Synthesis and Structure–Activity Relationships of TAN-1511 Analogues as Potent Hematopoietic Agents

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A series of TAN-1511 analogues bearing a non-peptide spacer in place of the Gly–Gly–Gly sequence in the peptide moiety was synthesized, and the effects of these compounds on the proliferation of bone marrow cells in culture and experimental leukocytopenia in mice were examined. The structure–activity relationships obtained were as follows. As the substituent at the 2-position of the 4-thiaheptanoic acid framework, an amino group, methyl group or hydrogen was preferable; as a spacer in place of the Gly–Gly–Gly sequence, a 4-aminobenzoyl or 4-aminomethylbenzoyl group was suitable; and as the fatty acids bonded to the 6,7-dihydroxy groups, C_{16} fatty acid was best. Compounds 12f, 30d and 30i potently promoted the proliferation of bone marrow cells in culture and the restoration of leukocyte counts in a murine leukocytopenia model.

Key words lipopeptide; TAN-1511; hematopoietic activity; bone marrow cell; non-peptide spacer; structure–activity relationship

Bone marrow suppression is a major side effect of cancer chemotherapy and radiotherapy,[1] and bacterial, fungal or viral infection during the associated leukopenia can result in the death of cancer patients.[2] Therefore, drugs that can increase the number of leukocytes in leukopenic patients and restore the body's resistance to infection are clinically useful.

The novel lipopeptides TAN-1511A (**1a**), B (**1b**) and C (**1c**), which potently promote the proliferation of bone marrow cells (BMC), were isolated from the culture broth of *Streptsporangium amethystogenes* subsp. *fukuiense* AL-23456.[3] Each component is a mixture of lipopeptides having fatty acids of different lengths on the hydroxyl and amino groups of 2-amino-6,7-dihydroxy-4-thiaheptanoic acid. The main component of TAN-1511A is (2*R*,6*R*)-2-tetradecanoylamino-6,7-bis(hexadecanoyloxy)-4-thiaheptanoyl–Gly–Gly–Gly–Glu–Thr–Thr–OH (**2**). Preliminary chemical modification of TAN-1511 revealed that the terminal peptide moiety could be shortened to $(Gly)_n$–Glu ($n=2$ or 3) without a marked decrease in activity and that 2-amino-4-thiaheptanoic acid analogues (**3**) more potently stimulate the proliferation of bone marrow cells than do the 2-acylamino-4-thiaheptanoic acid congeners.[4]

In order to clarify further the structure–activity relationships of TAN-1511 analogues and to find more potent compounds, we chemically modified the peptide, 4-thiaheptanoic acid and lipid moieties of the 2-amino analogue (**3**). Since the Gly–Gly–Gly sequence of the peptide moiety of TAN-1511 could be regarded as just a spacer, we designed compounds with a non-peptide spacer such as a methylene chain or benzene ring in place of the Gly–Gly–Gly sequence. As for the substituent at the 2 position of the 4-thiaheptanoic acid, the 2-amino and acylamino derivatives showed no remarkable differences in activity.[4] Therefore, we considered the 2-amino group not to be essential for activity, and we synthesized various 2-desamino TAN-1511 analogues, derivatives methylated at various positions and derivatives having a sulfinyl, sulfonyl, oxy or methylene group in place of the sulfur atom at the 4-position. Moreover, since the natural TAN-1511 is a mixture of lipopeptides having alkyl chains of different lengths, we also synthesized compounds having fixed chain lengths which were bonded to heptanoic acid *via* an ester, ether, urethane or amide linkage.

This paper describes the synthesis, BMC proliferation-stimulating activity, accelerating effect on the recovery from experimental leukocytopenia and structure–activity

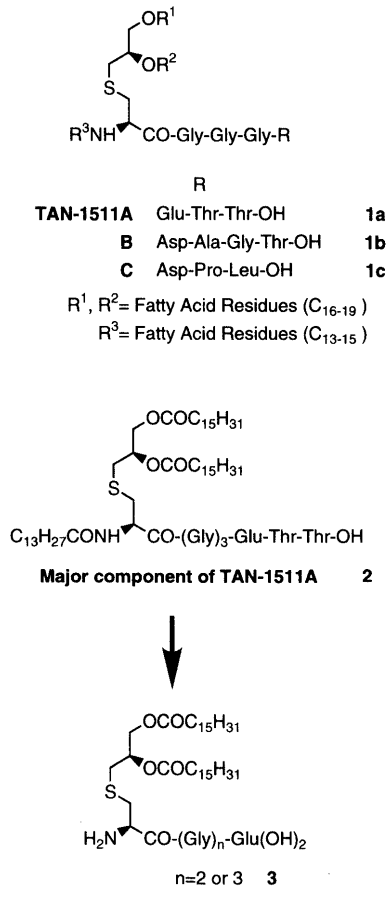

Fig. 1

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relationships of novel TAN-1511 analogues with a non-peptide spacer in place of the Gly–Gly–Gly sequence of the peptide moiety.

Chemistry

The terminal glutamates **6a—h**, **8a—d** and **8e** acylated with a spacer were prepared as shown in Chart 1. The treatment of benzyloxycarbonyl (Z)-protected ω-amino spacers **4a—g** with di-*tert*-butyl L-glutamate in the presence of diethyl phosphorocyanide (DEPC), followed by removal of the Z-group of **5a—g** by catalytic hydrogenation over Pd–C, gave the glutamate derivatives **6a—g**. The urea type compound **6h** was prepared by the reaction of di-*tert*-butyl L-glutamate with *p*-nitrophenyl carbamate, which was produced by the reaction of 6-Z-amino-1-hexylamine with *p*-nitrophenyl chloroformate in the presence of triethylamine, followed by catalytic hydrogenation of resultant **5h**. In the case of aniline-type spacers, **8a—d** were prepared by amidation of **7a—d** with di-*tert*-butyl L-glutamate using DEPC, without protecting the amino group of the spacer.

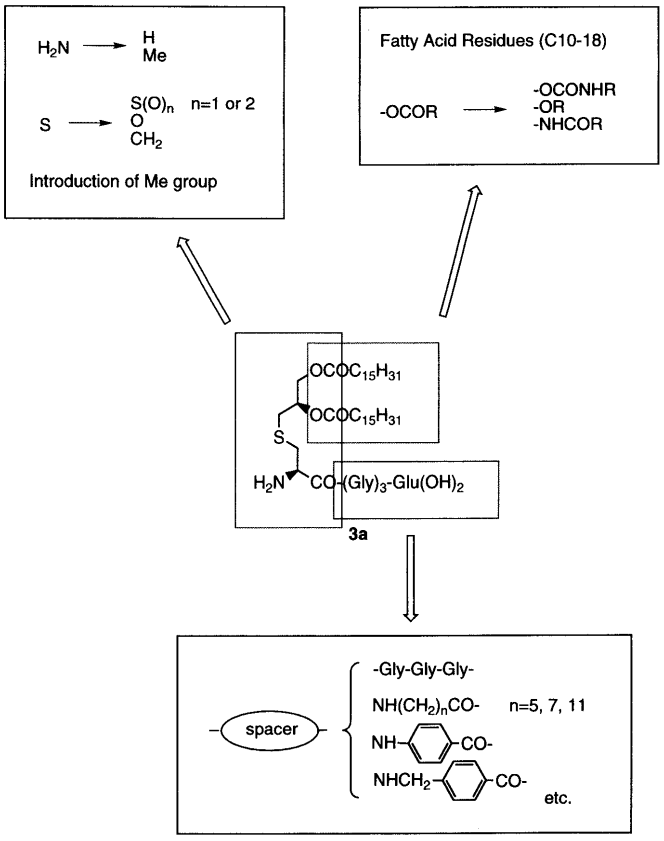


Fig. 2. Modifications of TAN-1511

The synthetic route to 2-amino-4-thiaheptanoic acid analogues **12** is shown in Chart 2. The 2-(9-fluorenyl)-methyloxycarbonyl (Fmoc) amino derivatives **10a—h** were prepared by the condensation of the glutamate derivatives **6b—h** and **8a** with 2-Fmoc-amino-4-thiaheptanoic acid **9**,[5] derived from L-cystine, using DEPC for **6b—h** or PCl_3 for **8a**. The treatment of **10a—h** with piperidine afforded the corresponding 2-amino derivatives **11a—g**. Finally, removal of the *tert*-butyl ester of **11a—g** with hydrogen chloride in ethyl acetate or trifluoroacetic acid (TFA) gave **12a—g**.

The diazotization of the 2-amino analogue **11e** with sodium nitrite in acetic acid gave the corresponding 2-acetoxy analogue **13**. This substitution seemed to proceed *via* an episulfonium intermediate as reported previously.[6] The treatment of **13** with TFA afforded the 2-acetoxy analogue **14**. Compound **10h**, which was prepared by amidation of **9** with **6g**, was treated with piperidine to give the dioxopiperazine **15**, and the subsequent deprotection with TFA afforded the dioxopiperazine analogue **16** of TAN-1511. The reaction of **11a** with *p*-nitrophenyl chloroformate produced the cyclic product **17**. The treatment of **17** with TFA gave the imidazolidinedione analogue **18** (Chart 2).

As for the synthesis of 2-desamino derivatives **30**, the 6,7-dihydroxy-4-thiaheptanoic acid derivatives **24**, the main backbone of TAN-1511, were prepared by the methods depicted in Chart 3. The methods can be roughly classified into two groups: one is the Michael addition of thioglycerol (**20**) to the acrylates **19a—c** (method A) or the propiolate **35** (method H), and the other is reaction of the 3-thiopropionic acid derivatives **21a—d** and **32** with the glycidols **22a—c** (methods B, F) or 3-iodo-1,2-propanediol (**23**) (method C). The Michael addition of **20**

