Synthesis and Biological Activities of TAN-1511 Analogues

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TAN-1511 analogues were synthesized and their effects on the proliferation of bone marrow cells were examined. To exert potent activity the following conditions are necessary: the configuration of the 2-amino-6,7-dihydroxy-4-thiaheptanoic acid moiety must be (2*R*,6*R*), long chain acyl groups (C_{14} to C_{18}) must be bound to both hydroxyl groups, the amino group must be free or acylated with the long chain fatty acid (*ca.* C_{14}) and the peptide moiety must have glutamic acid as a component. Among the synthesized compounds, trisodium (2*R*,6*R*)-2-amino-6,7-bis (hexadecanoyloxy)-4-thiaheptanoyl glycyl glutamyl glutamate, which has improved solubility, was effective in experimental leukocytopenia in mice.

During a screening program for microbial metabolites that promote the proliferation of bone marrow cells, we isolated three lipopeptides, TAN-1511 A (**1**), B (**2**) and C (**3**), from the culture broth of *Streptsporangium amethystogenes* subsp. *fukuiense* AL-23456[1].

We revealed the structure of these components, except for the configuration of the 2-amino-6,7-dihydroxy-4-thiaheptanoic acid (ADTA) moiety[1]. They were obtained from the culture broth in very low yields and are mixtures of molecules containing fatty acids of different lengths; mainly *O*,*O'*-diacylated with palmitic acid and *N*-acylated with isomyristic acid or myristic acid[1]. Therefore, it was necessary to synthesize them as a single molecule in large amounts for further investigations of their biological activities. In this paper, we describe the synthesis, structure-activity relationships and *in vivo* effects of TAN-1511 analogues.

Chemistry

Synthesis of ADTA Moiety

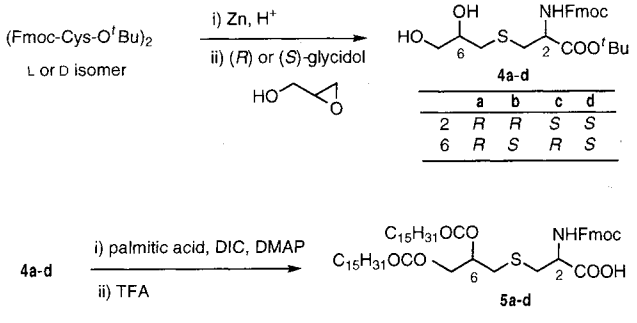
We first targeted the synthesis of TAN-1511 A analogues having palmitoyl and myristoyl residues as the ester and amide fatty acids, respectively. The synthesis of ADTA derivatives in high diastereomeric purity has been reported by METZGER *et al.*[2] and ACHIWA *et al.*[3~5]. Among them, we adopted METZGER's method using an optically active glycidol and the *N*-9-fluorenylmethoxycarbonyl-L-cysteine *tert*-butyl ester, Fmoc-L-Cys-O^{t}Bu[2], because this procedure was convenient and the reaction yields were high. After the reduction of *N*,*N'*-bis-Fmoc-L-cystine bis-*tert*-butyl ester, (Fmoc-L-Cys-O^{t}Bu)$_2$, into Fmoc-L-Cys-O^{t}Bu with zinc, the addition of (*R*)-(+)-glycidol to the mixture should yield (2*R*,6*R*)-2-(9-fluorenylmethoxycarbonyl)amino-6,7-dihydroxy-4-thiaheptanoic acid *tert*-butyl ester (**4a**) according to the reaction mechanism.

Four diastereomers of ADTA derivatives (**4a**~**4d**) were prepared from (*R*)-(+) or (*S*)-(−)-glycidol and L or D-cystine as described[2] and shown in Fig. 1. The hydroxyl groups were acylated with excess palmitic acid in the presence of *N*,*N'*-diisopropylcarbodiimide (DIC) and a catalytic amount of 4-dimethylaminopyridine (DMAP). Subsequent treatment with trifluoroacetic acid (TFA) to remove the *tert*-butyl ester afforded the desired 2-(9-fluorenylmethoxycarbonyl)amino-6,7-bis(palmitoyloxy)-4-thiaheptanoic acids (**5a**~**5d**).

Synthesis of the Peptide Moiety

The peptide moiety of **1** was synthesized starting from

Fig. 1. Synthesis of ADTA moieties.



This article is a special contribution in honour of Professor SATOSHI ŌMURA's 60th birthday.

H-Thr(^{t}Bu)-O^{t}Bu. Benzyloxycarbonyl (*Z*) -Thr(^{t}Bu)-OH, *Z*-Glu(O^{t}Bu)-OH, and *Z*-Gly-Gly-Gly-OH were coupled to H-Thr(^{t}Bu)-O^{t}Bu sequentially by the *N*,*N*'-dicyclohexylcarbodiimide(DCC)-*N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB) method to afford the protected peptide (**6**) as shown in Fig. 2. The *Z*-group in each step was removed by catalytic hydrogenation.

Synthesis of TAN-1511 A Analogues

The peptide (**6**) was coupled with each diastereomer of **5a**~**5d** by the DIC-HONB method and subsequent removal of the Fmoc-group with piperidine afforded amino analogues (**7a**~**7d**) (Fig. 3). After *N*-acylation with myristic acid in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (water soluble carbodiimide, WSC) and 1-hydroxybenzotriazole (HOBT), the *tert*-butyl groups were finally removed with TFA to obtain the desired analogues (**8a**~**8d**). *N*-Free analogues (**9a**~**9d**) were also prepared by deprotecting **7a**~**7d**.

Stereochemistry of TAN-1511

Although ADTA was scarcely detected in the 6 M hydrochloric acid hydrolysates of **1**, **2** and **3**, it was readily detected after hydrolysis with 4 M methane sulfonic acid (110°C, 12 hours). ADTA derived from TAN-1511 complex was observed on HPLC at retention time of 20.3 minutes after treatment with *o*-phthalaldehyde and *N*-acetyl-L-cysteine. When four diastereomers of ADTA obtained from **4a**~**4d** were analyzed under the same conditions, the (2*R*,6*R*), (2*R*,6*S*), (2*S*,6*R*) and (2*S*,6*S*) isomers gave peaks at 20.3, 20.2, 21.0 and 21.0 minutes, respectively, indicating that the C-2 position of the ADTA residue has the *R* cofiguration.

Whereas the ^{1}H NMR spectrum of **1** closely resembled those of the 2*R*,6*R* and 2*S*,6*S* diastereomers (**8a** and **8d**) except for the fatty acid residue in which the natural sample includes a variety of fatty acids, there were some

Fig. 2. Synthesis of the peptide moiety of TAN-1511 A.

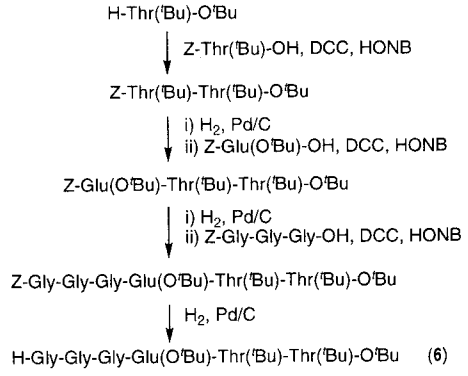


Fig. 3. Synthesis of TAN-1511 A analogues.

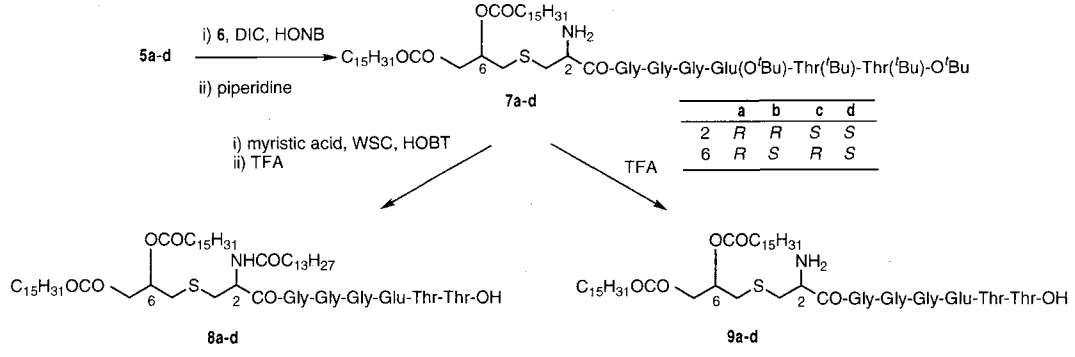
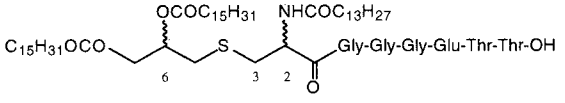


Table 1. ^{1}H NMR chemical shifts of TAN-1511 (A) (**1**) and **8a**~**8d**.



	1[b]	8a[a]	8b[a]	8c[a]	8d[a]
		2*R*,6*R*	2*R*,6*S*	2*S*,6*R*	2*S*,6*S*
H-3	2.69, dd (13.6, 8.9)	2.69, dd (13.7, 9.0)	2.74, dd (13.6, 8.6)	2.74, dd (13.6, 8.4)	2.69, dd (13.6, 8.7)
	2.94, dd (13.6, 5.1)	2.94, dd (13.7, 5.0)	2.91, dd (13.6, 5.2)	2.91, dd (13.6, 5.3)	2.94, dd (13.6, 5.1)

[a] 300 MHz, in DMSO-d_6, δ ppm, 50°C. *J* values in Hz are in parentheses.
[b] 500 MHz.

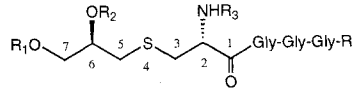
differences in those of the 2*R*,6*S* and 2*S*,6*R* diastereomers (**8b** and **8c**). The apparent inconsistency is one of the H-3 protons of the ADTA residue. As shown in Table 1, these protons of **1**, **8a** and **8d** were observed at δ 2.69 ppm. However, those of **8b** and **8c** were observed at δ 2.74 ppm. Since the 2*R* configuration of the ADTA residue of **1** was already determined, this finding revealed that the configuration of the C-6 position is also *R*.

Therefore, the structures of **1**, **2** and **3** were deduced as shown in Fig. 4.

Modification of the Peptide Moiety

At the beginning of the investigation concerning structure-activity relationships, we first synthesized derivatives with various peptide sequences. Like **6**, the *tert*-butyl group was adopted in protection of hydroxyl and carboxyl groups of peptide moieties. Compounds **11a**~**11g** were obtained by coupling **5a**, having the 2*R*,6*R* configuration, with protected peptides **10a**~**10g** and subsequent deprotection of the Fmoc-group, as shown in Fig. 5. After *N*-acylating **11a**~**11g**, all *tert*-butyl groups were finally deprotected to give *N*-myristoyl derivatives (**12a**~**12g**). *N*-Free derivatives (**13a**~**13g**) were prepared by deprotecting **11a**~**11g** with TFA.

Fig. 4. Structures of TAN-1511 A, B and C.



