer with the following specific rotation.

(+)-8-[*N*-(7-Guanidinoheptanoyl)-α-methoxyglycyl]-1-cyano-3,8-diazaoctane ((+)-**Xa**)
$[\alpha]_D$ +38.5° (*c* 1, H_2O).

(+)-10-[*N*-(7-Guanidinoheptanoyl)-α-methoxyglycyl]-1,5,10-triazadecane ((+)-**IIa**)
$[\alpha]_D$ +29.5° (*c* 1, H_2O).

(II) Synthesis of Optically Active 15-Deoxyspergualin (**II**)

(±)-*N*-(7-Guanidinoheptanoyl)-α-benzyloxyglycine (**VIIb**)

A solution of 22.3 g (100 mmol) of 7-guanidinoheptanamide (**V**) and 10.2 g (110 mmol) of glyoxylic acid in DMF (150 ml) was heated for 4 hours at 65~70°C. To the cooled solution were added 110 ml of benzyl alcohol and 6 ml of concentrated HCl, and the solution was stirred for 42 hours. The reaction mixture was adjusted to pH 12.0 with 6 N NaOH, and stirred at room temp for 40 minutes. The reaction mixture was adjusted to pH 7.0 with 6 N HCl, and diluted with water to 1 liter. The resulting precipitate was filtered off, and the filtrate was purified with a Diaion SP-207 (700 ml) column with elution of the 50~60% aq MeOH. The fractions containing the desired product were combined and evaporated. The residue was crystallized from aq MeOH to give 9.45 g (27%) of (±)-*N*-(7-guanidinoheptanoyl)-α-benzyloxyglycine (**VIIb**): MP 197~199°C (dec); ^1H NMR (CD_3OD) δ 1.2~1.8 (8H, m, CH_2), 2.25 (2H, t, CH_2), 3.04 (2H, t, NCH_2), 4.58 (2H, s, CH_2Ph), 5.39 (1H, s, CH), 7.28 (5H, s, C_6H_5).

(±)-*N*-(7-Guanidinoheptanoyl)-α-benzyloxyglycyl-L-arginine (**VIIIb**)

A solid of 7.92 g (22.6 mmol) of **VIIb** was dissolved in MeOH (30 ml) and 1 N HCl - MeOH. The solution was evaporated and dried under reduced pressure to give the HCl salt of **VIIb**. To a solution of 7.9 g (20.4 mmol) of **VIIb** (HCl salt) in DMF (80 ml) were added 3.12 g (27.1 mmol) of *N*-hydroxysuccinimide and 5.60 g (27.1 mmol) of DCC and the solution was stirred overnight at room temp. To the reaction mixture were added a solution of 4.72 g (27.1 mmol) of L-arginine and 3.8 ml (27.1 mmol) of triethylamine in water (40 ml), and the solution was stirred at room temp for 7 hours. To the reaction mixture was added 100 ml of water, and the solution was adjusted to pH 12 with 6 N NaOH and stirred for 1 hour. The resulting precipitate was filtered off, and the filtrate was purified with a Diaion HP-20 column (500 ml) with elution of 30% MeOH to give 5.41 g (44%) of (±)-*N*-(7-guanidinoheptanoyl)-α-benzyloxyglycyl-L-arginine (**VIIIb**, HCl salt): ^1H NMR (CD_3OD) δ 1.4 (4H, m, CH_2), 1.5~2.0 (8H, m, CH_2), 2.3 (2H, t, CH_2CO), 3.15 (4H, m, NCH_2), 4.23 (1H, t, CH), 4.66 (2H, dd, CH_2Ph), 5.44 and 5.56 (1H, s, CH), 7.36 (5H, Ph).

(−)-*N*-(7-Guanidinoheptanoyl)-α-benzyloxyglycine ((−)-**IXb**)

The residue of 10 g (18.4 mmol) of **VIIIb** was dissolved in 0.05 M AcONa - HCl buffer (pH 4.0; 1 liter). To the solution was added 10 mg of CPase W (1,500 units; one unit is the amount of enzyme required to liberate 1 μmol of L-alanine from Z-Phe-Ala per minute at 30°C, pH 4.0), and the mixture was shaken at 30°C for 22 hours. The mixture was diluted with water (1 liter), and the solution was purified with a CM-Sephadex C-25 (Na^+ form, 400-ml) column by elution with water. The fractions containing the desired product were combined and purified with a Diaion SP-207 column with elution of 50% MeOH to give 2.04 g (32%) of (−)-*N*-(7-guanidinoheptanoyl)-α-benzyloxyglycine ((−)-**IXb**): $[\alpha]_D$ +15.2° (*c* 1, MeOH) [Note that the sign of **IXb** obtained here is minus in spite of the specific rotation observed. See details in the introduction]; ^1H NMR (CD_3OD) δ 1.3~1.4 (4H, m, CH_2), 1.4~1.7 (4H, m, CH_2), 2.22 (2H, t, CH_2CO), 3.04 (2H, t, NCH_2), 4.56 (2H, s, CH_2Ph), 5.36 (1H, s, CH), 7.2 and 7.4 (5H, Ph).