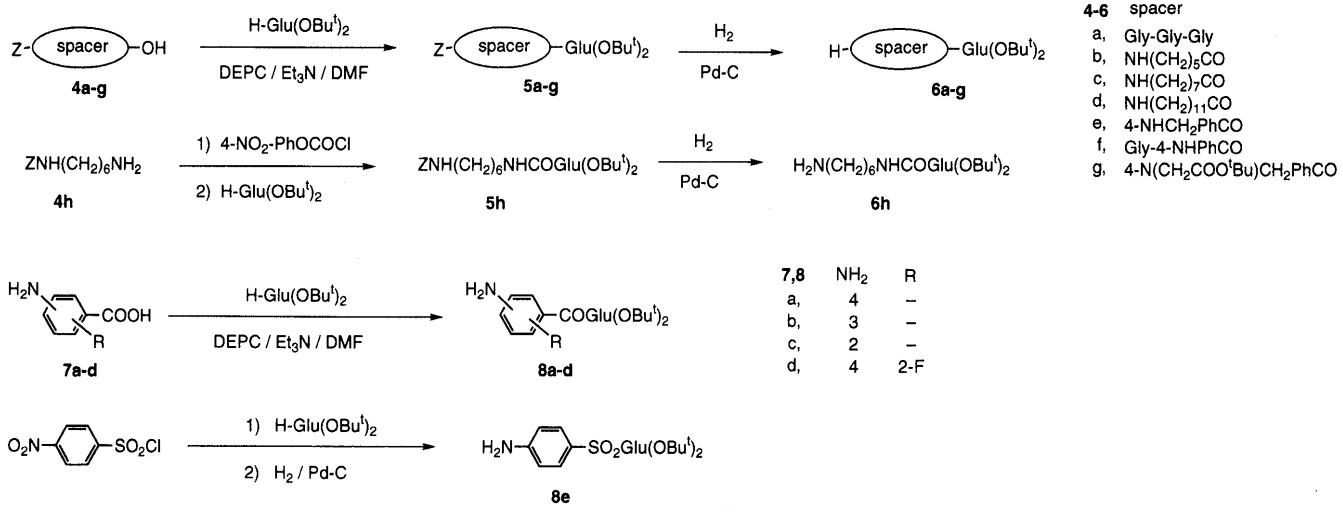


Chart 1

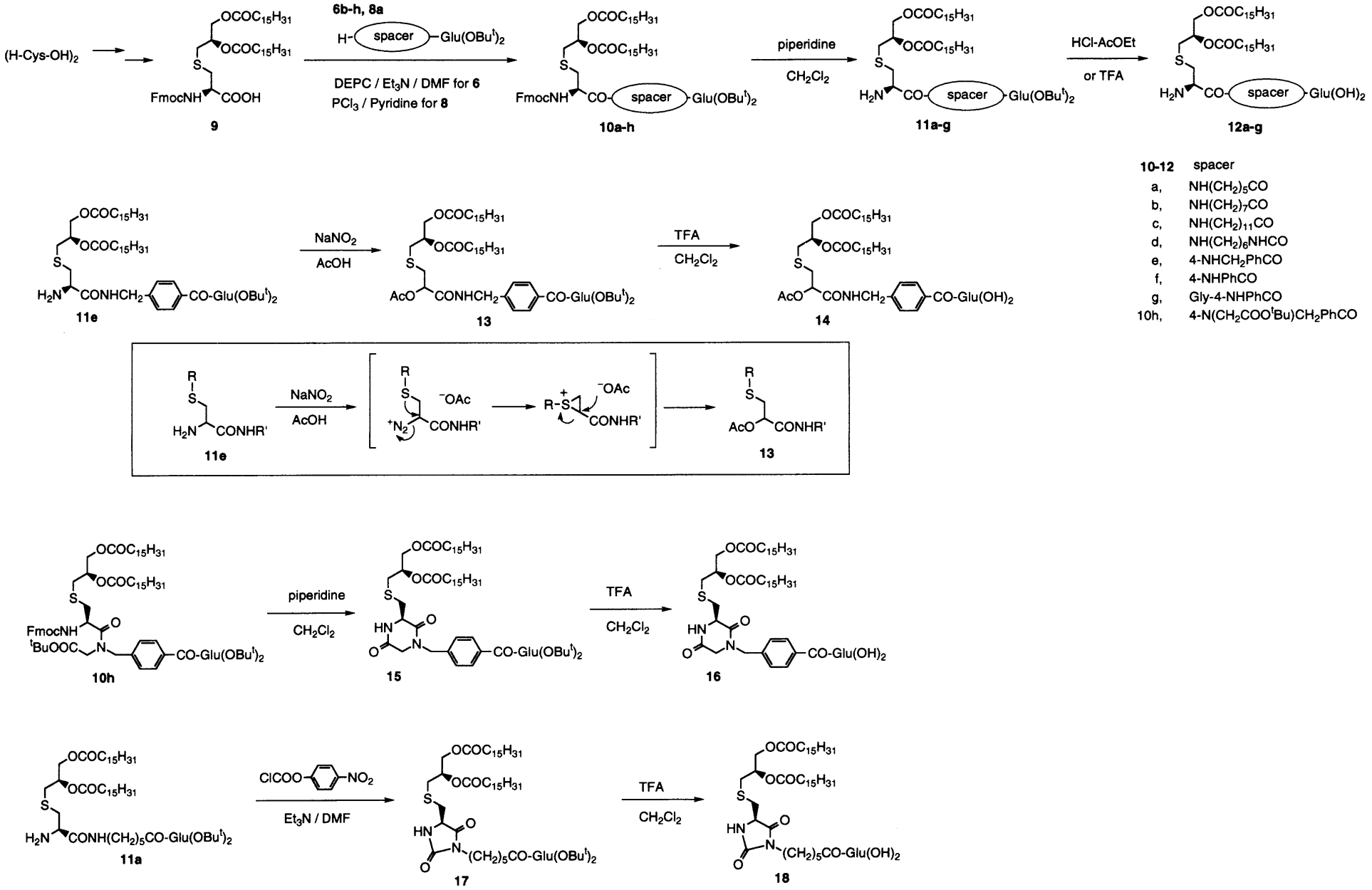


Chart 2

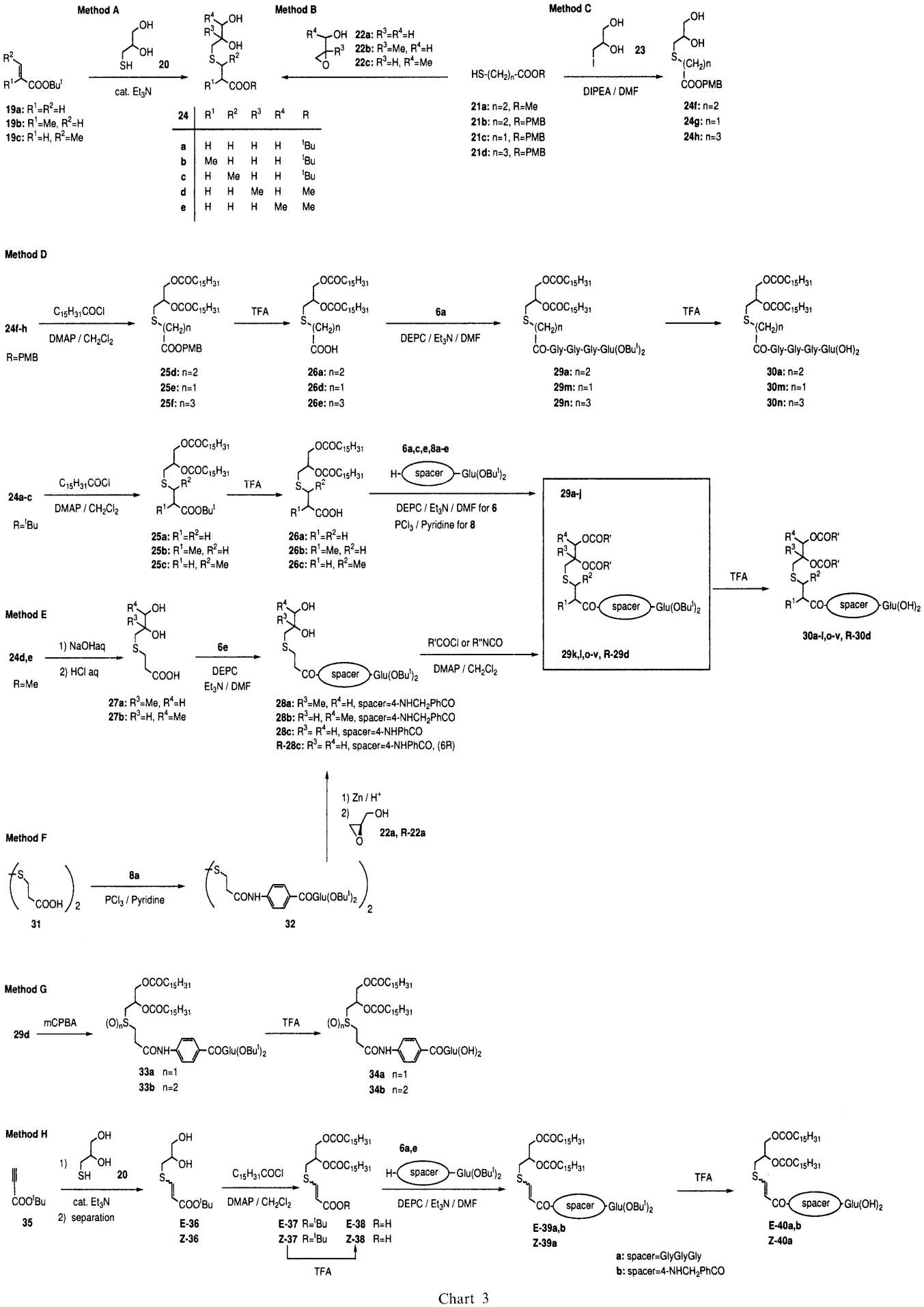


Chart 3

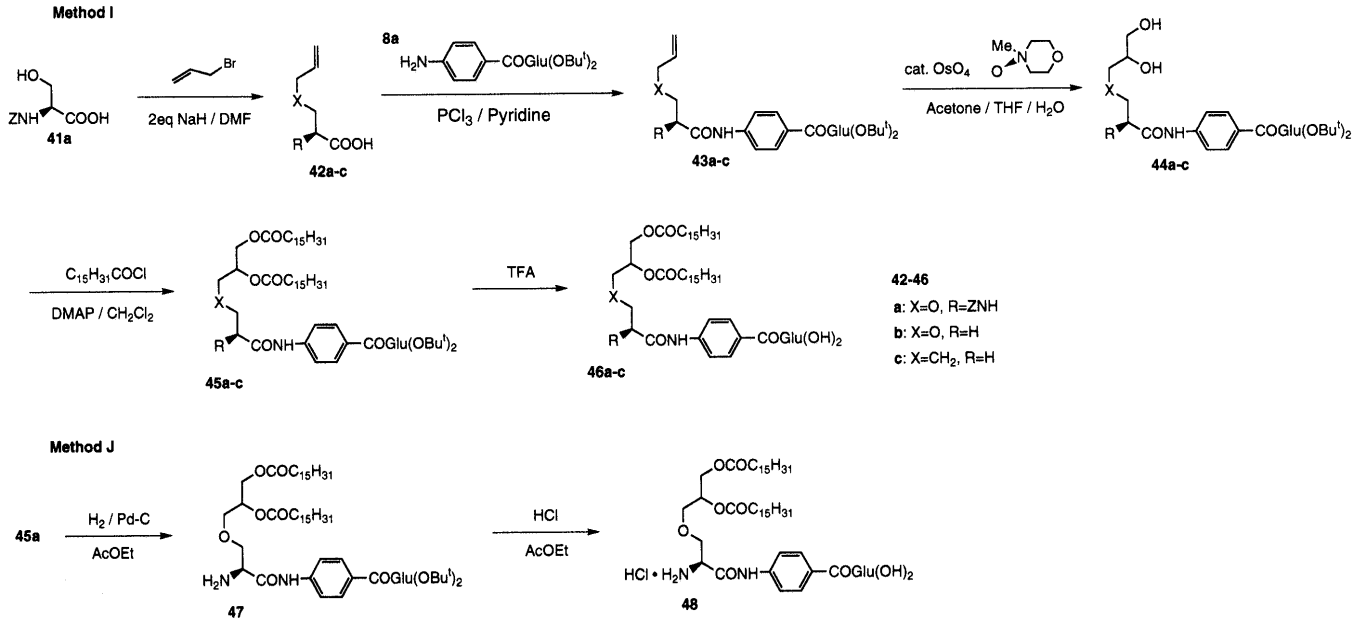


Chart 4

to **19a**—**c** in the presence of a catalytic amount of triethylamine without a solvent efficiently afforded **24a**—**c**. In order to prepare the 6- or 7-substituted analogues, the synthetic route of method B was utilized. The reaction of methyl 3-mercaptopropionate (**21a**) with **22b, c** in the presence of a catalytic amount of sodium methoxide gave the methyl 6,7-dihydroxy-4-thiaheptanoates **24d, e**. In the case of *tert*-butyl or *p*-methoxybenzyl (PMB) ester (method D), acylation of the diol of **24** with palmitoyl chloride in the presence of a stoichiometric amount of 4-dimethylaminopyridine (DMAP), followed by removal of the *tert*-butyl or PMB ester of the resultant 6,7-bis(palmitoyloxy)-4-thiaheptanoates **25a**—**f** with TFA, gave the 6,7-bis(palmitoyloxy)-4-thiaheptanoic acid derivatives **26a**—**e**. As described above, the condensation of **26a**—**e** with the terminal moiety **6a, c, e** and **8a**—**e** using DEPC or PCl_3 in pyridine, followed by deprotection of the resultant **29a**—**j, m, n**, furnished the corresponding TAN-1511 analogues **30**. On the other hand, in the case of methyl esters (method E), alkaline hydrolysis of **24d, e**, followed by amidation of the resultant carboxylic acids **27** with **6e** using DEPC, gave the diols **28a, b**. Compounds **28** were converted to the desired compounds **30** by acylation of the diol with acid chloride in the presence of DMAP and the subsequent deprotection with TFA. Compounds **28** were also prepared by the alternative route shown in method F. The disulfide derivative **32** were derived by amidation of the dithiodicarboxylic acid **31** with **8a**. The reductive cleavage of the disulfide group of **32** with zinc powder under acidic conditions, followed by treatment of the resultant thiol with glycidol (**22a**), gave the 6,7-dihydroxy-4-thiaheptanoic acid derivatives **28c**. This method was useful for preparation of the optically active analogues *R*-**30d**, by using optically active glycidol (*R*-**22a**), and the various fatty acid ester analogues **30o**—**t**. By using octadecyl isocyanate in place of acid chlorides, the carbamate derivatives **30u, v** were synthesized. In this case, treatment of **28c** with 1 mol eq of isocyanate gave the 7-carbamoyloxy-6-hydroxy derivative, and subsequent acylation with palmitoyl chloride afforded the 7-carbamoyloxy-6-palmitoyloxy analogue **30u**. The oxidation of **29d** with 1.5 mol eq of *m*-chloroperbenzoic acid (*m*CPBA) gave the sulfoxide **33a** and the sulfone **33b**, which were treated with TFA to afford the sulfur-oxidized analogues **34a** and **34b**, respectively (method G). The Michael addition of thioglycerol to the propiolate **35** gave the 6,7-dihydroxy-4-thia-2-heptenoates **36** as a separable mixture of *E*- and *Z*-forms in a ratio of 7 : 3. The isolated (*E*)-**36** was acylated with palmitoyl chloride in the presence of DMAP to give the 6,7-bis(palmitoyloxy)-4-thia-2(*E*)-heptenoate (*E*)-**37**, which was treated with TFA, followed by amidation of the resultant carboxylic acid (*E*)-**38** and subsequent deprotection to afford the 4-thia-2(*E*)-heptenoic acid analogues of TAN-1511 (*E*)-**40a, b**. The *Z*-isomer (*Z*)-**36** was converted to (*Z*)-**40a** in the same manner as the *E*-series (method H).

The synthetic routes for preparation of the 6,7-dihydroxy-4-oxaheptanoic acid derivatives **46a, b** and **48** and the 6,7-dihydroxyheptanoic acid derivatives **46c** are shown in Chart 4. The allylation of *Z*-Ser-OH (**41a**) with allyl bromide in the presence of 2 mol eq of sodium hydride in dimethylformamide (DMF) gave *O*-allyl-*Z*-serine (**42a**). The commercially available compounds **42b, c** and compound **42a** thus prepared were condensed with **8a** using PCl_3 in pyridine to give the amides **43a**—**c**, which were dihydroxylated with a catalytic amount of OsO_4 and *N*-methylmorpholine *N*-oxide (NMO) to afford the 6,7-dihydroxy-4-oxaheptanoic acid derivatives **44a, b** and the 6,7-dihydroxyheptanoic acid derivative **44c**. The bispalmitoylation of the diols **44a**—**c**, followed by deprotection of the resultant **45a**—**c**, gave the 4-oxaheptanoic acid derivatives **46a, b** and the heptanoic acid derivative **46c** containing an oxygen atom or a methylene group in place of the sulfur atom at the 4-position (method I). The Z group of compound **45a** was removed by hydrogenolysis, and subsequent treatment with acid gave the 2-amino analogue **48** (method J).

The palmitoyloxy group at the 6- or 7-position of the

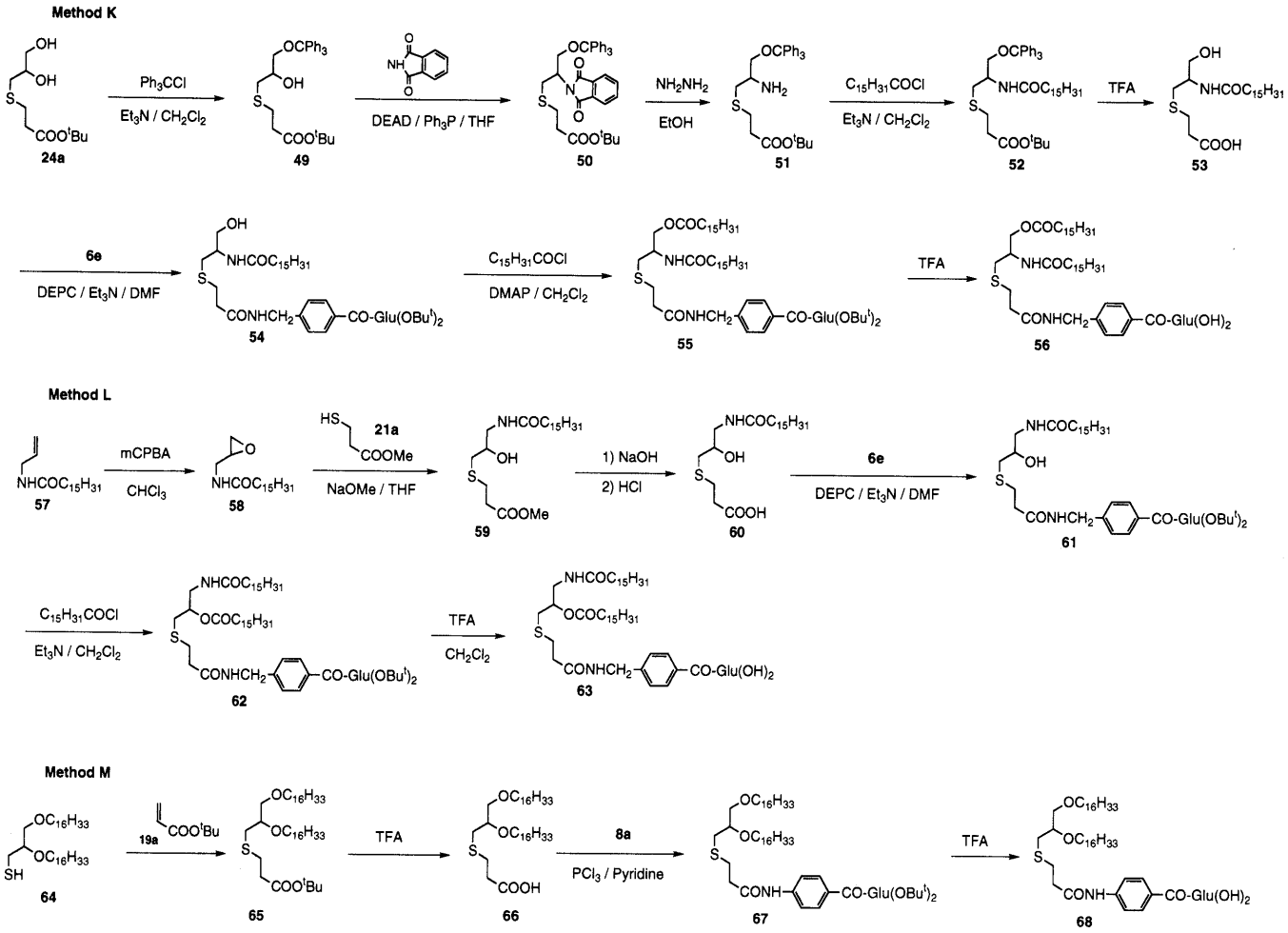


Chart 5

4-thiaheptanoic acid framework was converted to a palmitoylamino group as shown in Chart 5. The protection of the primary alcohol of **24a** with trityl chloride and subsequent Mitsunobu's reaction using phthalimide in the presence of diethyl azodicarboxylate and triphenylphosphine gave the 6-phthalimido compound **50**. Compound **50** was subjected successively to removal of the phthaloyl group, palmitoylation of the amino group, removal of both the trityl group and the *tert*-butyl ester using TFA, amidation with **6e**, palmitoylation of the hydroxyl group and deprotection with TFA to afford the 6-amido analogue **56** (method K). The epoxidation of *N*-allyl palmitoylamide (**57**) gave the epoxide **58**, which was ring-opened with **21a** followed by alkaline hydrolysis to afford 6-hydroxy-7-palmitoylamino-4-thiaheptanoic acid (**60**). The carboxylic acid **60** was converted to the desired compound **63** by the successive amidation, palmitoylation and deprotection (method L). The 6,7-bis(hexadecanoxy) derivative **68** was prepared by Michael addition of **64**[7] to **19a** followed by treatment similar to that as described in method D (method M).