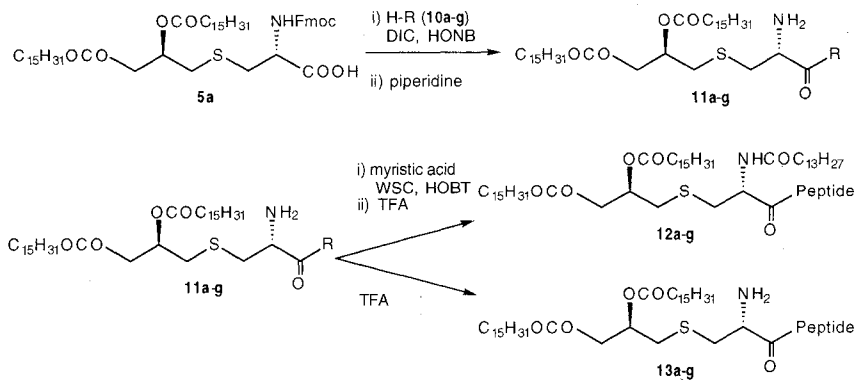
	R
TAN-1511 A (1)	L-Glu-L-Thr-L-Thr-OH
B (2)	L-Asp-L-Ala-Gly-L-Thr-OH
C (3)	L-Asp-L-Pro-L-Leu-OH

R_1, R_2 = Fatty Acid Residues (C_{16}-C_{19})
R_3 = Fatty Acid Residues (C_{13}-C_{15})

Modification of the *O*-acyl Groups

To modify the *O*-acyl groups, compound **4a** was used as the starting material (Fig. 6). By acylation of **4a** with excess stearic, myristic and hexanoic acids, compounds **14**~**16** were obtained, respectively, after deprotecting the *tert*-butyl ester with TFA. In a similar manner to synthesize **13d** (Fig. 5), **14**~**16** were coupled with H-Gly-Glu(O^{t}Bu)-Glu(O^{t}Bu)-O^{t}Bu, then the protecting groups were removed by treatment with piperidine and TFA to obtain compounds **17**~**19**. When diol **4a** was acylated with an equivalent amount of hexanoic acid, monohexanoate **20** was produced. This compound was subsequntly acylated with palmitic acid, and the *tert*-butyl ester was removed with TFA to afford **21**. Monopalmitate **22** was synthesized by a reaction with (Fmoc-L-Cys-O^{t}Bu)$_2$ and (*S*)-glycidyl palmitate. The secondary hydroxyl group was acylated with hexanoic acid and the *tert*-butyl ester was removed with TFA to afford **23**. Compounds **24** and **25** were synthesized from **21** and **23**, respectively, by the same method described above.

Fig. 5. Synthesis of TAN-1511 analogues (modification of the peptide moiety).



Compound	R
10a, 11a	Gly-Gly-Gly-Glu(O^{t}Bu)-O^{t}Bu
10b, 11b	Gly-Gly-Gly-O^{t}Bu
10c, 11c	Gly-Gly-Glu(O^{t}Bu)-O^{t}Bu
10d, 11d	Gly-Glu(O^{t}Bu)-Glu(O^{t}Bu)-O^{t}Bu
10e, 11e	Glu(O^{t}Bu)-Gly-Glu(O^{t}Bu)-O^{t}Bu
10f, 11f	Gly-Glu(O^{t}Bu)-O^{t}Bu
10g, 11g	Gly-D-Glu(O^{t}Bu)-O^{t}Bu

Fig. 6. Synthesis of TAN-1511 analogues (modification of the *O*-acyl groups).

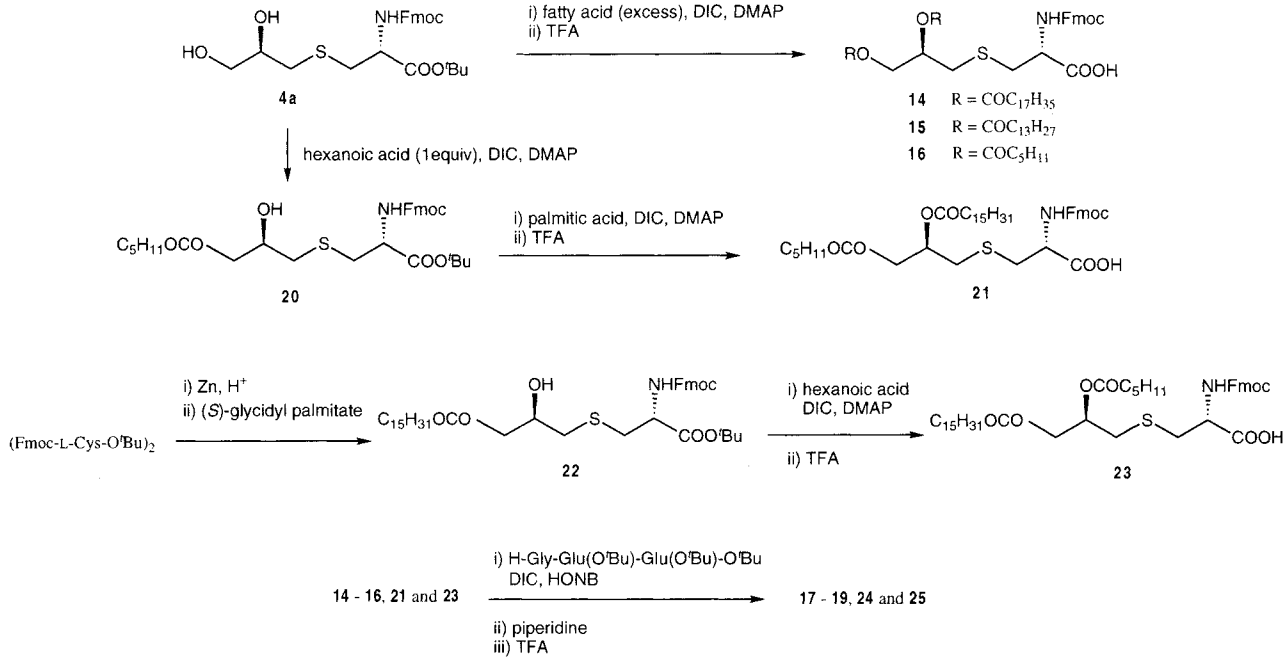
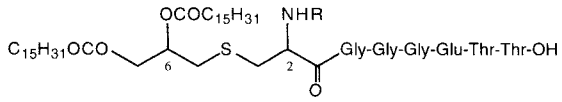


Table 2. Effect of TAN-1511 analogues on the proliferation of bone marrow cells.



Configuration	R = Myristoyl		R = H	
	Compound	MEC (ng/ml)	Compound	MEC[1] (ng/ml)
2*R*, 6*R*	**8a**	0.078	**9a**	0.078
2*R*, 6*S*	**8b**	> 10	**9b**	0.625
2*S*, 6*R*	**8c**	> 10	**9c**	1.25
2*S*, 6*S*	**8d**	> 10	**9d**	> 10

[1] MEC (minimal effective concentration) indicates the concentration received for a 30% increase in the proliferation compared with the drug free control culture.

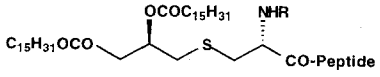
Modification of the Amino Group of the ADTA Moiety

To modify the amino group of the ADTA moiety, **11d** was used as the starting material. Compound **11d** was *N*-acylated or *N*-alkylated, following the deprotection of the *tert*-butyl groups with TFA, to obtain the desired compounds. *N*-acylation with hexanoic acid in the presence of WSC and HOBT gave an *N*-hexanoyl derivative (**26**), and treatment of **11d** with acetic anhydride yielded the *N*-acetyl derivative (**27**). Reductive alkylation produced the *N*-hexyl and *N*,*N*-dimethyl analogues (**28** and **29**). Reaction with chloroacetyl isocyanate and methyl chloroformate gave compounds **30** and **31**, respectively.

Biological Activity and Discussion

The effect of the synthesized compounds on the proliferation of bone marrow cells (BMC) was examined (Tables 2 and 3). Among the compounds (**8a**～**8d**) which have different configurations of the ADTA moiety, only the 2*R*,6*R*-analogue (**8a**) showed activity of the same potency as natural TAN-1511A (**1**). The configuration of **1** is supported by this result. The corresponding *N*-free derivative (**9a**) also had high activity. When the peptide sequence was shortened (**12a** and **12b**), the activity was rather weaker than that of **8a**. The activity, however, was increased by replacing the glycine moieties of **12b** with glutamic acids (**12c**～**12e**). The *N*-free derivatives (**13a**～**13c** and **13e**) were more potent than the

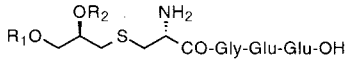
Table 3. Biological activities of TAN-1511 analogues.



Peptide	R = Myristoyl			R = H		
	Compound	BMC (ng/ml)	WBC (μg/kg)	Compound	BMC (ng/ml)	WBC (μg/kg)
Gly-Gly-Gly-Glu-OH	**12a**	0.313	130	**13a**	0.039	130
Gly-Gly-Gly-OH	**12b**	5.0	500	**13b**	0.156	130
Gly-Gly-Glu-OH	**12c**	2.5	130	**13c**	0.039	31
Gly-Glu-Glu-OH	**12d**	0.156	N.D.	**13d**	0.313	<31
Glu-Gly-Glu-OH	**12e**	0.156	130	**13e**	0.039	<7.8
Gly-Glu-OH	**12f**	0.156	130	**13f**	0.156	130
Gly-D-Glu-OH	**12g**	0.313	N.D.	**13g**	0.156	31

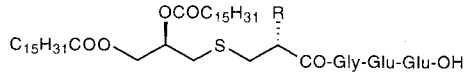
N.D. No Data

Table 4. Effect of *O*, *O'*-diacyl derivatives on the proliferation of bone marrow cells.



Compound	R_1	R_2	BMC (ng/ml)
17	$C_{17}H_{35}CO$	$C_{17}H_{35}CO$	0.313
18	$C_{13}H_{27}CO$	$C_{13}H_{27}CO$	0.156
19	$C_5H_{11}CO$	$C_5H_{11}CO$	>100
24	$C_{15}H_{31}CO$	$C_5H_{11}CO$	>100
25	$C_5H_{11}CO$	$C_{15}H_{31}CO$	>100
13d	$C_{15}H_{31}CO$	$C_{15}H_{31}CO$	0.313

Table 5. Effect of *N*-modified derivatives on the proliferation of bone marrow cells.



Compound	R	BMC (ng/ml)
13d	NH_2	0.313
12d	$C_{13}H_{27}CONH$	0.156
26	$C_5H_{11}CONH$	31.3
27	CH_3CONH	15.6
28	$C_6H_{13}NH$	31.3
29	$(CH_3)_2N$	6.25
30	$ClCH_2CONHCONH$	100
31	CH_3OCONH	6.25

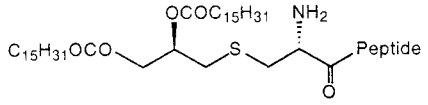
corresponding *N*-myristoyl derivatives and the introduction of glutamic acids was also effective. Introducing the D-isomer of glutamic acid to **12f** and **13f** did not change the activity compared with that of **12g** and **13g**.