(+)-*N*-(7-Guanidinoheptanoyl)-α-benzyloxyglycine ((+)-**IXb**)

The fractions containing (+)-*N*-(7-guanidinoheptanoyl)-α-benzyloxyglycyl-L-arginine (**VIIIb**) were recovered from the CM-Sephadex column with elution of 0.3 M NaCl, and purified with a Diaion SP-207 column (400-ml). The solid obtained of 4.55 g (8.4 ml) of **VIIIb** was dissolved in 0.005 M phosphate buffer (pH 7.8, 500 ml). To the solution was added 8 mg of CPase B (1,200 units) and the solution stirred for 18 hours at room temp. The mixture was purified with a Diaion SP-207 column (400-ml) by elution with 30% MeOH to give 2.51 g (43%) of (+)-*N*-(7-guanidinoheptanoyl)-α-benzyloxyglycine ((+)-**IXb**): $[\alpha]_D$ −14.2° (*c* 1, MeOH); ^1H NMR (CD_3OD) δ 1.3~1.4 (4H, m, CH_2), 1.4~1.7 (4H, m, CH_2), 2.22 (2H, t, CH_2CO), 3.06 (2H, t, NCH_2), 4.56 (2H, s, CH_2Ph), 5.36 (1H, s, CH), 7.2 and 7.4 (5H, Ph).

(−)-9-*N*-[(7-Guanidinoheptanoyl)-α-benzyloxyglycyl]-1-benzyloxycarbonylamino-4,9-diazanonane ((−)-**Xb**)

A solid of 1.9 g (5.42 mmol) of (−)-**IXb** was dissolved in a solution of MeOH (10 ml) and 1 N HCl - MeOH (5.42 ml), and concentrated under reduced pressure to give the HCl salt of (−)-**IXb**. The residue was dissolved in DMF (30 ml), 1.82 g (6.5 mmol) of 8-amino-1-benzyloxycarbonyl-4-azaoctane (mono HCl salt), 878 mg (6.5 mmol) of 1-benzotriazole (HOBt) and 1.4 g (7 mmol) of DCC were added. The solution was stirred for 16 hours at room temp. The reaction mixture was diluted with water (400 ml) and the resulting solid was filtered off. The filtrate was purified with a CM-Sephadex C-25 (Na^+ form) column (100 ml) with gradient elution of H_2O and 1 M NaCl (each 700 ml). The active fractions were combined and evaporated. The residue was desalted with MeOH to give 3.79 g of (−)-**Xb**: ^1H NMR (CD_3OD) δ 1.3~1.46 (4H, m, CH_2), 1.5~1.75 (8H, t, CH_2), 1.86 (2H, quintet, CH_2), 2.27 (2H, t, CH_2CO), 2.95 (2H, t, NCH_2), 3.1~3.3 (8H, m, NCH_2), 4.6 (2H, q, CH_2Ph), 5.04 (2H, s, CH_2Ph), 5.44 (1H, s, CH), 7.3 (10H, Ph).

(−)-15-Deoxyspergualin ((−)-**II**)

A solution of 3.5 g (5.11 mmol) of (−)-**Xb** in 1 N acetic acid was hydrogenated in the presence of 1 g of Pd black under a hydrogen pressure of 10 kg/cm² for 15 hours at room temp. The catalyst was removed by filtration; the filtrate was purified with a Diaion SP-207 column (200-ml), a CM-Sephadex C-25 (Na^+ form, 70-ml) column and Sephadex LH-20 (60-ml) column, and then freeze-dried to give 1.61 g (60%) of (−)-15-deoxyspergualin ((−)-**II**): $[\alpha]_D$ −14.3° (*c* 1, H_2O); ^1H NMR (D_2O) δ 1.35 (4H, m, CH_2), 1.5~1.9 (8H, m, CH_2), 2.1 (2H, quintet, CH_2), 2.30 (2H, t, CH_2CO), 3.1~3.4 (10H, m, NCH_2), 5.46 (1H, s, CH).

A procedure similar to that for the (−)-enantiomer (**II**) gave the (+)-enantiomer with the following specific rotation.

(+)-15-Deoxyspergualin ((+)-**II**)

$[\alpha]_D$ +14.7° (*c* 1, H_2O).

Assay of Optical Purities

Preparation of GITC derivative: A sample (0.5 mg) was dissolved in 1 ml of 50% aq aceto-

nitrile, and to the solution was added 2.8 μl of 10% triethylamine in acetonitrile. A portion (100 μl) of the sample solution was mixed with 20 μl of GITC solution (5 mg of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate dissolved in 1 ml of acetonitrile), and left for 1 hour at room temp. The resulting solution (20 μl) was directly injected into the reversed-phase HPLC system.

Chromatographic conditions: An analytical column (150×4.6 mm i.d.) was packed with Cosmosil $5C_{18}$-P (Nakarai Chemicals, Ltd.), and thermostated at 40°C throughout the analysis. The mobile phase was 0.2 M NaH_2PO_4(pH 5) - MeOH (60:40). A flow rate of 1 ml/minute was used. Chromatography was monitored with a UV detector at 205 nm and the optical purity was estimated by comparison of peak areas.

Acknowledgment

This work was partly supported by a grant-in-aid for New Drug Development Research from the Ministry of Health and Welfare, Japan.

References

1) 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
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
3) IWASAWA, H.; S. KONDO, D. IKEDA, T. TAKEUCHI & H. UMEZAWA: Synthesis of (−)-15-deoxyspergualin and (−)-spergualin-15-phosphate. J. Antibiotics 35: 1665~1669, 1982
4) YOKOYAMA, S. & E. ICHISHIMA: A new type of acid carboxypeptidase of molds of the genus *Penicillium*. Agric. Biol. Chem. 36: 1259~1261, 1972
5) NIMURA, N.; H. OGURA & T. KINOSHITA: Reversed-phase liquid chromatographic resolution of amino acid enantiomers by derivatization with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate. J. Chromatogr. 202: 375~379, 1980