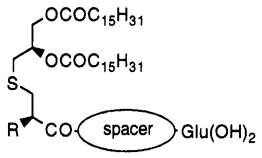
Biological Activity and Discussion

The stimulating effects of the prepared TAN-1511A analogues on the proliferation of bone marrow cells were examined in comparison with those of the synthetic TAN-1511A (**2**) and the 2-amino peptide analogue **3a** (Tables 1—4).

All of the prepared 2-amino analogues (**12a—g**) with a non-peptide spacer were found to stimulate the proliferation of bone marrow cells with potency similar to that of the synthetic TAN-1511A (**2**) or the 2-amino analogue **3a** with a Gly–Gly–Gly sequence (Table 1). In the case of the 2-amino analogues, the spacer part did not affect the activity. Therefore, it was demonstrated that the Gly–Gly–Gly sequence of the peptide moiety could be replaced with a non-peptide spacer.

Structure–activity relationships of the 2-desamino-4-thiaheptanoic acid analogues were examined. Deamination at the 2-position of the 4-thiaheptanoic acid frame gave interesting results. Compounds with a peptide or an aliphatic spacer such as the Gly–Gly–Gly sequence (**30a**) or a 7-aminoheptanoyl group (**30b**) had diminished BMC proliferation-stimulating activity. On the other hand, compounds with a *p*-substituted aromatic spacer such as a 4-aminobenzoyl (**30d**), 4-aminomethylbenzoyl (**30c**), 4-amino-2-fluorobenzoyl (**30g**) or 4-aminobenzenesulfonyl group (**30h**) exhibited as potent activity as the 2-amino analogues **12**. However, compounds **30e, f** with an *o*- or *m*-substituted spacer, such as a 2-aminobenzoyl or 3-aminobenzoyl group, showed no activity. Therefore, the activity of the 2-desamino analogues seems to depend on the structure of the spacer to a much greater extent than the activity of the 2-amino analogues (Table 2).

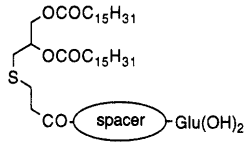
Table 1. Physicochemical and Biological Data for TAN-1511 Derivatives with Various Spacers



Compd.	R	Spacer	mp (°C) Solvent	Formula	Analysis (%) Calcd (Found) C	H	N	BMC MEC (ng/ml)
12a	$H_2N \cdot HCl$	$NH(CH_2)_5CO$	48—50 AcOEt	$C_{49}H_{91}N_3O_{10}S \cdot HCl \cdot 0.25H_2O$	61.61 (61.67	9.76 9.70	4.40 4.26)	0.020
12b	$H_2N \cdot HCl$	$NH(CH_2)_7CO$	53—56 AcOEt	$C_{51}H_{95}N_3O_{10}S \cdot HCl \cdot H_2O$	61.45 (61.17	9.91 9.81	4.22 4.26)	0.078
12c	$H_2N \cdot HCl$	$NH(CH_2)_{11}CO$	61—64 AcOEt	$C_{55}H_{103}N_3O_{10}S \cdot HCl \cdot 1.5H_2O$	62.20 (62.02	10.16 9.83	3.96 3.73)	0.625
12d	$H_2N \cdot TFA$	$NH(CH_2)_6NHCO$	82—84 AcOEt	$C_{50}H_{94}N_4O_{10}S \cdot TFA \cdot H_2O$	58.08 (58.19	9.09 8.77	5.21 5.24)	0.313
12e	$H_2N \cdot HCl$	4-$NHCH_2PhCO$	79—80 AcOEt	$C_{51}H_{87}N_3O_{10}S \cdot HCl \cdot H_2O$	61.95 (61.66	9.17 8.93	4.25 4.24)	0.039
12f	$H_2N \cdot HCl$	4-NHPhCO	94—96 AcOEt	$C_{50}H_{85}N_3O_{10}S \cdot HCl \cdot 2H_2O$	60.49 (60.39	9.14 8.98	4.23 4.19)	0.156
12g	$H_2N \cdot HCl$	Gly-4-NHPhCO	100—101 AcOEt	$C_{52}H_{88}N_4O_{11}S \cdot HCl \cdot 2H_2O$	59.49 (59.62	8.93 8.77	5.34 5.37)	0.078
14	AcO	4-$NHCH_2PhCO$	64—65 MeOH–H_2O	$C_{53}H_{88}N_2O_{12}S \cdot H_2O$	63.95 (63.89	9.11 9.46	2.81 2.84)	1.56
16	*–CH_2CONH[a]	**–NCH_2PhCO[a]	78—80 $CHCl_3$–MeOH–H_2O	$C_{53}H_{87}N_3O_{11}S \cdot 1.5H_2O$	63.57 (63.48	9.06 9.09	4.20 4.04)	12.5
18	*–CONH[a]	**–$N(CH_2)_5CO$[a]	72—73 MeOH–H_2O	$C_{50}H_{89}N_3O_{11}S \cdot H_2O$	62.66 (62.38	9.57 9.35	4.38 4.28)	6.25
2	$C_{13}H_{27}CONH$	Gly–Gly–Gly–Glu–Thr–Thr–OH						0.078
3a	$H_2N \cdot HCl$	Gly–Gly–Gly						0.078

a) Bond formed between * and **.

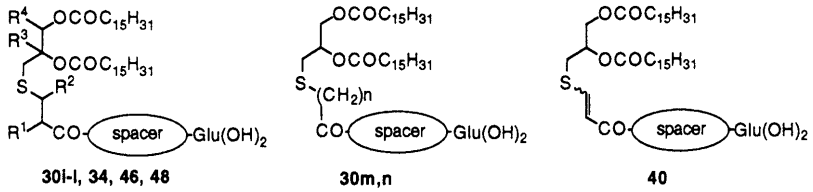
Table 2. Physicochemical and Biological Data of 2-Desamino Derivatives with Various Spacers



Compd.	Spacer	Method[a]	mp (°C) Solvent	Formula	Analysis (%) Calcd (Found) C	H	N	BMC MEC (ng/ml)
30a	Gly–Gly–Gly	D	92—94 TFA–MeCN–H_2O	$C_{49}H_{88}N_4O_{12}S \cdot 0.25TFA \cdot 0.25H_2O$	60.03 (60.24	9.03 8.81	5.66 5.74)	3.9
30b	$NH(CH_2)_7CO$	D	44—46 TFA–MeCN–H_2O	$C_{51}H_{94}N_2O_{10}S \cdot 0.5TFA \cdot 0.5H_2O$	62.87 (63.07	9.69 9.84	2.82 2.97)	15.6
30c	4-$NHCH_2PhCO$	D	58—59 MeOH–H_2O	$C_{51}H_{86}N_2O_{10}S \cdot 0.5H_2O$	65.99 (66.05	9.45 9.37	3.02 3.12)	0.156
30d	4-NHPhCO	D	110—111 MeOH–H_2O	$C_{50}H_{84}N_2O_{10}S \cdot H_2O$	65.04 (65.43	9.39 9.28	3.03 2.88)	0.156
30e	3-NHPhCO	D	35—36 MeCN–H_2O	$C_{50}H_{84}N_2O_{10}S \cdot 1.5H_2O$	64.42 (64.19	9.41 9.27	3.00 2.89)	>100
30f	2-NHPhCO	D	Syrup	$C_{50}H_{84}N_2O_{10}S \cdot 0.5TFA \cdot 2H_2O$	61.36 (61.39	8.94 8.86	2.81 2.94)	>100
30g	4-NHPh-2-FCO	D	60—61 MeCN–H_2O	$C_{50}H_{83}N_2O_{10}SF$[b]				0.078
30h	4-$NHPhSO_2$	D	93—94 MeCN–H_2O	$C_{49}H_{84}N_2O_{11}S_2 \cdot 0.5H_2O$	61.93 (62.06	9.02 8.71	2.95 3.01)	0.39
2								0.078
3a								0.078

a) See Chart. *b*) Determined by SIMS, 923 (MH^+).

Table 3. Physicochemical and Biological Data for Derivatives Modified in the 4-Thiaheptanoic Acid Moiety



Compd.	R^2	R^3	X	R^6	R^7	Spacer	Method[a]	mp (°C) Solvent	Formula	Analysis (%) Calcd (Found) C	H	N	BMC MEC (ng/ml)
30m	$n=1$					Gly–Gly–Gly	C, D	53—55 TFA–MeCN–H_2O	$C_{48}H_{86}N_4O_{12}S$ ·0.25TFA·H_2O	58.85 (58.46)	8.99 (8.75)	5.66 (5.66)	>100
30n	$n=3$					Gly–Gly–Gly	C, D	141—142 TFA–MeCN–H_2O	$C_{50}H_{90}N_4O_{12}S$ ·$2H_2O$	59.61 (59.86)	9.41 (9.23)	5.56 (5.39)	>100
E-**40a**						Gly–Gly–Gly	H	152—153 $CHCl_3$–MeOH–H_2O	$C_{49}H_{86}N_4O_{12}S$	61.61 (61.57)	9.07 (8.93)	5.86 (5.82)	3.9
Z-**40a**						Gly–Gly–Gly	H	142—145 $CHCl_3$–MeOH–H_2O	$C_{49}H_{86}N_4O_{12}S$	61.61 (61.47)	9.07 (9.09)	5.86 (5.58)	7.8
E-**40b**						4-NHCH$_2$PhCO	H	66—68 MeOH–H_2O	$C_{51}H_{84}N_2O_{10}S$ ·H_2O	65.49 (65.63)	9.27 (9.29)	3.00 (2.63)	1.25
30i	Me	H	S	H	H	4-NHPhCO	A, D	50—52 MeCN–H_2O	$C_{51}H_{86}N_2O_{10}S$ ·H_2O	65.35 (65.12)	9.46 (9.21)	2.99 (3.13)	0.039
30j	H	Me	S	H	H	4-NHPhCO	A, D	62—64 MeCN–H_2O	$C_{51}H_{86}N_2O_{10}S$ ·H_2O	65.35 (65.16)	9.46 (9.11)	2.99 (3.27)	1.56
30k	H	H	S	Me	H	4-NHCH$_2$PhCO	B, E	70—71 MeOH–H_2O	$C_{52}H_{88}N_2O_{10}S$ ·$2H_2O$	64.43 (64.28)	9.57 (9.37)	2.89 (2.79)	>100
30l	H	H	S	H	Me	4-NHCH$_2$PhCO	B, E	85 MeOH–H_2O	$C_{52}H_{88}N_2O_{10}S$ ·$2H_20$	64.43 (64.35)	9.57 (9.65)	2.89 (2.85)	5.0
34a	H	H	SO	H	H	4-NHPhCO	G	122—124 MeOH–H_2O	$C_{50}H_{84}N_2O_{11}S$ ·H_2O	63.94 (63.67)	9.23 (8.97)	2.98 (2.92)	12.5
34b	H	H	SO_2	H	H	4-NHPhCO	G	91—93 MeOH–H_2O	$C_{50}H_{84}N_2O_{12}S$ ·H_2O	62.86 (63.15)	9.07 (8.90)	2.93 (2.89)	100
46c	H	H	CH_2	H	H	4-NHPhCO	I	126—127 MeOH–H_2O	$C_{51}H_{86}N_2O_{10}$ ·$0.5H_2O$	68.35 (68.48)	9.78 (9.83)	3.13 (3.20)	0.078
48	H_2N·HCl	H	O	H	H	4-NHPhCO	J	115—117 IPE	$C_{50}H_{85}N_3O_{11}$ ·HCl	63.84 (63.72)	9.21 (9.23)	4.47 (4.42)	0.156
46a	ZNH	H	O	H	H	4-NHPhCO	I	170—171 MeOH–H_2O	$C_{58}H_{91}N_3O_{13}$ ·$0.5H_2O$	66.51 (66.57)	8.85 (8.99)	4.01 (3.76)	6.25
46b	H	H	O	H	H	4-NHPhCO	I	135—136 MeOH–H_2O	$C_{50}H_{84}N_2O_{11}$ ·$0.5H_2O$	66.86 (66.77)	9.54 (9.41)	3.12 (3.18)	3.9
2													0.078
3a													0.078

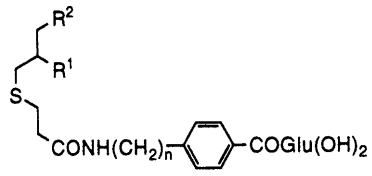
a) See Chart.

The 2-methyl analogue **30i** showed potent activity. However, the introduction of a methyl group at the 3- or 7-position (**30j** or **30l**) resulted in decreased activity, and compound **30k** with a methyl group at the 6-position showed no activity. Oxidation of the sulfur at the 4-position to sulfoxide (**34a**) or sulfone (**34b**) also caused reduction of the activity, but the substitution of oxygen (**46b**, **48**) or methylene (**46c**) for the sulfur did not affect the potent activity of the corresponding compounds (**12f**, **30d**). Interestingly, only compounds with a chain of two carbons connected by a double bond (**40**) or a single bond (**30a**, **c**, **d**) between the sulfur atom and the carbonyl group at the 1-position stimulated the proliferation of bone marrow cells, whereas the compounds with one carbon (**30m**) or a chain of three carbons (**30n**) were inactive. Conversion of either of the ester bonds connecting the fatty acid with the diol function at the 6- and 7-position of 4-thiaheptanoic acid into an ether (**68**) or amide (**56**, **63**) bond resulted in loss of activity. Nevertheless, the carbamoyloxy analogues **30u** and **30v** had potent activity. These findings suggest that the distance between the ester bonds at the 6- and 7-position and the carbonyl group at the 1-position and each functional group are severely restricted for high activity.

With regard to the length of the fatty acid, we found that a length of more than twelve carbons was necessary for potent activity and that a length of sixteen carbons was optimum. Compounds (**30t**, **30s**) having a phenyl or a cyclohexyl ring at the end of the fatty acid were also active (Table 4).