The compounds were further evaluated in mice with leukocytopenia (WBC). The effects of the *N*-myristoyl derivatives were similar (Table 3). As shown in BMC assay, however, replacing *N*-myristoyl with *N*-free was also effective *in vivo* (Table 3). In addition, the activities of these compounds with glutamic acid at the first position of the peptide sequence and at the carboxyl terminal (**13d** and **13e**) were 16 to 64 fold more potent than that of **9a**. On the other hand, the MEC values of the listed compounds did not differ from **9a** by more than 4-fold *in vitro*. Although the mechanism responsible for the discrepancy in the *in vivo* and the *in vitro* activities remained to be further studied, an important role of solubility in the pharmacokinetics was suggested.

We examined the effect of the synthesized compounds with *O*-acyl and amino groups on the BMC. Among the *O*-modified derivatives (**17**~**19**, **24** and **25**), Table 4 shows that the *O*,*O'*-distearoyl and -myristoyl derivatives (**17** and **18**) had the same degree of activity as the *O*,*O'*-dipalmitoyl derivative (**13d**). When one of the *O*-acyl groups was changed to a hexanoyl group, the activity was greatly diminished. These results revealed that both of the long chain acyl groups (C_{14} to C_{18}) are necessary for the activity. Besides, as shown in Table 5, among the *N*-modified compounds, only the *N*-myristoyl derivative (**12d**) had high activity similar to that of the *N*-free derivative (**13d**).

Consequently, the following conditions are required

Table 6. Acute toxicities of sodium salts.



Compound	Peptide	LD_{50} (mg/kg) iv	sc
32	Gly-Gly-Glu 2Na	ca.50	ca.100
33	Gly-Glu-Glu 3Na	50-100	50-100
34	Glu-Gly-Glu 3Na	12.5 [a)]	3.13 [a)]
35	Gly-D-Glu 2Na	ca.100	ca.100

[a)] Minimum Lethal Dose

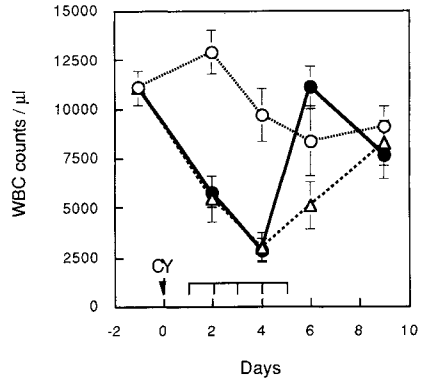
for satisfactory levels of activity: the configuration of ADTA moiety must be (2*R*,6*R*), both the hydroxyl groups of ADTA moiety should be esterified with the long chain acyl groups (C_{14} to C_{18}), the amino group of the ADTA moiety must be free or acylated with the long chain fatty acid (*ca.* C_{14}) and the peptide moiety must have glutamic acid as the component.

Though some compounds satisfied the above conditions, the solubilities of these compounds were too low to accurately determine their biological activities. To improve the low solubility, sodium salts of the four derivatives (**13c**, **13d**, **13e** and **13g**) were prepared and their properties were examined. Solutions of the derivatives in 5% acetonitrile-0.5% aqueous sodium hydrogen carbonate was desalted by column chromatography using Diaion HP-20 to afford the sodium salt (**32**~**35**). While the disodium salts (**32** and **35**) were soluble in 5% aqueous glucose in concentrations up to 10 mg/ml, the trisodium salts (**33** and **34**) were readily soluble in 5% aqueous glucose even at a concentration of 100 mg/ml. Moreover, though the particle diameter of the disodium salts (**32** and **35**) was 24.6 and 15.7 nm in distilled water at a concentration of 1 mg/ml, respectively, that of the trisodium salts (**33** and **34**) was below the limit of measurement. Thus the trisodium salts were highly soluble in water. The acute toxicities (LD_{50}) of these sodium salts are summarized in Table 6. Though **13e** (sodium free compound of **34**) was the most potent compound in the BMC and WBC assay, the toxicity of **34** was high in comparison with that of other compounds (**32**, **33** and **35**). Accordingly, taking into account the high solubility and the low toxicity, **33** was further evaluated using the mouse leukocytopenia model.

We examined the effect of **33** upon the peripheral leukocyte counts after inducing experimental leukopenia *in vivo* using cyclophosphamide (CY). The leukocyte

Fig. 7. Time course of the leukocyte counts after the administration of compound **33** to mice with CY-induced leukopenia.

Compound **33** treated (●), vehicle control (○) and CY control (△).

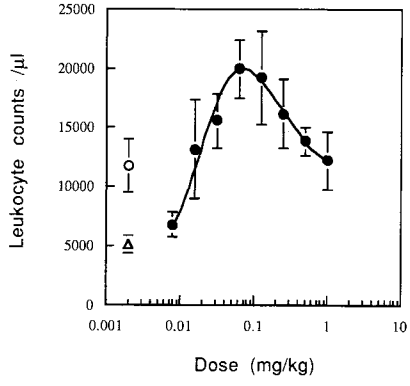


Mice (n=5) were administered orally with 150 mg/kg of CY on day 0. Compound **33** was administered subcutaneously to the mice once a day from days 1 to 5. Physiological saline and 5% glucose (0.2 ml/20 g body weight) were administered to the vehicle control group instead of CY and compound **33** solution, respectively. CY and 5% glucose were administered to the CY control group in the same manner.

Blood samples were collected from the orbital angular vein. Means and standard deviations are presented.

Fig. 8. Dose-response of the leukocyte counts after the administration of compound **33** to mice with CY-induced leukopenia.

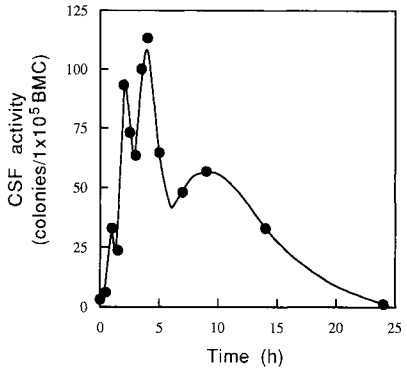
Compound **33** treated (●), vehicle control (○) and CY control (△).



CY and compound **33** were administered to mice as described in the legend to Fig. 7. The leukocytes in the peripheral blood were counted on day 6.

counts in CY-treated mice decreased to 25~30% of control level on day 4, and recovered to the control level on day 9. However, compound **33** administered at a dose of 0.031 mg/kg once a day from days 1 to 5 restored the leukocyte counts to the control level on day 6; three days before the counts spontaneously recovered in CY-treated mice (Fig. 7). The relationship between the dose of

Fig. 9. CSF activity in serum after a single administration of compound **33** in CY-treated mice.



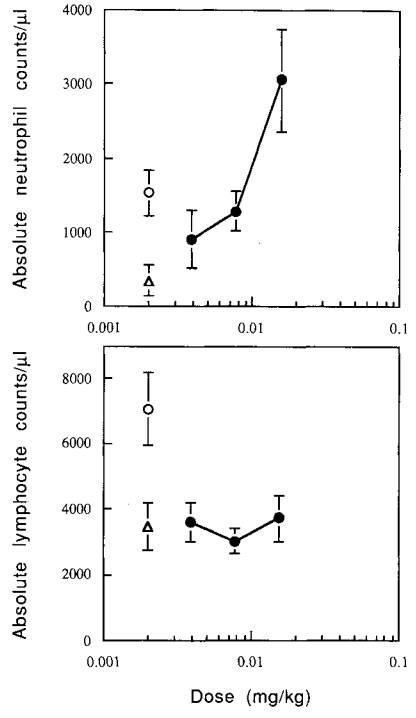
Mice (n=3) were administered orally with 150 mg/kg of CY on day 0. Compound **33** was administered subcutaneously at a dose of 0.078 mg/kg on day 1. Blood samples were obtained by cardiac puncture at the indicated time after the administration of compound **33**. Bone marrow cells (1×10^5 cells) from normal mice were cultured for 7 days in the presence of 0.1 ml of serum in 1.0 ml of agar medium in duplicate.

compound **33** and leukocyte counts was a bell curve as shown in Fig. 8. The minimum effective dose was 0.015 mg/kg/day, and the activity was maximal at a dose of 0.062 mg/kg/day. The maximal leukocyte counts increased to double the control level, whereas those in mice treated with the maximum dose (1.0 mg/kg/day) decreased to the control level. This was not derived from the different time courses of recovery of the leukocyte counts after various treatments of compound **33**, because the time courses in the lower (0.0078 ~ 0.062 mg/kg/day) and higher dose groups (0.12 ~ 1.0 mg/kg/day) were similar (data not shown). Therefore, an unknown mechanism which counteracts the overshoot of leukocyte counts might occur spontaneously *in vivo*, especially in mice treated with compound **33** at higher doses.

We examined the effect of compound **33** on colony-stimulating factor (CSF) activity in serum and granulocytopoiesis *in vivo* (Fig. 9). High levels of CSF activity were detected 2 ~ 5 hours after a single administration of compound **33** in CY-treated mice. Various CSFs might be produced because there were three peaks of CSF activity during 2 ~ 15 hours. This remains to be clarified. Compound **33** exhibited a selective restorative effect on the neutrophil count on day 6, at a dose of 0.0078 mg/kg/day. This was half of the minimum effective dose for the restoration of leukocyte counts. On the other hand, compound **33** did not restore lymphocyte counts (Fig. 10). These findings suggested that the stimulation of bone marrow accessory cells by compound **33** plays an important role in granulocytopoiesis and in the restoration of leukocyte counts in CY-induced leukopenia.

Fig. 10. Effect of compound **33** on the absolute neutrophil and lymphocyte counts.

Compound **33** treated (●), vehicle control (○) and CY control (△).



CY and compound **33** were administered to mice as described in the legend to Fig. 7. Differential leukocyte counts were obtained on day 6. Absolute neutrophil and lymphocyte counts were calculated from the differential and total leukocyte counts.

We found that compound **33** was also effective in other types of chemotherapy-induced experimental leukopenia using etoposide or a combination of CY, doxorubicin and vincristine, and in CY-induced leukopenia in colon carcinoma 26-bearing mice. The effective dose in these models was similar to that in CY-induced leukopenia described here (data not shown).

Experimental

General

IR spectra were measured with a Horiba FT-200 IR spectrophotometer using KBr pellets. Optical rotations were obtained with a JASCO DIP-181 digital polarimeter. The ^{1}H NMR spectra were recorded on a Bruker AC-300 (300 MHz) or AM-500 (500 MHz) instrument. Chemical shifts (δ) are reported in ppm downfield from tetramethylsilane (TMS).

2-(9-Fluorenylmethoxycarbonyl)amino-6,7-bis(palmitoyloxy)-4-thiaheptanoic acid *tert*-butyl esters (**5a** ~ **5d**)

(Fmoc-L-Cys-O^{t}Bu)$_2$ and (Fmoc-D-Cys-O^{t}Bu)$_2$ were

prepared from L-cystine and D-cystine, respectively, according to the literature[2].

(Fmoc-L-Cys-O^{t}Bu)$_2$: MP 151.5~152°C; $[\alpha]_D^{23}$ −6.4° (*c* 0.56, $CHCl_3$); IR (KBr) ν cm^{-1} 3360, 2980, 1720, 1705; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.48 (18H, s), 3.20 (4H, m), 4.20 (2H, br t, J=7.0 Hz), 4.36 (4H, br d, J=7.0 Hz), 4.57 (2H, m), 5.74 (2H, br d, J=7.3 Hz), 7.28 (4H, t, J=7.5 Hz), 7.38 (4H, t, J=7.5 Hz), 7.58 (4H, d, J=7.5 Hz), 7.74 (4H, d, J=7.5 Hz).

Anal Calcd for $C_{44}H_{48}N_2O_8S_2$:
C 66.31, H 6.07, N 3.51, S 8.05.
Found:
C 66.24, H 6.10, N 3.39, S 8.00.

(Fmoc-D-Cys-O^{t}Bu)$_2$: MP 149.5~150°C; $[\alpha]_D^{23}$ +5.9° (*c* 0.52, $CHCl_3$).

Anal Calcd for $C_{44}H_{48}N_2O_8S_2$:
C 66.31, H 6.07, N 3.51, S 8.05.
Found:
C 66.55, H 6.13, N 3.43, S 7.97.

Zinc powder (20.8 g, 300 mmol) and a mixture of methanol, conc. HCl and conc. H_2SO_4 (100 : 7 : 1, 240 ml) were added to an ice-cooled solution of (Fmoc-L-Cys-O^{t}Bu)$_2$ (63.4 g, 80 mmol) in CH_2Cl_2 (480 ml). After stirring for 30 minutes at 0°C, (*R*)-(+)-glycidol (52.9 ml, 800 mmol) was added to the reaction mixture and stirred for 3 hours at 40°C. The mixture was concentrated to a small volume (350 ml) and precipitates were removed by filtration. Saturated NaCl (1.0 liter) was added to the filtrate and extracted with CH_2Cl_2 (2×1.0 liter). The organic layers were combined, dried over anhydrous Na_2SO_4 and concentrated. The oily residue was chromatographed on a silica gel column, eluting with EtOAc-hexane (1 : 2 and 3 : 1) to yield **4a** (55.3 g, 73%) as a white powder: $[\alpha]_D^{21}$ −8.8° (*c* 0.65, $CHCl_3$); IR (KBr) ν cm^{-1} 3415, 2980, 2930, 1720; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.49 (9H, s), 2.30 (1H, br t, J=5.4 Hz), 2.63 (1H, dd, J=8.4, 13.8 Hz), 2.80 (1H, dd, J=3.9, 13.8 Hz), 2.93 (1H, dd, J=6.0, 14.0 Hz), 3.03 (1H, dd, J=4.6, 14.0 Hz), 3.26 (1H, d, J=3.1 Hz), 3.53 (1H, m), 3.68 (1H, m), 3.78 (1H, m), 4.23 (1H, t, J=6.9 Hz), 4.40 (2H, d, J=6.9 Hz), 4.52 (1H, m), 5.84 (1H, d, J=7.9 Hz), 7.32 (2H, t, J=7.5 Hz), 7.41 (2H, t, J=7.3 Hz), 7.61 (2H, br d, J=7.3 Hz), 7.76 (2H, d, J= 7.5 Hz).

Anal Calcd for $C_{25}H_{31}NO_6S \cdot 0.3H_2O$:
C 62.69, H 6.65, N 2.92, S 6.69.
Found:
C 62.77, H 6.45, N 2.90, S 6.81.

The same method produced **4b** from (Fmoc-L-Cys-O^{t}Bu)$_2$ and (*S*)-(−)-glycidol as a white powder: Yield 92%; $[\alpha]_D^{21}$ +6.7° (*c* 0.56, $CHCl_3$).

Anal Calcd for $C_{25}H_{31}NO_6S \cdot H_2O$:
C 61.08, H 6.77, N 2.85, S 6.52.
Found:
C 60.95, H 6.62, N 2.70, S 6.31.

The same method yielded **4c** from (Fmoc-D-Cys-O^{t}Bu)$_2$ and (*R*)-(+)-glycidol as a white powder: Yield 92%; $[\alpha]_D^{23}$ −7.6° (*c* 0.67, $CHCl_3$).

Anal Calcd for $C_{25}H_{31}NO_6S$:
C 63.40, H 6.60, N 2.96, S 6.77.
Found:
C 63.12, H 6.55, N 2.90, S 6.81.

The same method produced **4d** from (Fmoc-D-Cys-O^{t}Bu)$_2$ and (*S*)-(−)-glycidol as a white powder: Yield 84%; $[\alpha]_D^{23}$ +8.4° (*c* 0.67, $CHCl_3$).

Anal Calcd for $C_{25}H_{31}NO_6S$:
C 63.40, H 6.60, N 2.96, S 6.77.
Found:
C 63.15, H 6.47, N 2.88, S 6.67.

Palmitic acid (95.8 g, 370 mmol), DIC (58.5 ml, 370 mmol) and DMAP (5.71 g, 47 mmol) were added to a solution of **4a** (55.3 g, 120 mmol) in tetrahydrofuran (1.0 liter) and the mixture was stirred for 16 hours at room temperature. The mixture was concentrated and the residue was suspended in EtOAc (2.0 liters). This suspension was washed with 10% citric acid and water, and then concentrated. The residue was chromatographed on a silica gel column, eluting with EtOAc-hexane to afford crystals. Recrystallization from hexane gave colorless crystals (88.8 g).

A solution of the crystals (88.2 g, 92.8 mmol) in TFA (750 ml) was allowed to stand for 1 hour at room temperature. The reaction mixture was concentrated to give crude crystals. Recrystallization from EtOAc gave colorless crystals of **5a** (74.0 g, 71% from **4a**): MP 87.0~88.0°C; $[\alpha]_D^{23}$ +12.3° (*c* 0.58, $CHCl_3$); IR (KBr) ν cm^{-1} 3415, 2920, 2850, 1730; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (6H, t, J=6.7 Hz), 1.26 (48H, br s), 1.60 (4H, m), 2.30 (4H, m), 2.77 (2H, m), 3.12 (2H, m), 4.15 (1H, dd, J=6.0, 12.0 Hz), 4.24 (1H, t, J=7.1 Hz), 4.35 (1H, dd, J=3.1, 12.0 Hz), 4.40 (2H, d, J=7.1 Hz), 4.65 (1H, m), 5.17 (1H, m), 5.80 (1H, br d, J=7.2 Hz), 7.31 (2H, dt, J=1.1, 7.4 Hz), 7.40 (2H, t, J=7.4 Hz), 7.61 (2H, br d, J=7.4 Hz), 7.76 (2H, d, J=7.4 Hz).

Anal Calcd for $C_{53}H_{83}NO_8S$:
C 71.18, H 9.35, N 1.57, S 3.59.
Found:
C 71.05, H 9.43, N 1.51, S 3.42.

Following the same method, **5b**~**5d** were obtained from **4b**~**4d** each as colorless crystals.

5b: Yield 59% from **4b**; mp 82.5~83.5°C; $[\alpha]_D^{23}$ +14.9° (*c* 0.55, $CHCl_3$).

Anal Calcd for $C_{53}H_{83}NO_8S$:
C 71.18, H 9.35, N 1.57, S 3.59.
Found:
C 70.96, H 9.36, N 1.57, S 3.58.

5c: Yield 77% from **4c**; mp 82.5~83.0°C; $[\alpha]_D^{23}$ −16.0° (*c* 0.51, $CHCl_3$).

Anal Calcd for $C_{53}H_{83}NO_8S$:
C 71.18, H 9.35, N 1.57, S 3.59.
Found:
C 71.20, H 9.38, N 1.45, S 3.53.

5d: Yield 69% from **4d**; mp 88.5~89.0°C; $[\alpha]_D^{23}$ −13.1° (*c* 0.56, $CHCl_3$).

Anal Calcd for $C_{53}H_{83}NO_8S$:
C 71.18, H 9.35, N 1.57, S 3.59.
Found:
C 71.20, H 9.23, N 1.46, S 3.56.

Determination of the Configuration at the C-2 Position of the ADTA Moiety

TAN-1511 complex (0.73 mg) was hydrolyzed with 4 M CH_3OSO_3H (0.5 ml) for 12 hours at 110°C. The reaction mixture was neutralized with 1 M NaOH (2.0 ml). An aliquot of the solution (5 μl) was derivatized with *o*-phthalaldehyde in the presence of *N*-acetyl-L-cysteine according to the literature[6], then analyzed by HPLC (column, YMC-ODS-5; solvent, 20% MeOH - 50 mM AcONa; flow rate, 1.0 ml/minute; detection, fluorescence; excitation at 360 nm, emission at 440 nm). Piperidine (0.1 ml) was added to a solution of **4a**~**4d** (10 mg, 0.02 mmol). The reaction mixture was stirred for 1 hour at room temperature, and concentrated. The residue was dissolved in TFA (0.5 ml) and left for 30 minutes at room temperature. The reaction mixture was concentrated and suspended in EtOAc (3.0 ml). The suspension was extracted with water and the aqueous layer was concentrated. The residue was analyzed after derivatization as described above.

Configuration	(2*R*,6*R*)	(2*R*,6*S*)	(2*S*,6*R*)	(2*S*,6*S*)
Retetion time	20.3	20.2	21.0	21.0 minutes

Protected Peptide **6**

Sulfuric acid (1 M, 27.5 ml) was added to an ice-cooled suspension of *Z*-Thr(^{t}Bu)-OH dicyclohexylamine salt (12.0 g, 25 mmol) in a mixture of EtOAc (200 ml) and water (200 ml) with stirring. The organic layer was concentrated after drying over anhydrous Na_2SO_4 and the residue was dissolved in CH_3CN. HONB (4.93 g, 27.5 mmol) and DCC (5.67 g, 27.5 mmol) were added to the ice-cooled solution and the mixture was stirred for 2 hours at 0°C, then the precipitates were removed by filtration.

A solution of *Z*-Thr(^{t}Bu)-O^{t}Bu (11.0 g, 30 mmol) in MeOH (300 ml) was hydrogenated over 10% Pd-C as a catalyst for 2 hours at room temperature to give H-Thr(^{t}Bu)-O^{t}Bu as a colorless oil. The filtrate described above and diisopropylethylamine (5.48 ml, 31.5 mmol) were added to an ice-cooled solution of H-Thr(^{t}Bu)-O^{t}Bu in CH_3CN (200 ml) and the mixture was stirred overnight at room temperature. The mixture was concentrated, suspended in EtOAc, then successively washed with 10% citric acid, saturated $NaHCO_3$ and water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was chromatographed on a silica gel column, eluting with EtOAc - hexane (1 : 3), to afford *Z*-Thr(^{t}Bu)-Thr(^{t}Bu)-O^{t}Bu (12.7 g, 97%) as colorless crystals: MP 92.5~93.5°C; $[\alpha]_D^{25}$ +25.4° (*c* 1.0, DMF).