As regards *in vitro* BMC-stimulating activity, the compounds having a 4-aminobenzoyl group as a spacer were found to show superior activity. The *in vivo* hematopoietic activities of the 2-amino analogue **12f**, 2-nonsubstituted analogue **30d** and 2-methyl analogue **30i** were further examined using cyclophosphamide (CY)-induced leukocytopenia in mice. In the CY-induced leukocytopenia model, the peripheral leukocyte counts in

Table 4. Physicochemical and Biological Data for Derivatives Modified in the Lipid Moiety



Compd.	R^1	R^2	n	Method[a]	mp (°C) Solvent	Formula	Analysis (%) Calcd (Found) C	H	N	BMC MED (ng/ml)
30o	$OCO(CH_2)_8CH_3$	$OCO(CH_2)_8CH_3$	0	F	Syrup	$C_{38}H_{60}N_2O_{10}S \cdot 1.0H_2O$	60.45 (60.26)	8.28 8.22	3.71 3.62)	>100
30p	$OCO(CH_2)_{10}CH_3$	$OCO(CH_2)_{10}CH_3$	0	F	Syrup	$C_{42}H_{68}N_3O_{10}S \cdot 0.25TFA \cdot 0.75H_2O$	61.13 (61.12	8.42 7.97	3.35 3.95)	6.25
30q	$OCO(CH_2)_{12}CH_3$	$OCO(CH_2)_{12}CH_3$	0	F	48—49 MeCN–H_2O	$C_{46}H_{76}N_2O_{10}S \cdot 0.25H_2O$	64.72 (65.10	9.03 9.15	3.28 2.81)	0.625
30d	$OCO(CH_2)_{14}CH_3$	$OCO(CH_2)_{14}CH_3$	0	D	110—111 MeCN–H_2O	$C_{50}H_{84}N_2O_{10}S \cdot 1.0H_2O$	65.04 (65.43	9.39 9.28	3.03 2.88)	0.156
***R*-30d**	$OCO(CH_2)_{14}CH_3$	$OCO(CH_2)_{14}CH_3$	0	F	108—109 MeOH–H_2O	$C_{50}H_{84}N_2O_{10}S \cdot 1.0H_2O$	65.04 (65.21	9.39 9.39	3.03 2.94)	0.078
30r	$OCO(CH_2)_{16}CH_3$	$OCO(CH_2)_{16}CH_3$	0	F	54—56 MeCN–H_2O	$C_{54}H_{92}N_2O_{10}S$[b]				0.625
30s	$OCO(CH_2)_{11}$-c-Hex	$OCO(CH_2)_{11}$-c-Hex	0	F	58—60 MeOH–H_2O	$C_{54}H_{88}N_2O_{10}S \cdot 0.5H_2O$	67.12 (67.05	9.28 9.35	2.90 2.87)	0.625
30t	$OCO(CH_2)_{11}Ph$	$OCO(CH_2)_{11}Ph$	0	F	Syrup	$C_{54}H_{76}N_2O_{10}S$[c]				0.078
30u	$OCO(CH_2)_{14}CH_3$	$OCONH(CH_2)_{17}CH_3$	0	F	119—121 $CHCl_3$–MeOH–H_2O	$C_{53}H_{91}N_3O_{10}S \cdot 0.5H_2O$	65.53 (65.45	9.55 9.63	4.33 4.24)	3.13
30v	$OCONH(CH_2)_{17}CH_3$	$OCONH(CH_2)_{17}CH_3$	0	F	146—147 $CHCl_3$–MeOH–H_2O	$C_{56}H_{98}N_4O_{10}S \cdot 1.5H_2O$	64.27 (64.28	9.73 9.55	5.35 5.33)	3.13
68	$O(CH_2)_{15}CH_3$	$O(CH_2)_{15}CH_3$	0	M	76—78 TFA–MeCN–H_2O	$C_{50}H_{88}N_2O_8S \cdot 0.25TFA \cdot 0.5H_2O$	66.30 (66.02	9.83 9.51	3.06 3.29)	>100
56	$NHCO(CH_2)_{14}CH_3$	$OCO(CH_2)_{14}CH_3$	1	K	66—67 MeOH–H_2O	$C_{51}H_{87}N_3O_9S \cdot 0.5H_2O$	66.06 (65.94	9.56 9.32	4.53 4.22)	>100
63	$OCO(CH_2)_{14}CH_3$	$NHCO(CH_2)_{14}CH_3$	1	L	83—85 MeOH–H_2O	$C_{51}H_{87}N_3O_9S \cdot 1.0H_2O$	65.42 (65.47	9.58 9.41	4.49 4.21)	>100
2										0.078
3a										0.078

a) See Chart. *b*) Determined by FAB-MS, 959 $[M-H]^-$. *c*) Determined by HR-MS (FAB); Calcd: 943.5142 $[M-H]^-$, Found: 943.5128.

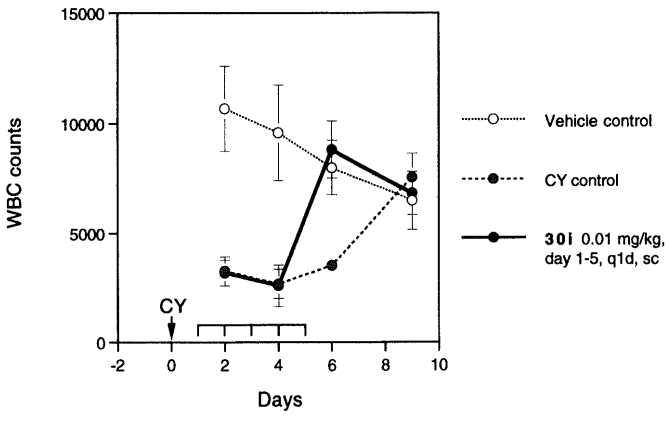
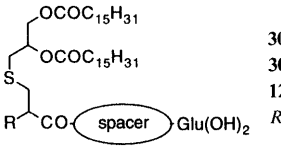


Fig. 3. WBC-Restorine Effect of TAN-1511 Derivatives in the Mouse Leukocytopenia Model

CY-treated mice decreased to 25—30% of the control level on day 4 and recovered to the normal level on day 9 (Fig 3). We examined the minimum effective doses (MED) at which the leukocyte count was restored to the normal level on day 6, three days before spontaneous recovery in CY-treated mice, and the results are shown in Table 5.

In spite of having almost the same potency *in vitro*,

Table 5. *In Vivo* Hematopoietic Activity of TAN-1511 Analogues



Compd.	R	Spacer	MED (mg/kg/day)
30i	Me	4-NHPhCO	0.010
30d	H	4-NHPhCO	0.0078
12f	H_2N	4-NHPhCO	0.0078—0.031
***R*-30d**	H	4-NHPhCO	0.0078
3a	H_2N	Gly–Gly–Gly	0.13
1a		TAN-1511A	1.0

compounds **12f**, **30d** and **30i** with aromatic non-peptide spacers showed more potent *in vivo* activity than the peptide derivatives **1a** and **3a**, with an MED range of 0.0078—0.031 mg/kg/d in comparison with an MED of 1 and 0.13 mg/kg/d, respectively. This result suggests that the introduction of an aromatic spacer in place of the Gly–Gly–Gly sequence might increase the stability of the compound *in vivo*. Since compounds **12f**, **30d** and **30i** were found to show the same activity, we concluded that the substituent at the 2-position had no influence on the activity, and that the simplest compound **30d** was the best

among the three compounds. Compound **30d** is a mixture of diastereoisomers with *RS*-configuration at the 6-position, so we next examined the *in vivo* activity of *R*-**30d**, because the compound with 6-(*R*) configuration was more potent than that with the 6-(*S*)-configuration in the case of synthetic TAN-1511A (**2**).[3] As a result, *R*-**30d** was found to show as potent activity as **30d**.

The previous work on TAN-1511 revealed that TAN-1511A induces the production of granulocyte-colony stimulating factor (G-CSF) and granulocyte macrophage-colony stimulating factor (GM-CSF) in BMC *in vitro* and high CSF activity in serum *in vivo* and that these hematopoietic cytokines contribute to the hematopoietic effects of TAN-1511A.[3] Therefore, the analogues prepared in this study should show hematopoietic activity *in vitro* and *in vivo via* a similar mechanism; that is, the stimulation of bone marrow accessory cells such as stroma cells and macrophages, followed by the induction of hematopoietic cytokines such as G-CSF and GM-CSF.

Many microbial lipopeptides and their derivatives with structures similar to that of TAN-1511 have been reported, and their biological properties have been intensively studied, including, for example, mitogenic effect on B-lymphocytes and activation of macrophages.[8,9] Recently, the novel lipopeptide WS1279, which was isolated from the culture broth of *Streptomyces willmorei* No. 1279, was found to stimulate the proliferation of BMC and to accelerate the recovery of granulocyte counts in mitomycin-induced leukopenic mice.[10] These lipopeptides, including TAN-1511, consist of the 2-amino-6,7-dihydroxy-4-thiaheptanoic acid moiety acylated with long chain fatty acids and a peptide moiety.[3,8-10] Derivatives in which non-peptide spacers have been introduced into the peptide moiety have never before been synthesized. The physicochemical characteristics of these derivatives are considerably different from those of peptide derivatives, and it is therefore very interesting that the derivatives with aromatic non-peptide spacers showed more potent *in vivo* activity than the peptide derivatives.

In conclusion, through the chemical modification of TAN-1511 we determined the following: the amino group at the 2-position of the 4-thiaheptanoic acid frame is not necessary; 4-aminobenzoyl and 4-aminomethylbenzoyl groups can be used as a spacer in place of the Gly–Gly–Gly sequence; and as for the lipid part, it is best that the 6,7-diol groups are acylated with a C_{16} fatty acid. Compound **30d** showed potent hematopoietic activity *in vitro* and *in vivo*.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus without correction. IR spectra were obtained on a JASCO IR-810 or a Horiba FT-200 spectrometer. ^{1}H-NMR spectra were recorded on a Varian Gemini-200 spectrometer; chemical shifts are given in ppm with tetramethylsilane as the internal standard, and coupling constants (J) are measured in hertz (Hz). High-resolution mass spectra (HR-MS) and fast atom bombardment mass spectra (FAB-MS) were measured on a JEOL JMS-AX505W instrument. Column chromatography was carried out using Silica gel 60 (E. Merck, Darmstadt, Germany).

Di-*tert*-butyl 4-(Aminomethyl)benzoyl-L-glutamate Hydrochloride (6e). Typical Procedure A stirred solution of **4e** (1.0 g) and di-*tert*-butyl L-glutamate hydrochloride (1.15 g) in DMF (20 ml) was treated with DEPC (860 mg) at 0 °C, then Et_3N (1.06 g) was added. The reaction mixture was stirred at room temperature for 1 h, then concentrated under reduced pressure and the residue was chromatographed on silica gel (hexane : AcOEt = 2 : 1) to give **5e** (1.86 g, 100%) as a colorless wax. IR (neat): 1720, 1700, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (9H, s), 1.49 (9H, s), 1.90—2.55 (4H, m), 4.43 (2H, d, J=6.0), 4.66 (1H, m), 5.15 (3H, s), 7.01 (1H, d, J=7.0), 7.30—7.40 (7H, m), 7.79 (2H, d, J=8.4).

A mixture of **5e** (1.85 g) and 10% Pd–C (200 mg) in MeOH (13 ml) was hydrogenated until 80 ml of hydrogen gas was consumed. The catalyst was filtered off and a 1 N HCl solution of AcOEt (3.52 ml) was added to the filtrate, and then the solution was concentrated under reduced pressure to give **6e** (1.38 g, 92%) as an amorphous solid. IR (KBr): 3400, 1731, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.41 (9H, s), 1.48 (9H, s), 1.90—2.40 (4H, m), 4.13 (2H, s), 4.59 (1H, m), 7.48 (2H, d, J=8.0), 7.69 (3H, d, J=8.0).

Compounds **6a—d, f, g** were prepared by a similar method to that described for **6e**.

6a: Yield, 95% (96% for **5a**). Colorless amorphous solid. IR (KBr): 3217, 3061, 1732, 1714, 1682, 1666 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (9H, s), 1.43 (9H, s), 1.80—2.20 (2H, m), 2.22—2.38 (2H, m), 3.90—4.16 (6H, m), 4.40 (1H, m), 7.78 (1H, br s), 7.96 (3H, br s), 8.22 (1H, br s), 8.62 (1H, br s).

6b: Yield, 100% (100% for **5b**). Colorless wax. IR (neat): 3270, 1725, 1645 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.39 (2H, m), 1.44 (9H, s), 1.47 (9H, s), 1.50—1.75 (4H, m), 1.75—2.15 (2H, m), 2.15—2.38 (4H, m), 2.80 (2H, t, J=6.6), 3.32 (2H, br s), 4.46 (1H, m), 6.40 (1H, d, J=7.4).

6c: Yield, 83% (85% for **5c**). Colorless oil. IR (neat): 3280, 1720, 1660 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.20—1.40 (6H, m), 1.44 (9H, s), 1.47 (9H, s), 1.52—2.38 (10H, m), 2.38—2.65 (2H, m), 2.73 (2H, t, J=7.0), 4.42—4.55 (1H, m), 6.21 (1H, d, J=8.2).

6d: Yield, 100% (39% for **5d**). Colorless oil. IR (neat): 3280, 1730, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.05—1.40 (16H, m), 1.44 (9H, s), 1.47 (9H, s), 1.55—1.72 (4H, m), 1.72—2.50 (10H, m), 2.68 (2H, t, J=7.0), 3.19 (2H, t, J=7.6), 4.43—4.57 (1H, m), 6.14 (1H, d, J=7.8).

6f: Yield, 94% (73% for **5f**). Colorless powder. IR (KBr): 3425, 3400, 1740, 1720, 1680, 1640, 1610 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.41 (9H, s), 1.48 (9H, s), 1.90—2.55 (4H, m), 3.59 (2H, br s), 4.58—4.72 (1H, m), 7.02—7.15 (1H, m), 7.65 (2H, d, J=8.8), 7.77 (2H, d, J=8.8), 9.65—9.77 (1H, m).

6g: Yield, 67% (94% for **5g**). Colorless solid. IR (KBr): 3400, 1730, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s), 1.47 (9H, s), 1.49 (9H, s), 1.90—2.50 (4H, m), 3.53 (2H, s), 4.41 (2H, s), 4.65 (1H, m), 7.18 (1H, d, J=7.2), 7.70 (2H, d, J=8.0), 7.88 (2H, d, J=8.0).

Di-*tert*-butyl *N*-(6-Aminohexylcarbamoyl)-L-glutamate (6h) *p*-Nitrophenyl chloroformate (971 mg) was added to a stirred mixture of 6-benzyloxycarbonylamino-1-hexylamine (**4h**, 1.2 g) and Et_3N (1.34 ml) in CH_2Cl_2 (10 ml) at 0 °C and the whole was stirred at 0 °C for 1 h. It was then diluted with $CHCl_3$, washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 (10 ml), and di-*tert*-butyl L-glutamate hydrochloride (825 mg), then DMAP (680 mg) and Et_3N (0.77 ml) were added. The reaction mixture was stirred at room temperature for 2 h and concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane : AcOEt = 3 : 2) to give di-*tert*-butyl *N*-[6-(benzyloxycarbonylamino)hexylcarbamoyl]-L-glutamate (**5h**, 1.495 g, 58%) as a colorless solid. IR (KBr): 3350, 1730, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.20—1.38 (8H, m), 1.44 (9H, s), 1.45 (9H, s), 1.70—2.22 (2H, m), 2.33 (2H, m), 3.17 (4H, m), 4.34 (1H, br s), 4.87 (1H, br s), 5.05 (1H, d, J=7.6), 5.10 (2H, s), 7.35 (5H, s).