Anal Calcd for $C_{28}H_{46}N_2O_7$: C 64.34, H 8.87, N 5.36.
Found: C 64.26, H 9.01, N 5.33.

HONB (3.60 g, 20 mmol) and DCC (4.15 g, 20 mmol) were added to an ice-cooled solution of *Z*-Glu(O^{t}Bu)-OH (6.17 g, 18 mmol) in CH_3CN and the mixture was stirred for 2 hours at 0°C, then the precipitates were removed by filtration.

A solution of *Z*-Thr(^{t}Bu)-Thr(^{t}Bu)-O^{t}Bu (11.5 g, 22 mmol) in MeOH (300 ml) was hydrogenated over 10% Pd-C as a catalyst for 2 hours at room temperature to give H-Thr(^{t}Bu)-Thr(^{t}Bu)-O^{t}Bu as a colorless oil. The filtrate described above and diisopropylethylamine (3.83 ml, 22 mmol) were added to an ice-cooled solution of H-Thr(^{t}Bu)-Thr(^{t}Bu)-O^{t}Bu in CH_3CN (200 ml) and the mixture was stirred overnight at room temperature. The mixture was concentrated, suspended in EtOAc, then successively washed with 10% citric acid, saturated $NaHCO_3$ and water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was chromatographed on a silica gel column, eluting with EtOAc - hexane (1 : 3), to afford *Z*-Glu(O^{t}Bu)-Thr(^{t}Bu)-Thr(^{t}Bu)-O^{t}Bu (10.4 g, 98%) as colorless crystals: MP 118~119.5°C; $[\alpha]_D^{25}$ +10.4° (*c* 1.0, DMF).

Anal Calcd for $C_{37}H_{61}N_3O_{10}$: C 62.78, H 8.69, N 5.94.
Found: C 62.88, H 8.89, N 5.68.

HONB (1.86 g, 10 mmol) and DCC (2.14 g, 10 mmol) were added to an ice-cooled solution of *Z*-Gly-Gly-Gly-OH (3.04 g, 9.4 mmol) in DMF and the mixture was stirred for 2 hours at 0°C, then the precipitates were removed by filtration.

A solution of *Z*-Glu(O^{t}Bu)-Thr(^{t}Bu)-Thr(^{t}Bu)-O^{t}Bu (6.66 g, 9.4 mmol) in MeOH (300 ml) was hydrogenated over 10% Pd-C as a catalyst for 2 hours at room temperature to yield H-Glu(O^{t}Bu)-Thr(^{t}Bu)-Thr(^{t}Bu)-O^{t}Bu as a colorless oil. The filtrate described above and diisobutylethylamine (1.80 ml, 10 mmol) were added to an ice-cooled solution of H-Glu(O^{t}Bu)-Thr(^{t}Bu)-Thr(^{t}Bu)-O^{t}Bu in DMF (150 ml) and the mixture was stirred overnight at room temperature. The mixture was concentrated, suspended in $CHCl_3$, then successively washed with 10% citric acid, saturated $NaHCO_3$ and water. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. The residue was chromatographed on a silica gel column, eluting with $CHCl_3$ - MeOH (95 : 5), to afford *Z*-Gly-Gly-Gly-Glu(O^{t}Bu)-Thr(^{t}Bu)-Thr(^{t}Bu)-O^{t}Bu (5.74 g, 75%) as colorless crystals: MP 167.5~168°C; $[\alpha]_D^{25}$ +7.3° (*c* 1.0, DMF).

Anal Calcd for $C_{43}H_{70}N_6O_{13}$: C 58.75, H 8.03, N 9.56.
Found: C 58.52, H 7.78, N 9.35.

A solution of *Z*-Gly-Gly-Gly-Glu(O^{t}Bu)-Thr(^{t}Bu)-Thr(^{t}Bu)-O^{t}Bu (1.97 g, 2.24 mmol) in MeOH (60 ml) was hydrogenated over 10% Pd-C as a catalyst for 2 hours at room temperature to give H-Gly-Gly-Gly-Glu(O^{t}Bu)-Thr(^{t}Bu)-Thr(^{t}Bu)-O^{t}Bu (**6**) as a white powder which was used in the following reactions without further purification.

Synthesis of the Diastereomers **8a**~**8d**

To a solution of **5a** (500 mg, 0.56 mmol) in DMF (5.0 ml) were added protected peptide **6** (458 mg, 0.62 mmol), HONB (110 mg, 0.61 mmol) and DIC (96 μl, 0.61 mmol) and the reaction mixture was stirred for 15 hours. The mixture was concentrated and the residue was dissolved in $CHCl_3$. The solution was successively washed with 10% citric acid, saturated $NaHCO_3$ and water, then dried over anhydrous Na_2SO_4. After concentration, the residue was suspended in CH_3CN and

the precipitates were collected as a white powder (873 mg).

Piperidine (0.70 ml) was added to a solution of the powder (770 mg, 0.47 mmol) in DMF (7.0 ml) and stirred for 1 hour at room temperature. The reaction mixture was concentrated and chromatographed on a silica gel column, eluting with $CHCl_3$ - MeOH (50 : 1 and 20 : 1), to yield **7a** (623 mg, 90% from **5a**) as a white powder: $[\alpha]_D^{20}$ +4.9° (*c* 0.55, $CHCl_3$).

Anal Calcd for $C_{73}H_{135}N_7O_{16}S \cdot 0.5H_2O$:
C 62.27, H 9.74, N 6.96, S 2.28.
Found:
C 62.31, H 9.73, N 6.99, S 2.19.

By the same method, **7b**~**7d** were obtained from **5b**~**5d** as white powders.

7b: Yield 90% from **5b**; $[\alpha]_D^{18}$ +7.1° (*c* 0.49, $CHCl_3$).

Anal Calcd for $C_{73}H_{135}N_7O_{16}S$:
C 62.68, H 9.73, N 7.01, S 2.29.
Found:
C 62.55, H 9.85, N 6.95, S 2.21.

7c: Yield 85% from **5c**; $[\alpha]_D^{20}$ +21.4° (*c* 0.64, $CHCl_3$).

Anal Calcd for $C_{73}H_{135}N_7O_{16}S$:
C 62.68, H 9.73, N 7.01, S 2.29.
Found:
C 62.75, H 9.41, N 7.05, S 2.40.

7d: Yield 89% from **5d**; $[\alpha]_D^{20}$ +24.4° (*c* 0.62, $CHCl_3$).

Anal Calcd for $C_{73}H_{135}N_7O_{16}S$:
C 62.68, H 9.73, N 7.01, S 2.29.
Found:
C 62.53, H 9.48, N 6.93, S 2.31.

Myristic acid (36 mg, 0.16 mmol), HOBT (21 mg, 0.16 mmol) and DIC (24 μl, 0.15 mmol) were added to a solution of **7a** (180 mg, 0.12 mmol) in DMF (2.0 ml), and the reaction mixture was stirred for 40 hours at room temperature. The mixture was concentrated and the residue was dissolved in $CHCl_3$. The solution was successively washed with 10% citric acid, saturated $NaHCO_3$ and water, then dried over anhydrous Na_2SO_4. The organic layer was concentrated and chromatographed on a silica gel column, eluting with $CHCl_3$ - MeOH (50 : 1), to give a white powder (193 mg).

A solution of the powder (138 mg, 0.09 mmol) in TFA (1.4 ml) was placed at room temperature for 1.5 hours. The solution was concentrated, and the residue was suspended in CH_3CN and the resulting precipitates were collected to afford **8a** (114 mg, 96% from **7a**) as a white powder: $[\alpha]_D^{21}$ −13.7° (*c* 0.52, 5% TFA - $CHCl_3$).

Anal Calcd for $C_{71}H_{129}N_7O_{17}S \cdot 1.5H_2O$:
C 60.40, H 9.42, N 6.94, S 2.27.
Found:
C 60.20, H 9.38, N 6.89, S 2.24.

By the same method, **8b**~**8d** were obtained from **7b**~**7d** as white powders.

8b: Yield 99%; $[\alpha]_D^{21}$ −10.2° (*c* 0.55, 5% TFA - $CHCl_3$).

Anal Calcd for $C_{71}H_{129}N_7O_{17}S \cdot 1.5H_2O$:
C 60.40, H 9.42, N 6.94, S 2.27.
Found:
C 60.24, H 9.17, N 6.90, S 2.23.

8c: Yield 97%; $[\alpha]_D^{21}$ +3.0° (*c* 0.56, 5% TFA - $CHCl_3$).

Anal Calcd for $C_{71}H_{129}N_7O_{17}S \cdot H_2O$:
C 60.79, H 9.41, N 6.99, S 2.29.
Found:
C 60.72, H 9.32, N 6.91, S 2.32.

8d: Yield 95%; $[\alpha]_D^{21}$ +7.7° (*c* 0.52, 5% TFA - $CHCl_3$).

Anal Calcd for $C_{71}H_{129}N_7O_{17}S \cdot H_2O$:
C 60.79, H 9.41, N 6.99, S 2.29.
Found:
C 60.80, H 9.40, N 6.87, S 2.18.

Synthesis of the *N*-Free Derivatives **9a**~**9d**

A solution of **7a** (150 mg, 0.11 mmol) in TFA (1.5 ml) was allowed to stand for 1.5 hours at room temperature, then concentrated. The residue was suspended in CH_3CN and the resulting precipitates were collected to afford **9a** (125 mg, 99%) as a white powder: $[\alpha]_D^{21}$ −2.3° (*c* 0.58, 5% TFA - $CHCl_3$).

Anal Calcd for $C_{57}H_{103}N_7O_{16}S \cdot 1.5H_2O$:
C 56.98, H 8.89, N 8.16, S 2.67.
Found:
C 56.72, H 8.62, N 8.11, S 2.63.

By the same method, **9b**~**9d** were obtained from **7b**~**7d** as white powders.

9b: Yield 98%; $[\alpha]_D^{21}$ −0.7° (*c* 0.55, 5% TFA - $CHCl_3$).

Anal Calcd for $C_{57}H_{103}N_7O_{16}S \cdot 1.5H_2O$:
C 56.98, H 8.89, N 8.16, S 2.67.
Found:
C 57.04, H 8.80, N 8.11, S 2.72.

9c: Yield 97%; $[\alpha]_D^{21}$ −21.7° (*c* 0.63, 5% TFA - $CHCl_3$).

Anal Calcd for $C_{57}H_{103}N_7O_{16}S \cdot H_2O$:
C 57.41, H 8.88, N 8.22, S 2.69.
Found:
C 57.38, H 8.66, N 8.27, S 2.59.

9d: Yield 98%; $[\alpha]_D^{21}$ −15.6° (*c* 0.50, 5% TFA - $CHCl_3$).

Anal Calcd for $C_{53}H_{103}N_7O_{16}S \cdot 1.5H_2O$:
C 56.98, H 8.89, N 8.16, S 2.67.
Found:
C 56.74, H 8.57, N 8.03, S 2.72.

Synthesis of Compounds **11a**~**11g**

Compounds **11a**~**11g** were obtained as white powders from **5a** and the corresponding protected peptides (**10a**~**10g**) as described above.

11a: Yield 80% from **5a**; $[\alpha]_D^{23}$ −6.7° (*c* 0.63, $CHCl_3$).