A mixture of **5h** (1.49 g) and 10% Pd–C (250 mg) in MeOH (20 ml) was hydrogenated at room temperature. The catalyst was filtered off and the filtrate was concentrated under reduced pressure to give **6h** (1.12 g, 100%) as a colorless solid. IR (KBr): 3350, 1730, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.39 (10H, m), 1.43 (9H, s), 1.45 (9H, s), 1.60—2.15 (4H, m), 2.90—3.20 (4H, m), 4.32 (1H, m), 6.01 (2H, m).

Di-*tert*-butyl *N*-(4-Aminobenzoyl)-L-glutamate (8a). Typical Procedure A stirred solution of **7a** (1.525 g) and di-*tert*-butyl L-glutamate hydrochloride (3.66 g) in DMF (30 ml) was treated with DEPC (2.70 g) at 0 °C, then Et_3N (4.40 g) was added and the mixture was stirred at room temperature for 1 h. It was concentrated under reduced pressure and the residue was dissolved in AcOEt. The solution was washed with water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was crystallized from hexane–AcOEt (1 : 1) to give **8a** (3.72 g, 88%) as a colorless solid. IR (KBr): 3356, 1728,

1633 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.41 (9H, s), 1.48 (9H, s), 1.90—2.50 (4H, m), 2.65 (2H, br), 4.66 (1H, m), 6.65 (2H, d, J=8.6), 6.75 (1H, d, J=8.0), 7.65 (2H, d, J=8.6).

Compounds **8b—d** were prepared by a similar method to that described for **8a**.

8b: Yield, 92%. Colorless solid. IR (KBr): 3440, 3360, 1715, 1650, 1620 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (9H, s), 1.49 (9H, s), 1.92—2.53 (4H, m), 3.79 (2H, br s), 4.60—4.73 (1H, m), 6.75—6.83 (1H, m), 6.88 (1H, d, J=7.6), 7.09—7.24 (3H, m).

8c: Yield, 100%. Yellow oil. IR (neat): 3450, 3350, 1715, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (9H, s), 1.49 (9H, s), 1.91—2.52 (4H, m), 4.57—4.69 (1H, m), 5.54 (1H, br s), 6.60—6.72 (2H, m), 6.85 (1H, d, J=7.2), 7.16—7.26 (1H, m), 7.38—7.47 (1H, m).

8d: Yield, 96%. Colorless solid. IR (KBr): 3445, 3395, 3350, 1715, 1620 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (9H, s), 1.49 (9H, s), 1.91—2.48 (4H, m), 4.13 (2H, br s), 4.66—4.80 (1H, m), 6.34 (1H, dd, J=2.2, 14.2), 6.48 (1H, dd, J=2.2, 8.6), 7.18 (1H, dd, J=7.6, 14.0), 7.88 (1H, t, J=8.6).

Di-*tert*-butyl *N*-(4-Aminobenzenesulfonyl)-L-glutamate (8e) 4-Nitrobenzenesulfonyl chloride (2.46 g) was added dropwise to a stirred mixture of pyridine (4 ml) and di-*tert*-butyl L-glutamate hydrochloride (2.96 g) in CH_2Cl_2 (20 ml) at 0 °C and the mixture was stirred at room temperature for 17 h. It was then concentrated under reduced pressure and the residue was dissolved in AcOEt. The solution was washed with water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane : AcOEt = 3 : 1) to give di-*tert*-butyl *N*-(4-nitrobenzenesulfonyl)-L-glutamate (3.78 g, 85%) as a pale yellow solid. IR (KBr): 3430, 3260, 1730 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (9H, s), 1.46 (9H, s), 1.60—1.92 (1H, m), 1.99—2.19 (1H, m), 2.35—2.52 (2H, m), 3.85—4.02 (1H, m), 5.48 (1H, d, J=9.2), 8.00—8.09 (2H, m), 8.30—8.39 (2H, m).

A mixture of di-*tert*-butyl *N*-(4-nitrobenzenesulfonyl)-L-glutamate (3.77 g) and 10% Pd–C (1.0 g) in MeOH (30 ml) was hydrogenated at room temperature until the starting material disappeared. The catalyst was filtered off and the filtrate was concentrated under reduced pressure to give **8e** (3.97 g, 84%) as a colorless solid. IR (KBr): 3470, 3390, 3290, 1730, 1720, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (9H, s), 1.45 (9H, s), 1.53—1.83 (1H, m), 1.92—2.12 (1H, m), 2.40 (2H, t, J=7.8), 3.74 (1H, dt, J=7.8, 9.2), 4.10 (1H, br s), 5.08 (1H, d, J=9.2), 6.59—6.68 (2H, m), 7.54—7.64 (2H, m).

Synthesis of 2-Fmoc-amino-4-thiaheptanoyl-spacer-L-glutamate Derivatives (10). (2*R*,6*R*) Di-*tert*-butyl *N*-{4-[6,7-Bis(hexadecanoyloxy)-2-(9-fluorenylmethoxycarbonylamino)-4-thiaheptanoylaminomethyl]benzoyl}-L-glutamate (10e). Typical Procedure A stirred solution of (2*R*,6*R*) 6,7-bis(hexadecanoyloxy)-2-(9-fluorenylmethyloxycarbonylamino)-4-thiaheptanoic acid[5] (**9**, 200 mg) and di-*tert*-butyl 4-(aminomethyl)benzoyl-L-glutamate (**6e**, 106 mg) in DMF (3 ml) was treated with DEPC (55 mg) at 0 °C, then Et_3N (44 mg) was added and the mixture was stirred at room temperature for 1 h. It was concentrated under reduced pressure and the residue was chromatographed on silica gel ($CHCl_3$: MeOH = 99 : 1) to give **10e** (247 mg, 87%) as a colorless wax. IR (neat): 3300, 1730, 1660 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.25 (48H, s), 1.42 (9H, s), 1.49 (9H, s), 1.40—1.65 (4H, m), 1.95—2.50 (8H, m), 2.77 (2H, d, J=6.6), 2.90—3.00 (2H, m), 4.05—4.55 (8H, m), 4.66 (1H, m), 5.24 (1H, m), 5.78 (1H, br s), 6.98 (1H, br s), 7.02 (1H, d, J=7.4), 7.25—7.45 (6H, m), 7.58 (2H, d, J=7.4), 7.76 (2H, d, J=7.2), 7.78 (2H, d, J=8.4).

Compounds **10a—d, g, h** were prepared by a similar method to that described for **10e**.

10a: Yield, 52%. Colorless oil. IR (neat): 3300, 1735, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.0), 1.25 (52H, s), 1.43 (9H, s), 1.46 (9H, s), 1.47—1.72 (6H, m), 1.72—2.15 (2H, m), 2.15—2.40 (16H, m), 2.79 (2H, d, J=5.8), 2.93 (2H, d, J=6.2), 3.27 (2H, m), 4.05—4.55 (7H, m), 5.23 (1H, br s), 5.83 (1H, d, J=8.2), 6.24 (1H, d, J=8.2), 6.60 (1H, br s), 7.37 (4H, m), 7.60 (2H, d, J=7.0), 7.77 (2H, d, J=7.0).

10b: Yield, 41%. Colorless wax. IR (neat): 3300, 1730, 1685, 1660, 1645 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=7.0), 1.03—1.38 (56H, m), 1.44 (9H, s), 1.47 (9H, s), 1.49—2.40 (12H, m), 2.79 (2H, d, J=5.2), 2.93 (2H, d, J=7.0), 3.17—3.32 (2H, m), 4.00—4.56 (7H, m), 5.17—5.32 (1H, m), 5.78—5.90 (1H, m), 6.19 (1H, d, J=8.0), 6.44—6.56 (1H, m), 7.26—7.46 (4H, m), 7.61 (2H, d, J=7.4), 7.78 (2H, d, J=7.4).

10c: Yield, 19%. Colorless wax. IR (neat): 3280, 1730, 1685, 1645 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=7.0), 0.95—1.38 (64H, m), 1.44 (9H, s), 1.47 (9H, s), 1.50—2.40 (12H, m), 2.79 (2H, d, J=5.2), 2.86—2.96 (1H, m), 3.18—3.32 (2H, m), 4.08—4.55 (6H, m), 5.18—5.33 (1H, m), 5.73—5.85 (1H, m), 6.14 (1H, d, J=7.8), 6.40—6.53 (1H, m), 7.25—7.45 (4H, m), 7.61 (2H, d, J=7.4), 7.78 (2H, d, J=7.0).

10d: Yield, 92%. Colorless oil. IR (neat): 3300, 1730, 1685, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=7.0), 1.25 (52H, s), 1.43 (9H, s), 1.45 (9H, s), 1.73 (8H, m), 1.75—2.20 (2H, m), 2.33 (6H, m), 2.78 (2H, d, J=5.0), 2.94 (2H, d, J=5.2), 3.05—3.50 (4H, m), 4.00—4.50 (9H, m), 4.86 (1H, t, J=6.0), 5.25 (2H, d, J=8.2), 6.08 (1H, br s), 6.71 (1H, br s), 7.26—7.55 (4H, m), 7.61 (2H, d, J=8.0), 7.77 (2H, d, J=8.0).

10g: Yield, 56%. Colorless wax. IR (neat): 3300, 1735, 1730, 1700, 1655, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.6), 1.02—1.84 (48H, m), 1.42 (9H, s), 1.49 (9H, s), 1.84—3.15 (13H, m), 4.00—4.73 (9H, m), 5.15—5.28 (1H, m), 5.80—5.92 (1H, m), 6.92—7.13 (2H, m), 7.26—7.83 (12H, m), 8.53 (1H, br s).

10h: Yield, 87%. Colorless wax. IR (neat): 1730, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.25 (48H, s), 1.40 (1/2 × 9H, s), 1.42 (9H, s), 1.44 (1/2 × 9H, s), 1.49 (9H, s), 1.45—1.70 (4H, m), 2.00—2.50 (8H, m), 2.65—3.20 (4H, m), 3.70—4.50 (8H, m), 4.60—4.80 (3H, m), 5.17 (1H, m), 5.70—5.80 (1H, m), 7.00—7.10 (1H, m), 7.25—7.45 (6H, m), 7.61 (2H, d, J=7.4), 7.75—7.90 (4H, m).

(2*R*,6*R*) Di-*tert*-butyl *N*-{4-[6,7-Bis(hexadecanoyloxy)-2-(9-fluorenylmethoxycarbonylamino)-4-thiaheptanoylamino]benzoyl}-L-glutamate (10f) A stirred solution of **9** (100 mg) and di-*tert*-butyl 4-aminobenzoyl-L-glutamate (**8a**, 85 mg) in pyridine (1.5 ml) was treated dropwise with PCl_3 (0.01 ml) and the reaction mixture was stirred at room temperature for 3 h. It was then concentrated under reduced pressure and the residue was chromatographed on silica gel ($CHCl_3$: hexane = 7 : 1) to give **10f** (107 mg, 84%) as a colorless wax. IR (KBr): 3300, 1730, 1685, 1660, 1640, 1600 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=7.0), 1.10—1.38 (48H, m), 1.42 (9H, s), 1.49 (9H, s), 1.55—1.83 (4H, m), 1.90—2.58 (8H, m), 2.75—2.86 (2H, m), 3.02 (2H, d, J=6.6), 4.12—4.72 (8H, m), 5.24—5.49 (1H, m), 5.74—5.86 (1H, m), 7.01 (2H, d, J=7.6), 7.32 (2H, d, J=7.6), 7.35—7.87 (8H, m), 8.70 (1H, br s).

(2*R*,6*R*) *N*-{4-[2-Amino-6,7-bis(hexadecanoyloxy)-4-thiaheptanoylaminomethyl]benzoyl}-L-glutamic Acid Hydrochloride (12e). Typical Procedure Piperidine (2 ml) was added to a stirred solution of **10e** (243 mg) in CH_2Cl_2 (0.5 ml) at 0 °C and the mixture was stirred at room temperature for 2 h. It was then concentrated under reduced pressure and the residue was chromatographed on silica gel ($CHCl_3$) to give (2*R*,6*R*) di-*tert*-butyl *N*-{4-[2-amino-6,7-bis(hexadecanoyloxy)-4-thiaheptanoylaminomethyl]benzoyl}-L-glutamate (**11e**, 195 mg, 97%) as a colorless wax. IR (neat): 3350, 1730, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.25 (48H, s), 1.42 (9H, s), 1.49 (9H, s), 1.45—1.65 (4H, m), 1.73 (2H, br s), 1.90—2.50 (8H, m), 2.75 (2H, d, J=6.4), 2.81 (1H, dd, J=8.4, 13.4), 3.14 (1H, dd, J=3.8, 13.4), 3.57 (1H, dd, J=4.0, 8.4), 4.14 (1H, dd, J=6.2, 12.0), 4.36 (1H, dd, J=3.2, 12.0), 4.49 (2H, d, J=6.2), 4.66 (1H, m), 5.16 (1H, m), 7.02 (1H, d, J=7.4), 7.35 (2H, d, J=8.2), 7.79 (2H, d, J=8.2), 7.83 (1H, br s).

A solution of **11e** (112 mg) in 4 N HCl in ethyl acetate (2 ml) was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and dried *in vacuo* to give **12e** (88 mg, 85%) as a colorless powder. IR (KBr): 3450, 1730, 1710, 1690, 1670, 1640, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$–TFA) δ: 0.88 (6H, t, J=6.8), 1.25 (48H, s), 1.40—1.65 (4H, m), 2.00—3.30 (12H, m), 4.00—4.80 (6H, m), 5.22 (1H, m), 7.00—8.10 (6H, m).

Compounds **12a—d, f, g** were prepared by a similar method to that described for **12e**.

12a: Yield, 100% (83% for **11a**). Colorless powder. IR (KBr): 3320, 1740, 1665 cm^{-1}. ^{1}H-NMR ($CDCl_3$–TFA) δ: 0.88 (6H, t, J=6.6), 1.26 (52H, s), 1.57 (6H, m), 1.90—2.20 (2H, m), 2.37 (8H, m), 2.60 (2H, t, J=6.4), 2.74 (2H, d, J=7.0), 3.10 (2H, m), 4.13 (1H, dd, J=6.0, 12.0), 4.28—4.50 (2H, m), 4.65 (1H, m), 5.17 (1H, m), 7.08 (1H, d, J=7.5), 7.42 (1H, br s).

12b: Yield, 100% (93% for **11b**). Colorless powder. IR (KBr): 3250, 1740, 1715, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.03—1.50 (56H, m), 1.50—1.80 (6H, m), 1.80—3.55 (16H, m), 4.03—4.70 (4H, m), 5.10—5.35 (1H, m).

12c: Yield, 100% (71% for **11c**). Colorless powder. IR (KBr): 3250, 1740, 1720, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.03—1.50 (64H, m), 1.50—1.80 (6H, m), 1.80—3.55 (16H, m), 4.03—4.70 (4H, m), 5.10—5.35 (1H, m).

12d: Yield, 100% (83% for **11d**). Colorless powder. IR (KBr): 3400, 1740, 1700, 1670 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.2), 1.26 (52H, s), 1.59 (8H, m), 2.00—2.20 (2H, m), 2.30 (6H, m), 2.78 (2H, m),

3.00—3.45 (3H, m), 3.52 (2H, m), 3.80—4.25 (6H, m), 4.34 (2H, m), 5.20 (1H, br s), 7.57 (1H, br s), 7.71 (1H, br s).

12f: Yield, 100% (68% for **11f**). Colorless powder. IR (KBr): 3400, 1750, 1630, 1610 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.02—1.48 (48H, s), 1.48—1.68 (4H, m), 2.00—2.85 (13H, m), 4.05—5.32 (4H, m), 7.40—8.00 (4H, m).

12g: Yield, 100% (72% for **11g**). Colorless powder. IR (KBr): 3400, 1735 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 0.93—1.47 (48H, s), 1.47—1.70 (4H, m), 1.70—3.32 (16H, m), 3.70—4.60 (4H, m), 4.60—4.78 (1H, m), 5.17—5.32 (1H, m), 7.47—7.90 (4H, m).

Di-*tert*-butyl *N*-{4-[2-Acetoxy-6(*R*),7-bis(hexadecanoyloxy)-4-thiaheptanoylaminomethyl]benzoyl}-L-glutamate (13) A solution of sodium nitrite (13 mg) in water (0.3 ml) was added to a solution of **11e** (243 mg) in AcOH (6 ml) and the mixture was stirred at room temperature overnight. It was concentrated under reduced pressure and the residue was chromatographed on silica gel (hexane : AcOEt = 2 : 1) to give **13** (94 mg, 54%) as a colorless wax. IR (neat): 3300, 1730, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=7.0), 1.26 (48H, s), 1.42 (9H, s), 1.49 (9H, s), 1.50—1.70 (4H, m), 1.90—2.50 (8H, m), 2.16 (3H, s), 2.70—2.90 (2H, m), 2.95—3.25 (2H, m), 4.00—4.70 (5H, m), 5.18 (1H, m), 5.39 (1H, m), 6.80—6.95 (1H, m), 7.07 (1H, d, J=7.4), 7.35 (2H, d, J=8.0), 7.79 (2H, d, J=8.0).

***N*-{4-[2-Acetoxy-6(*R*),7-bis(hexadecanoyloxy)-4-thiaheptanoylaminomethyl]benzoyl}-L-glutamic Acid (14)** A solution of **13** (92 mg) in CH_2Cl_2 (0.5 ml) was treated with TFA (2 ml) and the mixture was stirred at room temperature for 1 h. It was then concentrated under reduced pressure and the residue was crystallized from MeOH–H_2O, washed with water and dried *in vacuo* to give **14** (65 mg, 79%) as a colorless powder. IR (KBr): 3344, 1727, 1647 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.25 (48H, s), 1.50—1.70 (4H, m), 2.00—2.35 (6H, m), 2.15 (3H, s), 2.40—2.55 (2H, m), 2.70—2.80 (2H, m), 2.95—3.15 (2H, m), 4.00—4.70 (5H, m), 5.18 (1H, m), 5.37 (1H, m), 7.24 (2H, d, J=8.2), 7.50—7.80 (2H, m), 7.67 (2H, d, J=8.2).

Di-*tert*-butyl *N*-{4-[[3(*R*)-[2(*R*),3-Bis(hexadecanoyloxy)propyl]thiomethyl]-2,5-diketopiperazinyl]methyl]benzoyl}-L-glutamate (15) Piperidine (1 ml) was added to a stirred solution of **10h** (130 mg) in CH_2Cl_2 (0.5 ml) and the mixture was stirred at room temperature for 1 h, then concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane : AcOEt = 1 : 1) to give **15** (68 mg, 67%) as a colorless wax. IR (neat): 1730, 1660 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.25 (48H, s), 1.42 (9H, s), 1.49 (9H, s), 1.50—1.70 (4H, m), 1.95—2.50 (8H, m), 2.76 (2H, d, J=6.4), 3.02 (1H, dd, J=7.4, 14.0), 3.24 (1H, dd, J=3.4, 14.0), 3.83 (1H, d, J=17.6), 3.97 (1H, d, J=17.6), 4.13 (1H, dd, J=6.4, 12.0), 4.25 (1H, m), 4.36 (1H, dd, J=3.4, 12.0), 4.65 (2H, s), 4.67 (1H, m), 5.12 (1H, m), 6.60 (1H, s), 7.08 (1H, d, J=7.6), 7.35 (2H, d, J=8.2), 7.83 (2H, d, J=8.2).

***N*-{4-[[3(*R*)-[2(*R*),3-Bis(hexadecanoyloxy)propyl]thiomethyl]-2,5-diketopiperazinyl]methyl]benzoyl}-L-glutamic Acid (16)** Compound **16** (51 mg, 85%) was prepared from **15** (112 mg) by a similar method to that described for **14**. Colorless powder. IR (KBr): 1735, 1710, 1680, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.6), 1.25 (48H, s), 1.40—1.65 (4H, m), 2.00—2.60 (8H, m), 2.65—2.80 (2H, m), 3.00—3.20 (2H, m), 3.65—4.20 (4H, m), 4.30—4.40 (2H, m), 4.60—4.85 (2H, m), 5.12 (1H, m), 7.20—7.55 (2H, m), 7.60—7.90 (3H, m).

Di-*tert*-butyl *N*-{6-[5(*R*)-[[2(*R*),3-Bis(hexadecanoyloxy)propyl]thiomethyl]imidazolin-2,4-dion-3-yl]hexanoyl}-L-glutamate (17) *p*-Nitrophenyl chloroformate (13 mg) was added to a mixture of **11a** (60 mg) and Et_3N (20 mg) in CH_2Cl_2 (1 ml) and the mixture was stirred at room temperature for 2 h, then concentrated under reduced pressure. The residue was chromatographed on silica gel ($CHCl_3$) to give **17** (43 mg, 70%) as a colorless solid. IR (KBr): 3300, 1730, 1710, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.25 (50H, s), 1.44 (9H, s), 1.47 (9H, s), 1.64 (8H, m), 1.80—2.15 (2H, m), 2.15—2.40 (8H, m), 2.72—2.88 (3H, m), 3.16 (1H, dd, J=3.6, 14.2), 3.50 (2H, t, J=7.0), 4.08—4.30 (2H, m), 4.30—4.56 (2H, m), 5.12 (1H, m), 5.97 (1H, s), 6.19 (1H, d, J=8.4).

***N*-{6-[5(*R*)-[[2(*R*),3-Bis(hexadecanoyloxy)propyl]thiomethyl]imidazolin-2,4-dion-3-yl]hexanoyl}-L-glutamic Acid (18)** Compound **18** (30 mg, 100%) was prepared from **17** (43 mg) by a similar method to that described for **14**. Colorless powder. IR (KBr): 3350, 1730, 1710, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (6H, s), 1.26 (50H, s), 1.63 (8H, s), 2.00 (2H, br s), 2.34 (8H, m), 2.70—2.95 (4H, m), 3.00—3.20 (2H, m), 4.10—4.60 (4H, m), 5.15 (1H, br s).

Synthesis of 6,7-Dihydroxy-4-thiaheptanoic Acid Esters (24). Method A, *tert*-Butyl 6,7-Dihydroxy-4-thiaheptanoate (24a). Typical Procedure One drop of Et_3N was added to a mixture of thioglycerol (**20**, 15.0 g) and *tert*-butyl acrylate (**19a**, 15.0 g) and the whole was stirred at room temperature for 30 min. It was then diluted with AcOEt, washed with water, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give **24a** (25.79 g, 93%) as a colorless oil. IR (neat): 3380, 2970, 2920, 1720, 1360, 1245, 1145 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (9H, s), 2.40—2.85 (7H, m), 3.15—3.30 (1H, m), 3.50—3.65 (1H, m), 3.70—3.90 (2H, m).

tert-Butyl 6,7-dihydroxy-2-methyl-4-thiaheptanoate (**24b**) and *tert*-butyl 6,7-dihydroxy-3-methyl-4-thiaheptanoate (**24c**) were prepared by a similar method to that described for **24a**.

24b: Yield, 84%. Colorless oil. IR (neat): 3400, 1720 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.21 (3H, d, J=6.6), 1.47 (9H, s), 2.50—2.90 (5H, m), 3.50—3.63 (1H, m), 3.68-3.88 (2H, m).

24c: Yield, 73%. Colorless oil. IR (neat): 3400, 1725 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.34 (3H, d, J=6.8), 1.47 (9H, s), 2.34—2.84 (4H, m), 3.11—3.34 (1H, m), 3.50—3.93 (3H, m).

Method B, Methyl 6,7-Dihydroxy-6-methyl-4-thiaheptanoate (24d). Typical Procedure Methyl 3-mercaptopropionate (**21a**, 10 ml) and 28% sodium methylate (1.20 ml) were added to a stirred solution of 2-methylglycidol (**22b**, 5.0 g) in DMF (20 ml), and the mixture was stirred at room temperature for 3 d. It was then poured into 1 N HCl and extracted with AcOEt. The organic solution was successively washed with water, aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4 and concentrated *in vacuo* to give **24d** (4.80 g, 41%) as a pale yellow oil. IR (neat): 3404, 1736 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.22 (3H, s), 2.25 (1H, t, J=6.2), 2.64 (2H, t, J=6.8), 2.72 (1H, s), 2.78 (1H, s), 2.80 (1H, s), 2.87 (2H, t, J=6.8), 3.50 (1H, d, J=3.4), 3.55 (1H, d, J=3.4), 3.71 (3H, s).

Methyl 6,7-dihydroxy-4-thiaoctanoate (**24e**) was prepared by a similar method to that described for **24d**. Yield, 55%. Colorless oil. IR (neat): 3419, 1732 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (1/2 × 3H, d, J=6.6), 1.22 (1/2 × 3H, d, J=6.2), 1.84 (2H, br s), 2.60—2.68 (3H, m), 2.73—2.88 (3H, m), 3.40—3.65 (2H, m), 3.72 (3H, s).

Method C, *p*-Methoxybenzyl 6,7-Dihydroxy-4-thiaheptanoate (24f). Typical Procedure A mixture of 3-bromo-1,2-propanediol (**23**, 4.56 g), *p*-methoxybenzyl 3-mercaptopropionate (**21b**, 2.25 g) and Et_3N (3.5 ml) in DMF (20 ml) was stirred at 80 °C for 24 h. The reaction mixture was concentrated under reduced pressure and the residue was dissolved in AcOEt. The organic solution was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed on silica gel (AcOE) to give **24f** (2.0 g, 67%) as a colorless solid. IR (KBr): 3400, 1725, 1610 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.52—2.89 (6H, m), 3.43—3.95 (3H, m), 3.81 (3H, s), 5.08 (2H, s), 6.82—6.96 (2H, m), 7.25—7.40 (2H, m).

p-Methoxybenzyl 5,6-dihydroxy-3-thiahexanoate (**24g**) and *p*-methoxybenzyl 7,8-dihydroxy-5-thiaoctanoate (**24h**) were prepared by a similar method to that described for **24f**.

24g: Yield, 41%. Colorless oil. IR (neat): 3400, 1730, 1660, 1610 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.67 (1H, dd, J=8.0, 14.2), 2.80 (1H, dd, J=4.2, 14.2), 3.31 (2H, s), 3.43—3.86 (3H, m), 3.82 (3H, s), 5.12 (2H, s), 6.84—6.94 (2H, m), 7.25—7.36 (2H, m).

24h: Yield, 59%. Colorless oil. IR (neat): 3400, 1720, 1610 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.82—2.03 (2H, m), 2.40—2.75 (6H, m), 3.44—3.83 (3H, m), 3.81 (3H, s), 5.06 (2H, s), 6.84—6.94 (2H, m), 7.24—7.35 (2H, m).

Method D, 6,7-Bis(hexadecanoyloxy)-4-thiaheptanoic Acid (26a). Typical Procedure Palmitoyl chloride (4.66 g) was added to a stirred mixture of **24a** (2.0 g) and DMAP (2.27 g) in CH_2Cl_2 (20 ml), and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure and the residue was chromatographed on silica gel (hexane : AcOEt = 10 : 1) to give *tert*-butyl 6,7-bis(hexadecanoyloxy)-4-thiaheptanoate **25a** (4.848 g, 80%) as a colorless oil. IR (neat): 1725, 1245, 1150 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.26 (48H, s), 1.45 (9H, s), 1.50—1.70 (4H, m), 2.31 (4H, t, J=7.4), 2.52 (2H, t, J=6.8), 2.70—2.85 (4H, m), 4.19 (1H, dd, J=5.8, 12.0), 4.36 (1H, dd, J=3.4, 12.0), 5.16 (1H, m).

A stirred solution of **25a** (4.84 g) in CH_2Cl_2 (1 ml) was treated with TFA (4 ml) and the mixture was stirred at room temperature for 1 h, then concentrated under reduced pressure. The residual solid was washed with hexane to give **26a** (4.46 g, 100%) as a colorless solid. IR (KBr): 3450, 1735, 1700 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t. J=6.8), 1.12—1.43 (48H, m), 1.51—1.74 (4H, m), 2.25—2.38 (4H, m), 2.62—2.92 (6H, m), 4.17 (1H, dd, J=5.9, 11.8), 4.37 (1H, dd, J=3.5, 11.8), 5.08—5.21 (1H, m).

Compounds **25b—f** and **26b—e** were prepared by similar methods to those described for **25a** and **26a**, respectively.

tert-Butyl 6,7-Bis(hexadecanoyloxy)-2-methyl-4-thiaheptanoate (**25b**): Yield, 91%. Colorless oil. IR (neat): 1730, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.03—1.40 (51H, m), 1.45 (9H, s), 1.50—1.70 (4H, m), 2.31 (4H, t, J=7.4), 2.45—2.90 (5H, m), 4.18 (1H, dd, J=5.8, 11.8), 4.36 (1H, dd, J=3.4, 11.8), 5.08—5.23 (1H, m).

tert-Butyl 6,7-Bis(hexadecanoyloxy)-3-methyl-4-thiaheptanoate (**25c**): Yield, 99%. Colorless oil. IR (neat): 1730 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.05—1.41 (51H, m), 1.46 (9H, s), 1.52—1.72 (4H, m), 2.25—3.32 (9H, m), 4.12—4.42 (2H, m), 5.07—5.22 (1H, m).

p-Methoxybenzyl 6,7-Bis(hexadecanoyloxy)-4-thiaheptanoate (**25d**): Yield, 45%. Colorless solid. IR (KBr): 1730, 1620 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.12—1.48 (48H, m), 1.48—1.72 (4H, m), 2.31 (4H, t, J=7.6), 2.57—2.90 (6H, m), 3.81 (3H, s), 4.16 (1H, dd, J=5.8, 12.0), 4.35 (1H, dd, J=3.6, 12.0), 5.08 (2H, s), 5.04—5.20 (1H, m), 6.83—6.93 (2H, m), 7.24—7.33 (2H, m).

p-Methoxybenzyl 5,6-Bis(hexadecanoyloxy)-3-thiahexanoate (**25e**): Yield, 78%. Colorless solid. IR (KBr): 1740 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.03—1.48 (48H, m), 1.48—1.72 (4H, m), 2.30 (4H, t, J=8.0), 2.70—2.93 (2H, m), 3.24 (1H, d, J=14.8), 3.33 (1H, d, J=14.8), 3.81 (3H, s), 4.12 (1H, dd, J=5.8, 12.0), 4.30 (1H, dd, J=3.6, 12.0), 5.10 (2H, s), 5.13—5.26 (1H, m), 6.83—6.93 (2H, m), 7.23—7.35 (2H, m).

p-Methoxybenzyl 7,8-Bis(hexadecanoyloxy)-5-thiaoctanoate (**25f**): Yield, 84%. Colorless solid. IR (KBr): 1735 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.9), 1.10—1.42 (48H, m), 1.42—1.70 (4H, m), 1.91 (2H, m), 2.31 (4H, t, J=7.6), 2.45 (2H, t, J=7.3), 2.59 (2H, t, J=7.3), 2.67 (2H, d, J=6.6), 3.81 (3H, s), 4.16 (1H, dd, J=6.0, 11.9), 4.35 (1H, dd, J=3.4, 11.9), 5.05 (2H, s), 5.12 (1H, m), 6.83—6.92 (2H, m), 7.23—7.33 (2H, m).