Anal Calcd for $C_{57}H_{105}N_5O_{12}S \cdot H_2O$:
C 62.09, H 9.78, N 6.35, S 2.91.
Found:
C 62.12, H 9.59, N 6.36, S 2.87.

11b: Yield 82% from **5a**; $[\alpha]_D^{23}$ −14.3° (*c* 0.48, $CHCl_3$).

Anal Calcd for $C_{48}H_{90}N_4O_9S$:
C 64.11, H 10.09, N 6.23, S 3.57.
Found:
C 63.97, H 10.01, N 6.21, S 3.44.

11c: Yield 59% from **5a**; $[\alpha]_D^{23}$ −7.9° (*c* 0.60, $CHCl_3$).

Anal Calcd for $C_{55}H_{102}N_4O_{11}S \cdot 0.5H_2O$:
C 63.73, H 10.02, N 5.41, S 3.09.
Found:

C 63.88, H 10.22, N 5.48, S 3.09.

11d: Yield 84% from **5a**; $[\alpha]_D^{24}$ −10.6° (*c* 0.50, $CHCl_3$).

Anal Calcd for $C_{62}H_{114}N_4O_{13}S \cdot 0.5H_2O$:
C 63.94, H 9.95, N 4.81, S 2.75.
Found:
C 63.91, H 9.80, N 4.74, S 2.71.

11e: Yield 90% from **5a**; $[\alpha]_D^{24}$ −9.6° (*c* 0.53, $CHCl_3$).

Anal Calcd for $C_{62}H_{114}N_4O_{13}S \cdot H_2O$:
C, 63.45, H 9.96, N 4.77, S 2.73.
Found:
C 63.31, H 9.81, N 4.82, S 2.65.

11f: Yield 64% from **5a**; $[\alpha]_D^{23}$ −5.6° (*c* 0.57, $CHCl_3$).

Anal Calcd for $C_{53}H_{99}N_3O_{10}S$:
C 65.60, H 10.28, N 4.33, S 3.30.
Found:
C 65.51, H 10.31, N 4.20, S 3.25.

11g: Yield 81% from **5a**; $[\alpha]_D^{24}$ −16.4° (*c* 0.53, $CHCl_3$).

Anal Calcd for $C_{53}H_{99}N_3O_{10}S \cdot 0.8H_2O$:
C 64.64, H 10.30, N 4.27, S 3.26.
Found:
C 64.62, H 10.25, N 4.08, S 3.22.

Synthesis of *N*-Myristoyl Derivatives **12a**~**12g**

The *N*-myristoyl derivatives **12a**~**12g** were obtained as white powders from the corresponding compounds (**11a**~**11g**) by *N*-acylation followed by deprotection.

12a: Yield 86% from **11a**; $[\alpha]_D^{23}$ −8.9° (*c* 0.56, 5% TFA-$CHCl_3$).

Anal Calcd for $C_{63}H_{115}N_5O_{13}S \cdot 1.5H_2O$:
C 62.55, H 9.83, N 5.79, S 2.65.
Found:
C 62.74, H 9.68, N 5.91, S 2.56.

12b: Yield 88% from **11b**; $[\alpha]_D^{21}$ −14.8° (*c* 0.55, 5% TFA-$CHCl_3$).

Anal Calcd for $C_{58}H_{108}N_4O_{10}S \cdot 0.5H_2O$:
C 65.56, H 10.34, N 5.27, S 3.02.
Found:
C 65.57, H 10.17, N 5.15, S 2.90.

12c: Yield 87% from **11c**; $[\alpha]_D^{21}$ −11.5° (*c* 0.69, 5% TFA-$CHCl_3$).

Anal Calcd for $C_{61}H_{112}N_4O_{12}S \cdot 0.5H_2O$:
C 64.57, H 10.04, N 4.94, S 2.83.
Found:
C 64.74, H 9.97, N 4.83, S 2.76.

12d: Yield 71% from **11d**; $[\alpha]_D^{24}$ −19.1° (*c* 0.53, 5% TFA-$CHCl_3$).

Anal Calcd for $C_{64}H_{116}N_4O_{14}S \cdot 0.5H_2O$:
C 63.70, H 9.77, N 4.64, S 2.66.
Found:
C 63.80, H 9.76, N 4.76, S 2.66.

12e: Yield 79% from **11e**; $[\alpha]_D^{24}$ −17.9° (*c* 0.51, 5% TFA-$CHCl_3$).

Anal Calcd for $C_{64}H_{116}N_6O_{14}S \cdot 0.5H_2O$:
C 63.70, H 9.77, N 4.64, S 2.66.
Found:
C 63.79, H 9.61, N 4.75, S 2.65.

12f: Yield 73% from **11f**; $[\alpha]_D^{21}$ −13.3° (*c* 0.51, 5% TFA-$CHCl_3$).

Anal Calcd for $C_{59}H_{109}N_3O_{11}S \cdot 0.5H_2O$:
C 65.76, H 10.29, N 3.90, S 2.98.
Found:
C 65.84, H 10.32, N 3.92, S 2.97.

12g: Yield 77% from **11g**; $[\alpha]_D^{24}$ −19.5° (*c* 0.51, 5% TFA-$CHCl_3$).

Anal Calcd for $C_{59}H_{109}N_3O_{11}S$:
C 66.32, H 10.28, N 3.93, S 3.00.
Found:
C 66.41, H 10.19, N 3.95, S 2.91.

Synthesis of *N*-Free Derivatives **13a**~**13g**

The *N*-free derivatives **13a**~**13g** were obtained as white powders from the corresponding compounds (**11a**~**11g**) by deprotection with TFA.

13a: Yield 94%; $[\alpha]_D^{23}$ +8.3° (*c* 0.60, 5% TFA-$CHCl_3$).

Anal Calcd for $C_{49}H_{89}N_5O_{12}S \cdot H_2O$:
C 59.43, H 9.26, N 7.07, S 3.24.
Found:
C 59.19, H 8.96, N 6.96, S 3.26.

13b: Yield 98%; $[\alpha]_D^{23}$ +12.2° (*c* 0.63, 5% TFA-$CHCl_3$).

Anal Calcd for $C_{44}H_{82}N_4O_9S \cdot 2.5H_2O$:
C 59.50, H 9.87, N 6.31, S 3.61.
Found:
C 59.21, H 9.17, N 6.16, S 3.34.

13c: Yield 97%; $[\alpha]_D^{23}$ +14.8° (*c* 0.68, 5% TFA-$CHCl_3$).

Anal Calcd for $C_{47}H_{86}N_4O_{11}S \cdot 2.5H_2O$:
C 58.78, H 9.55, N 5.83, S 3.34.
Found:
C 58.91, H 8.83, N 5.67, S 3.06.

13d: Yield 88%; $[\alpha]_D^{21}$ +13.0° (*c* 0.52, 5% TFA-$CHCl_3$).

Anal Calcd for $C_{50}H_{90}N_4O_{13}S \cdot 1.5H_2O$:
C 59.20, H 9.24, N 5.52, S 3.16.
Found:
C 59.15, H 9.09, N 5.50, S 3.41.

13e: Yield 87%; $[\alpha]_D^{24}$ +8.7° (*c* 0.52, 5% TFA-$CHCl_3$).

Anal Calcd for $C_{50}H_{90}N_4O_{13}S \cdot 1.5H_2O$:
C 59.20, H 9.24, N 5.52, S 3.16.
Found:
C 59.34, H 9.13, N 5.53, S 3.21.

13f: Yield 82%; $[\alpha]_D^{21}$ +2.1° (*c* 0.53, 5% TFA-$CHCl_3$).

Anal Calcd for $C_{45}H_{83}N_3O_{10}S \cdot 0.5H_2O$:
C 62.32, H 9.76, N 4.85, S 3.70.
Found:
C 62.16, H 9.64, N 4.61, S 3.67.

13g: Yield 79%; $[\alpha]_D^{24}$ +14.2° (*c* 0.52, 5% TFA-$CHCl_3$).

Anal Calcd for $C_{45}H_{83}N_3O_{10}S \cdot 2.2H_2O$:
C 60.19, H 9.81, N 4.68, S 3.57.
Found:
C 60.05, H 9.44, N 4.35, S 3.61.

Compounds **14**~**16**

By the same method described in the synthesis of **5a**,

using stearic acid **14** was obtained as colorless crystals: Yield 97%; mp 92.0~92.8°C; $[\alpha]_D^{24}$ +11.0° (*c* 0.67, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.9 Hz), 1.25 (56H, br s), 1.60 (4H, m), 2.30 (2H, t, J=7.3 Hz), 2.32 (2H, t, J=7.3 Hz), 2.77 (2H, m), 3.06 (1H, dd, J=13.9, 5.8 Hz), 3.17 (1H, dd, J=13.9, 4.3 Hz), 4.15 (1H, dd, J=12.0, 6.1 Hz), 4.24 (1H, t, J=7.0 Hz), 4.35 (1H, dd, J=12.0, 3.3 Hz), 4.40 (2H, d, J=7.0 Hz), 4.51 (1H, br), 4.65 (1H, m), 5.17 (1H, m), 5.81 (1H, d, J=7.8 Hz), 7.31 (2H, t, J=7.4 Hz), 7.40 (2H, t, J=7.5 Hz), 7.61 (2H, d, J=7.4 Hz), 7.76 (2H, d, J=7.5 Hz).

Anal Calcd for $C_{57}H_{91}NO_8S$:
C 72.03, H 9.65, N 1.47, S 3.37.
Found:
C 72.21, H 10.05, N 1.57, S 3.24.

The same method yielded **15** and **16** from **4a**.

15: Yield 48%; colorless crystals; mp 82.8~83.5°C; $[\alpha]_D^{24}$ +12.7°(*c* 0.58, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.9 Hz), 1.25 (40H, br s), 1.59 (4H, m), 2.30 (2H, t, J=7.3 Hz), 2.32 (2H, t, J=7.3 Hz), 2.76 (2H, m), 3.06 (1H, dd, J=13.8, 5.7 Hz), 3.17 (1H, dd, J=13.8, 3.7 Hz), 4.16 (1H, dd, J=12.1, 6.2 Hz), 4.24 (1H, t, J=7.0 Hz), 4.35 (1H, dd, J=12.1, 3.4 Hz), 4.40 (2H, d, J=7.0 Hz), 4.65 (1H, m), 5.17 (1H, m), 5.80 (1H, d, J=7.8 Hz), 7.31 (2H, t, J=7.4 Hz), 7.40 (2H, t, J=7.3 Hz), 7.61 (2H, d, J=7.3 Hz), 7.76 (2H, d, J=7.4 Hz).

Anal Calcd for $C_{49}H_{75}NO_8S \cdot 0.25H_2O$:
C 69.84, H 9.03, N 1.66, S 3.81.
Found:
C 69.85, H 9.09, N 1.62, S 3.78.