6,7-Bis(hexadecanoyloxy)-2-methyl-4-thiaheptanoic Acid (**26b**): Yield, 100%. Colorless solid. IR (KBr): 1730 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.03—1.48 (51H, m), 1.48—1.72 (4H, m), 2.31 (4H, t, J=7.8), 2.32 (2H, t, J=7.8), 2.62—3.00 (5H, m), 4.16 (1H, dd, J=6.0, 12.0), 4.37 (1H, dd, J=3.2, 12.0), 5.07—5.22 (1H, m).

6,7-Bis(hexadecanoyloxy)-3-methyl-4-thiaheptanoic Acid (**26c**): Yield, 100%. Colorless solid. IR (KBr): 3450, 1730 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.6), 1.03—1.50 (51H, m), 1.50—1.74 (4H, m), 2.32 (4H, t, J=7.8), 2.37—2.86 (3H, m), 2.76 (2H, d, J=6.6), 4.18 (1H, dd, J=4.6, 11.8), 4.37 (1H, dd, J=3.4, 11.8), 5.07—5.23 (1H, m).

5,6-Bis(hexadecanoyloxy)-3-thiahexanoic Acid (**26d**): Yield, 90%. Colorless solid. IR (KBr): 1730, 1720, 1690 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.6), 1.03—1.50 (48H, m), 1.50—1.75 (4H, m), 2.32 (2H, t, J=7.6), 2.33 (2H, t, J=7.0), 2.70—3.02 (2H, m), 3.30 (2H, s), 4.10—4.43 (2H, m), 5.15—5.33 (1H, m).

7,8-Bis(hexadecanoyloxy)-5-thiaoctanoic Acid (**26e**): Yield, 91%. Colorless solid. IR (KBr): 1740, 1710 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.02—1.46 (48H, m), 1.46—1.72 (4H, m), 1.93 (2H, m), 2.32 (2H, t, J=7.8), 2.33 (2H, t, J=7.8), 2.49 (2H, t, J=7.1), 2.63 (2H, t, J=7.1), 2.70 (2H, d, J=6.6), 4.18 (1H, dd, J=6.2, 12.0), 4.38 (1H, dd, J=3.4, 12.0), 5.15 (1H, ddd, J=3.4, 6.2, 6.6).

Di-*tert*-butyl *N*-{*N*-[6,7-Bis(hexadecanoyloxy)-4-thiaheptanoyl]glycylglycylglycyl}-L-glutamate (29a) A stirred solution of **26a** (197 mg) and **6a** (168 mg) in DMF (3 ml) was treated with DEPC (73 mg) at 0 °C, then Et_3N (65 mg) was added and the mixture was stirred at room temperature for 1 h. It was then concentrated under reduced pressure and the residue was chromatographed on silica gel ($CHCl_3$: MeOH = 30 : 1) to give **29a** (291 mg, 91%) as a colorless wax. IR (KBr): 3330, 1740, 1700, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.6), 1.11—1.40 (48H, m), 1.44 (9H, s), 1.47 (9H, s), 1.51—1.70 (4H, m), 1.82—2.23 (2H, m), 2.31 (4H, t, J=7.8), 2.59 (2H, t, J=7.0), 2.73 (2H, d, J=6.6), 2.91 (2H, t, J=7.0), 3.88—4.35 (7H, m), 4.35—4.54 (2H, m), 5.07—5.25 (1H, m), 7.00—7.45 (4H, m).

Compounds **29b, c, m, n** were prepared by a similar method to that described for **29a**.

Di-*tert*-butyl *N*-{8-[6,7-Bis(hexadecanoyloxy)-4-thiaheptanoylamino]-octanoyl}-L-glutamate (**29b**): Yield, 81%. Colorless wax. IR (KBr): 1730, 1645 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.09—1.40 (56H, m), 1.45 (9H, s), 1.47 (9H, s), 1.50—2.38 (12H, m), 2.32 (2H, t, J=8.0), 2.33 (2H, t, J=7.4), 2.46 (2H, t, J=7.4), 2.72 (2H, d, J=6.6), 2.88 (2H, t, J=7.0), 3.24 (2H, m), 4.15 (1H, dd, J=6.4, 12.0), 4.40 (1H, dd, J=3.4, 12.0), 4.40—4.55 (1H, m), 5.08—5.24 (1H, m), 5.82 (1H, t, J=5.6), 6.15 (1H, d, J=8.0).

Di-*tert*-butyl *N*-{4-[6,7-Bis(hexadecanoyloxy)-4-thiaheptanoylaminomethyl]benzoyl}-L-glutamate (**29c**): Yield, 99%. Colorless wax. IR (neat): 3300, 1730, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.25 (48H, s), 1.42 (9H, s), 1.49 (9H, s), 1.50—1.70 (4H, m), 1.95—2.50 (8H, m), 2.55 (2H, t, J=7.4), 2.72 (2H, d, J=6.8), 2.93 (2H, t, J=7.4), 4.13 (1H, dd, J=6.4, 12.0), 4.38 (1H, dd, J=3.2, 12.0), 4.50 (2H, d, J=5.8), 4.66 (1H, m), 5.15 (1H, m), 6.32 (1H, br t, J=5.8), 7.04 (1H, d, J=7.6), 7.36 (2H, d, J=8.2), 7.79 (2H, d, J=8.2).

Di-*tert*-butyl *N*-{*N*-[5,6-Bis(hexadecanoyloxy)-3-thiahexanoyl]glycylglycylglycyl}-L-glutamate (**29m**): Yield, 67%. Colorless solid. IR (KBr): 3360, 1730, 1670 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.10—1.39 (48H, s), 1.44 (9H, s), 1.47 (9H, s), 1.50—1.70 (4H, m), 1.77—2.43 (4H, m), 2.31 (2H, t, J=8.0), 2.33 (2H, t, J=7.6), 2.82 (2H, d, J=6.4), 3.33 (2H, s), 3.88—4.20 (6H, m), 4.14 (1H, dd, J=6.4, 11.8), 4.25 (1H, dd, J=3.2, 11.8), 4.38—4.53 (1H, m), 5.12—5.30 (1H, m), 6.98—7.60 (4H, m).

Di-*tert*-butyl *N*-{*N*-[7,8-Bis(hexadecanoyloxy)-5-thiaoctanoyl]glycylglycylglycyl}-L-glutamate (**29n**): Yield, 76%. Colorless solid. IR (KBr): 3280, 1740, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.6), 1.02—1.40 (48H, s), 1.44 (9H, s), 1.46 (9H, s), 1.51—1.70 (4H, m), 1.70—2.56 (4H, m), 2.31 (2H, t, J=7.8), 2.32 (2H, t, J=7.8), 2.41 (2H, t, J=7.2), 2.62 (2H, t, J=6.8), 2.69 (2H, t, J=6.8), 3.90—4.55 (9H, m), 5.08—5.23 (1H, m), 6.90—7.00 (1H, m), 7.21—7.32 (1H, m), 7.39 (1H, d, J=7.6), 7.50—7.61 (1H, m).

Di-*tert*-butyl *N*-{4-[6,7-Bis(hexadecanoyloxy)-4-thiaheptanoylamino]-benzoyl}-L-glutamate (**29d**) A stirred solution of **26a** (100 mg) and **8a** (115 mg) in pyridine (10 ml) was treated dropwise with PCl_3 (25 mg) and the mixture was stirred at room temperature for 1 h, then concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane : AcOEt = 3 : 2) to give **29d** (120 mg, 77%) as a colorless wax. IR (KBr): 3350, 1730 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.6), 1.03—1.37 (48H, m), 1.42 (9H, s), 1.49 (9H, s), 1.53—1.80 (4H, m), 1.90—3.12 (14H, m), 4.15 (1H, dd, J=6.8, 12.0), 4.46 (1H, dd, J=3.0, 12.0), 4.58—4.72 (1H, m), 5.08—5.23 (1H, m), 7.00 (1H, d, J=7.4), 7.60 (2H, d, J=8.8), 7.80 (2H, d, J=8.8), 8.15 (1H, br s).

Compounds **29e—j** were prepared by a similar method to that described for **29d**.

Di-*tert*-butyl *N*-{3-[6,7-Bis(hexadecanoyloxy)-4-thiaheptanoylamino]-benzoyl}-L-glutamate (**29e**): Yield, 80%. Colorless wax. IR (KBr): 3350, 1735, 1645 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.6), 1.02—1.38 (48H, m), 1.42 (9H, s), 1.49 (9H, s), 1.53—1.80 (4H, m), 1.92—3.09 (12H, m), 4.16 (1H, dd, J=6.4, 12.0), 4.45 (1H, dd, J=3.2, 12.0), 4.60—4.72 (1H, m), 5.10—5.25 (1H, m), 7.04 (1H, d, J=7.8), 7.37—7.94 (4H, m), 8.02 (1H, br s).

Di-*tert*-butyl *N*-{2-[6,7-Bis(hexadecanoyloxy)-4-thiaheptanoylamino]-benzoyl}-L-glutamate (**29f**): Yield, 52%. Colorless wax. IR (KBr): 3400, 1740, 1645 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.81 (6H, t, J=6.6), 1.03—1.38 (48H, m), 1.43 (9H, s), 1.51 (9H, s), 1.53—1.75 (4H, m), 1.95—2.55 (4H, m), 2.31 (2H, t, J=7.6), 2.32 (2H, t, J=7.8), 2.70 (2H, t, J=8.8), 2.77 (2H, t, J=6.6), 2.96 (2H, t, J=8.8), 4.18 (1H, dd, J=5.8, 11.8), 4.37 (1H, dd, J=3.6, 11.8), 4.52—4.66 (1H, m), 5.10—5.25 (1H, m), 7.02—7.14 (1H, m), 7.20 (2H, d, J=6.6), 7.42—7.63 (2H, m), 8.59—8.66 (1H, m).

Di-*tert*-butyl *N*-{4-[6,7-Bis(hexadecanoyloxy)-4-thiaheptanoylamino]-2-fluorobenzoyl}-L-glutamate (**29g**): Yield, 87%. Colorless wax. IR (KBr): 1730 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.00—1.38 (48H, m), 1.42 (9H, s), 1.50 (9H, s), 1.53—1.72 (4H, m), 1.85—3.15 (14H, m), 4.14 (1H, dd, J=7.0, 12.0), 4.49 (1H, dd, J=3.0, 12.0), 4.67—4.81 (1H, m), 5.10—5.23 (1H, m), 7.16 (1H, dd, J=2.2, 8.8), 7.32 (1H, dd, J=7.2, 13.6), 7.81 (1H, dd, J=2.2, 14.4), 8.02 (1H, t, J=8.8), 8.26 (1H, br s).

Di-*tert*-butyl *N*-{4-[6,7-Bis(hexadecanoyloxy)-4-thiaheptanoylamino]-benzenesulfonyl}-L-glutamate (**29h**): Yield, 89%. Colorless wax. IR (KBr): 3370, 3270, 1730, 1700 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=7.0), 1.12—1.41 (48H, m), 1.45 (9H, s), 1.63 (9H, s), 1.51—3.18 (14H, m), 3.80 (1H, dt, J=4.7, 9.2), 4.13 (1H, dd, J=7.0, 12.1), 4.50 (1H, dd, J=3.1, 12.1), 5.09—5.23 (1H, m), 5.19 (1H, d, J=9.2), 7.66—7.81 (4H, m), 8.25 (1H, br s).

Di-*tert*-butyl *N*-{4-[6,7-Bis(hexadecanoyloxy)-2-methyl-4-thiaheptanoylamino]benzoyl}-L-glutamate (**29i**): Yield, 62%. Colorless wax. IR (neat): 1750 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.03—1.53 (51H, m), 1.42 (9H, s), 1.49 (9H, s), 1.53—1.82 (4H, m), 1.95—3.22 (13H, m), 4.00—5.30 (4H, m), 6.94—7.03 (1H, m), 7.64—8.53 (5H, m).

Di-*tert*-butyl *N*-{4-[6,7-Bis(hexadecanoyloxy)-3-methyl-4-thiaheptanoylamino]benzoyl}-L-glutamate (**29j**): Yield, 59%. Colorless wax. IR

(neat): 3350, 1730 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.03—1.53 (51H, m), 1.42 (9H, s), 1.49 (9H, s), 1.53—1.82 (4H, m), 1.94—3.50 (13H, m), 4.08—5.27 (4H, m), 6.93—7.20 (1H, m), 7.57—8.40 (6H, m).

Method E, Di-*tert*-butyl *N*-{4-[6,7-Bis(hexadecanoyloxy)-6-methyl-4-thiaheptanoylaminomethyl]benzoyl}-L-glutamate (29k). Typical Procedure Water (5 ml) and 28% sodium methylate (6.0 ml) were added to a stirred solution of **24d** (4.80 g) in MeOH (20 ml) and the mixture was stirred at room temperature overnight. It was then adjusted to pH 1 with concentrated HCl and extracted with AcOEt. The organic solution was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to give 6,7-dihydroxy-6-methyl-4-thiaheptanoic acid (**27a**; 4.33 g, 97%) as a colorless oil. IR (neat): 3315, 1718, 1406, 1342, 1248, 1128, 1049 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.23 (3H, s), 2.65—2.88 (8H, m), 3.53 (2H, m).

A stirred solution of **27a** (75 mg) and **6e** (182 mg) in DMF (3 ml) was treated with DEPC (101 mg) at 0 °C, then Et_3N (0.20 ml) was added and the mixture was stirred at room temperature overnight. It was then concentrated under reduced pressure and the residue was dissolved in AcOEt. The organic solution was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residual **28a** was dissolved in CH_2Cl_2 (5.0 ml), then DMAP (200 mg) and palmitoyl chloride (357 mg) were added. The mixture was stirred at room temperature overnight and concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane : AcOEt = 2 : 1) to give **29k** (86 mg, 24%) as a colorless oil. IR (neat): 1728, 1670, 1265, 1153 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.6), 1.06—1.34 (51H, m), 1.42 (9H, s), 1.45 (9H, s), 1.56—1.63 (4H, m), 2.00—2.20 (2H, m), 2.31 (2H, t, J=8.0), 2.35 (2H, t, J=8.0), 2.49 (2H, t, J=7.8), 2.50 (2H, t, J=7.6), 2.86 (2H, t, J=7.6), 3.04 (2H, br s), 4.34 (2H, m), 4.50 (2H, d, J=5.8), 4.65 (1H, m), 6.20 (1H, t, J=5.8), 7.03 (1H, d, J=7.0), 7.36 (2H, d, J=8.2), 7.80 (2H, d, J=8.2).

Compounds **27b** and **29l** were prepared by similar methods to those described for **27a** and **29k**, respectively.

6,7-Dihydroxy-4-thiaoctanoic Acid (**27b**): Yield, 73%. Colorless oil. IR (neat): 3400, 1720 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.18—1.30 (3H, m), 2.56—2.89 (6H, m), 3.48—3.78 (2H, m).