16: Yield 52%; colorless oil; $[\alpha]_D^{22}$ +15.6° (*c* 0.70, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.87 (3H, t, J=6.9 Hz), 0.88 (3H, t, J=6.9 Hz), 1.29 (8H, m), 1.60 (4H, m), 2.29 (2H, t, J=7.4 Hz), 2.31 (2H, t, J=7.4 Hz), 2.77 (2H, br d, J=5.6 Hz), 3.05 (1H, dd, J=13.7, 5.6 Hz), 3.17 (1H, dd, J=13.7, 4.3 Hz), 4.15 (1H, dd, J=12.0, 6.2 Hz), 4.23 (1H, t, J=7.0 Hz), 4.35 (1H, dd, J=12.0, 3.4 Hz), 4.40 (2H, d, J=7.0 Hz), 4.63 (1H, m), 4.91 (1H, br), 5.17 (1H, m), 5.81 (1H, d, J=7.8 Hz), 7.31 (2H, t, J=7.4 Hz), 7.40 (2H, t, J=7.5 Hz), 7.61 (2H, d, J=7.4 Hz), 7.76 (2H, d, J=7.5 Hz).

Anal Calcd for $C_{33}H_{43}NO_8S \cdot 0.5H_2O$:
C 63.64, H 7.12, N 2.25, S 5.15.
Found:
C 63.51, H 7.31, N 2.66, S 5.20.

Compound **20**

Hexanoic acid (724 μl, 5.91 mmol), DIC (925 μl, 5.91 mmol) and DMAP (516 mg, 4.22 mmol) were added to a solution of **4a** (2.00 g, 4.22 mmol) in CH_2Cl_2 (50 ml). The reaction mixture was stirred for 3 hours at room temperature, then concentrated. The residue was suspended in EtOAc, successively washed with 10% citric acid, 2% aqueous $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, then concentrated. The residue was chromatographed on a silica gel column, eluting with hexane - EtOAc (85 : 15 and 80 : 20), to afford **20** (1.19 g, 49%) as a colorless oil: $[\alpha]_D^{22}$ −2.6° (*c* 0.63, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.89 (3H, t, J=6.9 Hz), 1.29 (4H, m), 1.48 (9H, s), 1.62 (2H, m), 2.32 (2H, t, J=7.4 Hz), 2.62 (1H, dd, J=13.9, 8.0 Hz), 2.81 (1H, dd, J=13.9, 4.0 Hz), 2.97 (1H, dd, J=13.8, 5.7 Hz), 3.04 (1H, dd, J=13.8, 4.6 Hz), 3.94 (1H, m), 4.08 (1H, dd, J=11.4, 6.0 Hz), 4.16 (1H, dd, J=11.4, 4.2 Hz), 4.24 (1H, t, J=7.0 Hz), 4.40 (2H, d, J=7.0 Hz), 4.53 (1H, m), 5.78 (1H, d, J=7.7 Hz), 7.32 (2H, t, J=7.4 Hz), 7.41 (2H, t, J=7.5 Hz), 7.61 (2H, d, J=7.4 Hz), 7.77 (2H, d, J=7.5 Hz).

Anal Calcd for $C_{31}H_{41}NO_7S \cdot 1.5H_2O$:
C 62.19, H 7.41, N 2.34, S 5.36.
Found:
C 62.09, H 7.17, N 2.26, S 5.12.

Compound **21**

As described in the synthesis of compound **20**, **21** was obtained from **20** as a colorless oil: Yield 97%; $[\alpha]_D^{25}$ +14.2° (*c* 0.57, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.8 Hz), 1.25 (28H, br s), 1.60 (4H, m), 2.30 (2H, t, J=7.4 Hz), 2.32 (2H, t, J=7.4 Hz), 2.77 (2H, m), 3.12 (2H, m), 4.15 (1H, dd, J=11.9, 6.2 Hz), 4.24 (1H, t, J=6.9 Hz), 4.35 (1H, dd, J=11.9, 3.3 Hz), 4.40 (2H, d, J=6.9 Hz), 4.65 (1H, m), 5.17 (1H, m), 5.80 (1H, d, J=7.5 Hz), 7.32 (2H, t, J=7.4 Hz), 7.41 (2H, t, J=7.5 Hz), 7.61 (2H, d, J=7.4 Hz), 7.77 (2H, d, J=7.5 Hz).

Anal Calcd for $C_{43}H_{63}NO_8S \cdot H_2O$:
C 66.90, H 8.49, N 1.81, S 4.15.
Found:
C 67.05, H 8.20, N 1.74, S 4.01.

(*S*)-Glycidyl Palmitate

Palmitic acid (18.2 g, 67.5 mmol), DIC (11.1 ml, 70.9 mmol) and DMAP (423 mg, 3.46 mmol) were added to a solution of (*R*)-(+)-glycidol (5.00 g, 67.5 mmol) in THF (100 ml). The reaction mixture was stirred for 18 hours at room temperature, filtered, then the filtrate was concentrated. The residue was suspended in EtOAc, successively washed with 10% citric acid, 2% aqueous $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, then concentrated. The residue was chromatographed on a silica gel column, eluting with hexane - EtOAc (20 : 1), to afford (*S*)-glycidyl palmitate (16.0 g, 76%) as a white powder: $[\alpha]_D^{20}$ +13.9° (*c* 0.78, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, J=6.8 Hz), 1.25 (24H, br s), 1.63 (2H, m), 2.35 (2H, t, J=7.4 Hz), 2.65 (1H, dd, J=4.9, 2.6 Hz), 2.85 (1H, dd, J=4.9, 4.2 Hz), 3.21 (1H, m), 3.92 (1H, dd, J=12.3, 6.3 Hz), 4.42 (1H, dd, J=12.3, 3.1 Hz).

Anal Calcd for $C_{19}H_{36}O_3$: C 73.03, H 11.61.
Found: C 73.10, H 11.52.

Compound **22**

As described in the synthesis of compound **4a**, **22** was obtained as a white powder using (*S*)-glycidyl palmitate: Yield 74%; $[\alpha]_D^{20}$ −2.9° (*c* 0.55, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.9 Hz), 1.25 (24H, br s), 1.50 (9H, s), 1.60 (2H, br s), 2.32 (2H, t, J=7.4 Hz), 2.62 (1H, dd, J=13.9, 8.3 Hz), 2.81 (1H, dd, J=13.9, 3.4 Hz), 3.02 (2H, m), 3.93 (1H, br), 4.08 (1H, dd, J=11.5, 6.0 Hz),

4.16 (1H, dd, J=11.5, 4.2 Hz), 4.24 (1H, t, J=6.9 Hz), 4.40 (2H, d, J=6.9 Hz), 4.53 (1H, m), 5.75 (1H, d, J=7.6 Hz), 7.32 (2H, t, J=7.4 Hz), 7.40 (2H, t, J=7.5 Hz), 7.61 (2H, d, J=7.4 Hz), 7.77 (2H, d, J=7.5 Hz).

Anal Calcd for $C_{41}H_{61}NO_7S$:
C 69.16, H 8.64, N 1.97, S 4.50.
Found:
C 68.95, H 8.67, N 1.83, S 4.48.

Compound **23**

Hexanoic acid (290 mg, 2.5 mmol), DCC (615 mg, 2.5 mmol) and DMAP (49 mg, 0.40 mmol) were added to a solution of **22** (712 mg, 1.0 mmol) in THF (15 ml). After stirring for 40 hours at room temperature, the reaction mixture was concentrated and the residue was suspended in EtOAc. The suspension was washed with 10% citric acid and water, dried over anhydrous Na_2SO_4, then concentrated. The residue was chromatographed on a silica gel column, eluting with toluene - EtOAc (20 : 1), to afford a colorless oil (610 mg, 75%). The oil (500 mg, 0.62 mmol) was dissolved in TFA (5.0 ml). The solution was allowed to stand for 2 hours at room temperature, then concentrated. The residue was dissolved in EtOAc and washed with water. The organic layer was dried over anhydrous Na_2SO_4, then concentrated to give **23** (460 mg, 99%) as a colorless oil: $[\alpha]_D^{22}$ +14.0° (c 0.56, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.9 Hz), 1.25 (28H, m), 1.60 (4H, m), 2.30 (4H, q, J=7.4 Hz), 2.77 (2H, br), 3.11 (2H, m), 4.15 (1H, dd, J=11.9, 6.2 Hz), 4.23 (1H, t, J=7.0 Hz), 4.35 (1H, dd, J=11.9, 3.3 Hz), 4.40 (2H, d, J=7.0 Hz), 4.65 (1H, br), 5.17 (1H, br), 5.80 (1H, d, J=7.7 Hz), 7.31 (2H, t, J=7.4 Hz), 7.40 (2H, t, J=7.5 Hz), 7.61 (2H, d, J=7.4 Hz), 7.76 (2H, d, J=7.5 Hz).

Anal Calcd for $C_{43}H_{63}NO_8S$:
C 68.49, H 8.42, N 1.86, S 4.25.
Found:
C 68.27, H 8.34, N 1.83, S 4.26.

Compound **17**

The protected peptide, H-Gly-Glu(O^{t}Bu)-Glu(O^{t}Bu)-O^{t}Bu[3], (1.47 g, 2.93 mmol), *N*-hydroxy-5-norbornene-2,3-dicarboximide (HONB) (525 mg, 2.93 mmol) and DIC (459 μl, 2.93 mmol) were added to an ice-cooled solution of **14** (2.53 g, 2.66 mmol) in DMF (20 ml). The reaction mixture was stirred for 16 hours at room temperature, then concentrated. The residue was suspended in EtOAc, successively washed with 10% citric acid, 2% aqueous $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, then concentrated. The residue was suspended in CH_3CN and the precipitates were collected to afford a white powder (3.75 g, 98%). Piperidine (3.6 ml) was added to an ice-cooled solution of the powder (3.56 g, 2.48 mmol) in CH_2Cl_2 (30 ml) and stirred for 2 hours at room temperature. The reaction mixture was concentrated and chromatographed on a silica gel column, eluting with $CHCl_3$ - MeOH (50 : 1 and 20 : 1), to afford a white powder (2.78 g, 92%). The powder (593 mg, 0.49 mmol) was dissolved in TFA (6.0 ml), placed for 2 hours at room temperature, then concentrated. The residue was suspended in CH_3CN and the precipitates were collected to afford **17** (510 mg, 99%) as a white powder: $[\alpha]_D^{24}$ +10.8° (c 0.65, 5% TFA - $CHCl_3$).

Anal Calcd for $C_{54}H_{98}N_4O_{13}S \cdot 4H_2O$:
C 58.14, H 9.58, N 5.02, S 2.87.
Found:
C 58.48, H 9.20, N 5.20, S 2.82.

Compounds **18**, **19**, **24** and **25** were obtained from **15**, **16**, **21** and **23** as white powders by the same method.

18: Yield 69%; $[\alpha]_D^{22}$ +10.9° (c 0.86, 5% TFA - $CHCl_3$).

Anal Calcd for $C_{46}H_{82}N_4O_{13}S \cdot 4H_2O$:
C 55.07, H 9.04, N 5.58, S 3.20.
Found:
C 54.96, H 9.12, N 5.25, S 2.94.

19: Yield 59%; $[\alpha]_D^{25}$ +18.1° (c 0.48, 5% TFA - $CHCl_3$).

Anal Calcd for $C_{30}H_{50}N_4O_{13}S \cdot 2.5H_2O$:
C 47.93, H 7.37, N 7.45, S 4.26.
Found:
C 47.80, H 6.78, N 7.46, S 4.31.

24: Yield 61%; $[\alpha]_D^{22}$ +8.8° (c 0.50, 5% TFA - $CHCl_3$).

Anal Calcd for $C_{40}H_{70}N_4O_{13}S \cdot 3H_2O$:
C 53.32, H 8.50, N 6.22, S 3.56.
Found:
C 53.35, H 8.79, N 6.21, S 3.45.

25: Yield 57%; $[\alpha]_D^{22}$ +8.9° (c 0.58, 5% TFA - $CHCl_3$).

Anal Calcd for $C_{40}H_{70}N_4O_{13}S \cdot 3.5H_2O$:
C 52.79, H 8.53, N 6.16, S 3.52.
Found:
C 52.99, H 8.30, N 6.12, S 3.27.

Compound **26**

Hexanoic acid (24 μl, 0.19 mmol), HOBT (26 mg, 0.19 mmol) and WSC (36 mg, 0.19 mmol) were added to an ice-cooled solution of **11d** (200 mg, 0.17 mmol) in CH_2Cl_2 (5.0 ml). The reaction mixture was stirred for 17 hours at room temperature, then concentrated. The residue was suspended in EtOAc, successively washed with 2% aqueous $NaHCO_3$, 10% aqueous NH_4Cl and water, dried over anhydrous Na_2SO_4, then concentrated. The residue was suspended in CH_3CN and the precipitates were collected to afford a white powder (165 mg, 76%). The powder (110 mg, 0.09 mmol) was dissolved in TFA (1.0 ml). The solution was allowed to stand for 2 hours at room temperature, then concentrated. The residue was suspended in CH_3CN and the precipitates were collected to afford **26** (95 mg, 100%) as a white powder: $[\alpha]_D^{21}$ −17.1° (c 0.41, 5% TFA - $CHCl_3$).

Anal Calcd for $C_{56}H_{100}N_4O_{14}S \cdot 2H_2O$:
C 59.97, H 9.35, N 5.00, S 2.86.
Found:
C 59.79, H 9.19, N 5.03, S 2.89.

Compound **27**

Acetic anhydride (28 μl, 0.30 mmol) was added to a

solution of **11d** (230 mg, 0.20 mmol) in CH_2Cl_2 (2.0 ml). The reaction mixture was stirred for 2 hours at room temperature, then concentrated. The residue was suspended in CH_3CN and the precipitates were collected to afford a white powder (222 mg, 93%). The powder (165 mg, 0.14 mmol) was dissolved in TFA (1.7 ml), placed for 2 hours at room temperature, then concentrated. The residue was suspended in CH_3CN and the precipitates were collected to afford **27** (139 mg, 98%) as a white powder: $[\alpha]_D^{21}$ −14.1° (*c* 0.52, 5% TFA - $CHCl_3$).

Anal Calcd for $C_{52}H_{92}N_4O_{14}S \cdot 0.5H_2O$:
C 60.15, H 9.03, N 5.40, S 3.09.
Found:
C 60.02, H 9.08, N 5.72, S 3.19.

Compound **28**

Hexanal (41 μl, 0.34 mmol), acetic acid (42 μl, 0.73 mmol) and $NaBH_3CN$ (21 mg, 0.33 mmol) were added to a solution of **11d** (350 mg, 0.30 mmol) in MeOH (12 ml). The reaction mixture was stirred for 1 hour at room temperature, then concentrated. The residue was suspended in EtOAc, washed with 2% aqueous $NaHCO_3$ and water, dried over anhydrous Na_2SO_4, then concentrated. The residue was chromatographed on a silica gel column, eluting with hexane - EtOAc (7 : 3 and 6 : 4), to afford a white solid (244 mg, 65%). The solid (188 mg, 0.15 mmol) was dissolved in TFA (1.9 ml), placed for 2 hours at room temperature, then concentrated. The residue was suspended in CH_3CN and the precipitates were collected to afford **28** (165 mg, 100%) as a white powder: $[\alpha]_D^{21}$ +2.3° (*c* 0.41, 5% TFA - $CHCl_3$).

Anal Calcd for $C_{56}H_{102}N_4O_{13}S \cdot 0.5H_2O$:
C 62.25, H 9.61, N 5.19, S 2.97.
Found:
C 58.92, H 9.23, N 4.72, S 2.73.

Compound **29**

By the same method, **29** was obtained from **11d** as a white powder: Yield 37%; $[\alpha]_D^{21}$ +9.7° (*c* 0.48, 5% TFA - $CHCl_3$).

Anal Calcd for $C_{52}H_{94}N_4O_{13}S \cdot 4.5H_2O$:
C 56.96, H 9.47, N 5.11, S 2.92.
Found:
C 56.95, H 9.22, N 4.95, S 2.91.

Compound **30**

Chloroacetyl isocyanate (24 μl, 0.29 mmol) was added to an ice-cooled solution of **11d** (300 mg, 0.26 mmol) in CH_2Cl_2 (5.0 ml). The reaction mixture was stirred for 30 minutes at 0°C, then concentrated. The residue was suspended in EtOAc, successively washed with 2% aqueous $NaHCO_3$, 10% aqueous NH_4Cl and water, dried over anhydrous Na_2SO_4, then concentrated. The residue was suspended in CH_3CN and the precipitates were collected to afford a white powder (305 mg, 92%). The powder (220 mg, 0.17 mmol) was dissolved in TFA (3.0 ml), placed for 2 hours at room temperature, then concentrated. The residue was suspended in CH_3CN and the precipitates were collected to afford **30** (189 mg, 99%) as a white powder: $[\alpha]_D^{21}$ −5.3° (*c* 0.45, 5% TFA - $CHCl_3$).

Anal Calcd for $C_{53}H_{92}N_5O_{15}SCl \cdot 0.5H_2O$:
C 57.05, H 8.40, N 6.28, S 2.93, Cl 3.18.
Found:
C 57.10, H 8.55, N 6.30, S 2.93, Cl 3.14.

Compound **31**

By the same method, **31** was obtained from **11d** as a white powder: Yield 37%; $[\alpha]_D^{21}$ −14.5° (*c* 0.45, 5% TFA - $CHCl_3$).

Anal Calcd for $C_{52}H_{92}N_4O_{15}S \cdot 1.5H_2O$:
C 58.24, H 8.93, N 5.22, S 2.99.
Found:
C 58.32, H 8.84, N 5.40, S 2.93.

Compound **32**

Compound **13c** (5.0 g) was dissolved in 20% acetonitrile - 0.5% aqueous sodium hydrogen carbonate (5.0 liters) at 40°C and adjusted to pH 9.5. The solution was applied to a column of Diaion HP-20 (1.0 liter), washed with 20% acetonitrile, then eluted with 40% acetonitrile (4.0 liters) and 60% acetonitrile (5.0 liters). The eluate was concentrated to a small volume and lyophilized. The powder was suspended in acetonitrile (150 ml) and the resulting precipitates were collected to afford **32** (4.5 g, 85%) as a white powder: $[\alpha]_D^{25}$ +4.5° (*c* 0.57, H_2O).

Anal Calcd for $C_{47}H_{84}N_4O_{11}SNa_2 \cdot 3H_2O$:
C 55.71, H 8.95, N 5.53, S 3.16, Na 4.54.
Found:
C 55.90, H 9.39, N 5.34, S 3.16, Na 4.78.

Compounds **33**, **34**, and **35** were obtained from **13d**, **13e**, **13g** as white powders by the same method.

33: Yield 79%; $[\alpha]_D^{25}$ −9.5° (*c* 0.71, H_2O).

Anal Calcd for $C_{50}H_{87}N_4O_{13}SNa_3 \cdot 4H_2O$:
C 53.37, H 8.51, N 4.98, S 2.85, Na 6.13.
Found:
C 53.48, H 8.81, N 4.89, S 3.03, Na 6.00.

34: Yield 76%; $[\alpha]_D^{25}$ −19.9° (*c* 0.86, H_2O).

Anal Calcd for $C_{50}H_{87}N_4O_{13}SNa_3 \cdot 2.5H_2O$:
C 54.68, H 8.44, N 5.10, S 2.92, Na 6.28.
Found:
C 54.84, H 8.64, N 5.09, S 2.79, Na 6.13.

35: Yield 85%; $[\alpha]_D^{25}$ +5.6° (*c* 0.62, H_2O).

Anal Calcd for $C_{45}H_{81}N_3O_{10}SNa_2 \cdot 3H_2O$:
C 56.62, H 9.17, N 4.39, S 3.35, Na 4.81.
Found:
C 56.61, H 9.14, N 4.26, S 3.35, Na 5.25.

Effect of Derivatives on the Proliferation of Bone Marrow Cells (BMC, *in vitro*)

The BMC assay was performed as described[1].

Effect of Derivatives on the Number of White Blood Cells (WBC, *in vivo*)

The compounds were dissolved in 5% glucose containing an equivalent molar amount of NaOH at 2.0 mg/ml. After ultrasonic dispersion, the solution was

diluted in 5% glucose and stored at 4°C during the experimental period.

Experimental leukopenia was induced by cyclophosphamide (CY) according to HATTORI *et al.* with a slight modification[7]. Female CDF1/Crj mice (n=5) were administered orally with 150 mg/kg of CY in physiological saline on day 0. Solutions of derivatives were administered subcutaneously to the mice once a day from days 1 to 5. Physiological saline and 5% glucose (0.2 ml/20 g body weight) were administered to vehicle control groups instead of CY and derivative, respectively. CY and 5% glucose were administered to the CY control group in the same manner.

Blood samples were collected from the orbital angular vein on day 6 using EDTA-K_2 treated capillary tubes. The leukocytes were counted by a multiple automatic blood cell counter, Sysmex K-2000 (Toa Medical Electronics, Kobe). The ratio of the leukocyte counts (% control) was calculated from those of the vehicle control group as 100%, and the % control in CY control group was 41 ± 11% (mean $\pm$ sd) throughout the study.

The differential leukocyte counts were examined in some experiments. Blood samples were smeared on slide glasses, stained with Giemsa solution and a total 200 leukocytes were counted. Absolute neutrophil and lymphocyte counts were calculated from the differential counts of these cells and total leukocytes.

Colony-stimulating Factor Activity in Serum

Compound **33** was administered at a dose of 0.031 mg/kg on day 1 to female CDF1/Crj mice (n=3) treated with 150 mg/kg of CY on day 0. Blood sample was obtained by cardiac puncture and pooled at the indicated time. Serum samples were stored at -20°C until colony assay according to ZSEBO *et al.* with a slight modification[8]. Bone marrow cells (1×10^5 cells) from normal CDF1/Crj mice were cultured in 6-well plates for 7 days in the presence of 0.1 ml of serum in 1.0 ml of RPMI1640 medium containing 0.3% agar, 15% horse serum and 15% fetal bovine serum. Colony-stimulating factor activity (CSF activity) was expressed as the number of colonies containing more than 40 cells from 1×10^5 bone marrow cells.

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