Di-*tert*-butyl *N*-{4-[6,7-Bis(hexadecanoyloxy)-4-thiaoctanoylaminomethyl]benzoyl}-L-glutamate (**29l**): Yield, 72%. Colorless solid. IR (KBr): 3300, 1730, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.2), 1.18 (3H, d, J=6.4), 1.26 (48H, s), 1.42 (9H, s), 1.49 (9H, s), 1.57—1.64 (4H, m), 2.02—2.44 (8H, m), 2.49—2.58 (2H, m), 2.63—2.71 (2H, m), 2.84—3.00 (2H, m), 4.45 (2H, d, J=6.0), 4.65 (1H, m), 5.00 (1/2 × 1H, m), 5.13 (1/2 × 1H, m), 5.16 (1H, m), 6.25 (1/2 × 1H, m), 6.45 (1/2 × 1H, m), 6.99—7.04 (1H, m), 7.36 (2H, d, J=8.2), 7.79 (2H, d, J=8.2).

Method F, Di-*tert*-butyl *N*-{4-[6(*R*),7-Dihydroxy-4-thiaheptanoylamino]-benzoyl}-L-glutamate (*R*-28c) A stirred solution of 3,3′-dithiodipropionic acid (**31**) (420 mg) and **8a** (1.15 g) in pyridine (10 ml) was treated dropwise with PCl_3 (0.17 ml) and the mixture was stirred at room temperature for 5 h, then concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane : AcOEt = 2 : 3) to give 3,3′-dithiobis[4,4′-(propionylamino)benzoyl-L-glutamic acid di-*tert*-butyl ester] (**32**, 960 mg, 54%) as a colorless solid. IR (KBr): 3310, 1715, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (18H, s), 1.48 (18H, s), 1.95—2.47 (8H, m), 2.78 (4H, t, J=7.2), 3.05 (4H, t, J=7.2), 4.57—4.71 (2H, m), 7.15 (2H, d, J=7.2), 7.61 (4H, d, J=8.8), 7.75 (4H, d, J=8.8), 8.59 (2H, br s).

An acidic solution (MeOH : $CHCl_3$: cH_2SO_4 = 100 : 6.5 : 1, 8 ml) was added to a stirred mixture of **32** (2.35 g) and zinc powder (1.15 g) in CH_2Cl_2 (20 ml), and the mixture was stirred at room temperature for 15 min. Then, (*R*)-glycidol (*R*-**22a**, 1.88 g) was added and the reaction mixture was stirred at 40 °C for 5 h, diluted with CH_2Cl_2, washed with 5% aqueous $KHSO_4$ solution and brine, dried over Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed on silica gel ($CHCl_3$: MeOH = 30 : 1—10 : 1) to give *R*-**28c** (1.75 g, 64%) as a colorless syrup. IR (neat): 3300, 1725, 1635 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (9H, s), 1.48 (9H, s), 1.90—2.50 (4H, m), 2.50—2.75 (4H, m), 2.87 (2H, t, J=6.2), 3.30—3.95 (5H, m), 4.62 (1H, m), 7.30 (1H, d, J=7.4), 7.60 (2H, d, J=8.8), 7.73 (2H, d, J=8.8), 8.91 (1H, s).

Acylation of Diol Derivatives (28). Di-*tert*-butyl *N*-{4-[6(*R*),7-Bis(hexadecanoyloxy)-4-thiaheptanoylamino]benzoyl}-L-glutamate (*R*-29d). Typical Procedure Palmitoyl chloride (882 mg) was added to a stirred mixture of *R*-**28c** (825 mg) and DMAP (470 mg) in CH_2Cl_2 (15 ml). The reaction mixture was stirred at room temperature for 2 h, then concentrated under reduced pressure, and the residue was chromatographed on silica gel (hexane : AcOEt = 2 : 1) to give *R*-**29d** (1.377 g, 89%) as an amorphous solid. IR (KBr): 3330, 1730, 1635, 1595, 1520, 1500, 1360, 1250, 1150 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.25 (48H, s), 1.42 (9H, s), 1.49 (9H, s), 1.50—1.75 (4H, m), 1.90—2.50 (8H, m), 2.60—3.10 (6H, m), 4.15 (1H, dd, J=6.6, 12.0), 4.46 (1H, dd, J=3.0, 12.0), 4.66 (1H, m), 5.18 (1H, m), 7.00 (1H, d, J=7.2), 7.65 (2H, d, J=8.8), 7.80 (2H, d, J=8.8), 8.16 (1H, br s).

Compounds **29o—t** were prepared from **28c** and various acid chlorides by a similar method to that described for *R*-**29d**.

Di-*tert*-butyl *N*-{4-[6,7-Bis(decanoyloxy)-4-thiaheptanoylamino]benzoyl}-L-glutamate (**29o**): Yield, 16%. Colorless syrup. IR (neat): 3310, 1730, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.6), 1.05—1.55 (24H, m), 1.42 (9H, s), 1.49 (9H, s), 1.55—1.75 (4H, m), 1.95—3.15 (14H, m), 4.17 (1H, dd, J=6.6, 12.0), 4.47 (1H, dd, J=3.2, 12.0), 4.62—4.73 (1H, m), 5.12—5.27 (1H, m), 7.02 (1H, d, J=7.4), 7.65 (2H, d, J=8.8), 7.81 (2H, d, J=8.8), 8.18 (1H, br s).

Di-*tert*-butyl *N*-{4-[6,7-Bis(dodecanoyloxy)-4-thiaheptanoylamino]-benzoyl}-L-glutamate (**29p**): Yield, 74%. Colorless wax. IR (KBr): 3310, 1720, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=7.0), 1.07—1.57 (30H, m), 1.42 (9H, s), 1.49 (9H, s), 1.57—1.79 (4H, m), 1.95—3.16 (10H, m), 4.15 (1H, dd, J=6.8, 12.2), 4.46 (1H, dd, J=3.2, 12.2), 4.60—4.71 (1H, m), 5.09—5.23 (1H, m), 7.01 (1H, d, J=7.0), 7.64 (2H, d, J=8.8), 7.80 (2H, d, J=8.8), 8.13 (1H, br s).

Di-*tert*-butyl *N*-{4-[6,7-Bis(tetradecanoyloxy)-4-thiaheptanoylamino]-benzoyl}-L-glutamate (**29q**): Yield, 76%. Colorless wax. IR (KBr): 3400, 1730, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=7.0), 1.02—1.37 (44H, m), 1.42 (9H, s), 1.49 (9H, s), 1.52—1.73 (4H, m), 1.92—3.12 (14H, m), 4.15 (1H, dd, J=6.8, 12.0), 4.47 (1H, dd, J=3.0, 12.0), 4.60—4.73 (1H, m), 5.10—5.24 (1H, m), 7.00 (1H, d, J=7.6), 7.65 (2H, d, J=8.6), 7.65 (2H, d, J=8.6), 7.81 (2H, d, J=8.6), 8.10 (1H, br s).

Di-*tert*-butyl *N*-{4-[6,7-Bis(octadecanoyloxy)-4-thiaheptanoylamino]-benzoyl}-L-glutamate (**29r**): Yield, 76%. Colorless wax. IR (KBr): 3430, 1750, 1710, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.6), 1.04—1.30 (56H, m), 1.42 (9H, s), 1.49 (9H, s), 1.52—3.15 (18H, m), 4.15 (1H, dd, J=6.8, 12.2), 4.47 (1H, dd, J=3.2, 12.2), 4.59—4.73 (1H, m), 5.07—5.24 (1H, m), 6.99 (1H, d, J=7.6), 7.65 (2H, d, J=8.8), 7.81 (2H, d, J=8.8), 8.08 (1H, br s).

Di-*tert*-butyl *N*-{4-[6,7-Bis(12-cyclohexyldodecanoyloxy)-4-thiaheptanoylamino]benzoyl}-L-glutamate (**29s**): Yield, 28%. Colorless solid. IR (KBr): 3300, 1730, 1635, 1590, 1250, 1150 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.87 (4H, m), 1.18 (4H, m), 1.25 (42H, s), 1.42 (9H, s), 1.49 (9H, s), 1.68 (16H, m), 1.90—2.50 (8H, m), 2.60—2.80 (4H, m), 2.80—3.10 (2H, m), 4.16 (1H, dd, J=7.2, 11.6), 4.47 (1H, dd, J=3.0, 11.6), 4.53 (1H, m), 5.66 (1H, m), 6.99 (1H, d, J=7.6), 7.65 (2H, d, J=8.8), 7.81 (2H, d, J=8.8), 8.10 (1H, s).

Di-*tert*-butyl *N*-{4-[6,7-Bis(12-phenyldodecanoyloxy)-4-thiaheptanoylamino]benzoyl}-L-glutamate (**29t**): Yield, 93%. Colorless oil. IR (neat): 3300, 1730, 1700, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.26 (28H, s), 1.42 (9H, s), 1.49 (9H, s), 1.50—1.70 (8H, m), 1.90—2.50 (8H, m), 2.50—3.10 (10H, m), 4.15 (1H, dd, J=6.6, 12.0), 4.46 (1H, dd, J=3.2, 12.0), 4.65 (1H, m), 5.17 (1H, m), 6.98 (1H, d, J=7.2), 7.10—7.35 (10H, m), 7.64 (2H, d, J=8.8), 7.80 (2H, d, J=8.8), 8.07 (1H, s).

Di-*tert*-butyl *N*-[4-(6-Hexadecanoyloxy-7-octadecylcarbamoyloxy-4-thiaheptanoylamino)benzoyl]-L-glutamate (29u) Octadecyl isocyanate (90 mg) was added to a stirred mixture of **28c** (150 mg) and DMAP (102 mg) in dichloroethane (5 ml), and the mixture was stirred at 60 °C for 10 h. Then, palmitoyl chloride (90 mg) was added and the whole was stirred at room temperature for 3 h, and concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane : AcOEt = 2 : 1) to give **29u** (152 mg, 51%) as a colorless wax. IR (neat): 3310, 1730, 1700, 1640, 1600, 1250, 1150 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.25 (54H, s), 1.42 (9H, s), 1.49 (9H, s), 1.40—1.70 (4H, m), 2.00—2.50 (6H, m), 2.65—3.10 (6H, m), 3.18 (2H, q, J=6.4), 4.12 (1H, m), 4.48 (1H, dd, J=3.4, 12.4), 4.66 (1H, m), 4.79 (1H, br t, J=5.0), 5.18 (1H, m), 6.98 (1H, d, J=7.0), 7.67 (2H, d, J=8.8), 7.80 (2H, d, J=8.8), 8.45 (1H, br s).

Di-*tert*-butyl *N*-{4-[6,7-Bis(octadecylcarbamoyloxy)-4-thiaheptanoylamino]benzoyl}-L-glutamate (29v) Octadecyl isocyanate (200 mg) was added to a stirred mixture of **28c** (152 mg) and DMAP (102 mg) in dichloroethane (5 ml). The mixture was stirred at 80 °C for 15 h, then concentrated under reduced pressure, and the residue was chromatographed on silica gel (CH_2Cl_2 : MeOH = 50 : 1) to give **29v** (102 mg, 32%) as a colorless solid. IR (KBr): 3320, 1725, 1700, 1670, 1650, 1630, 1600, 1250, 1150 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.25

(60H, s), 1.42 (9H, s), 1.49 (9H, s), 1.40—1.60 (4H, m), 1.90—2.50 (4H, m), 2.60—3.25 (10H, m), 4.17 (1H, dd, J=6.4, 12.0), 4.42 (1H, dd, J=2.8, 12.0), 4.66 (1H, m), 4.79 (1H, br t, J=6.4), 4.90 (1H, br t, J=6.6), 5.00 (1H, m), 6.97 (1H, d, J=7.4), 7.69 (2H, d, J=8.8), 7.79 (2H, d, J=8.8), 8.70 (1H, br s).

***N*-{*N*-[6,7-Bis(hexadecanoyloxy)-4-thiaheptanoyl]glycylglycylglycyl}-L-glutamic Acid (30a). Typical Procedure** A solution of **29a** (291 mg) in CH_2Cl_2 (1 ml) was treated with TFA (3 ml) and the mixture was stirred at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure and the residue was crystallized from MeOH–H_2O, washed with water and dried *in vacuo* to give **30a** (252 mg, 97%) as a colorless powder. IR (KBr): 3270, 1735, 1660 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (6H, t, J=6.8), 1.04—1.49 (48H, m), 1.50—1.80 (4H, m), 1.95—2.26 (2H, m), 2.32 (2H, t, J=7.4), 2.33 (2H, t, J=7.6), 2.43 (2H, t, J=6.6), 2.57 (2H, t, J=7.2), 2.74 (2H, d, J=6.6), 2.87 (2H, t, J=7.2), 3.75—4.11 (6H, m), 4.16 (1H, dd, J=6.4, 12.2), 4.39 (1H, dd, J=3.2, 12.2), 4.44—4.56 (1H, m), 5.08—5.23 (1H, m).

Compounds **30b—v** were prepared by a similar method to that described for **30a**.

N-{8-[6,7-Bis(hexadecanoyloxy)-4-thiaheptanoylamino]octanoyl}-L-glutamic Acid (**30b**): Yield, 100%. Colorless powder. IR (KBr): 3360, 1735, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (6H, t, J=7.0), 1.03—1.41 (48H, m), 1.41—1.73 (6H, m), 1.95—2.38 (4H, m), 2.32 (2H, t, J=7.6), 2.33 (2H, t, J=7.4), 2.54 (2H, t, J=7.2), 2.71 (2H, d, J=6.6), 2.87 (2H, t, J=7.2), 3.17—3.35 (1H, m), 4.14 (1H, dd, J=6.6, 12.0), 4.42 (1H, dd, J=2.8, 12.0), 4.52—4.70 (1H, m), 5.06—5.22 (1H, m), 6.25—7.32 (2H, m).

N-{4-[6,7-Bis(hexadecanoyloxy)-4-thiaheptanoylaminomethyl]benzoyl}-L-glutamic Acid (**30c**): Yield, 80%. Colorless powder. IR (KBr): 1737, 1639, 1243, 1222, 1199, 1174 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.6), 1.25 (48H, s), 1.50—1.70 (4H, m), 1.90—2.65 (10H, m), 2.71 (2H, d, J=6.6), 2.87 (2H, t, J=6.2), 4.12 (1H, dd, J=5.8, 11.6), 4.25—4.50 (3H, m), 4.63 (1H, m), 5.13 (1H, m), 7.16 (2H, d, J=7.8), 7.31 (1H, brs), 7.61 (2H, d, J=7.8), 7.79 (1H, d, J=6.0).

N-{4-[6,7-Bis(hexadecanoyloxy)-4-thiaheptanoylamino]benzoyl}-L-glutamic Acid (**30d**): Yield, 98%. Colorless powder. IR (KBr): 3450, 1735, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (6H, t, J=6.9), 1.13—1.42 (48H, m), 1.51—1.72 (4H, m), 2.32 (2H, t, J=7.7), 2.34 (2H, t, J=7.5), 2.68 (2H, t, J=7.4), 2.75 (2H, d, J=6.8), 2.94 (2H, t, J=7.4), 4.17 (1H, dd, J=6.6, 12.0), 4.42 (1H, dd, J=3.2, 12.0), 4.63—4.78 (1H, m), 5.10—5.25 (1H, m), 7.64 (2H, d, J=8.7), 7.79 (2H, d, J=8.7).

N-{4-[6(*R*),7-Bis(hexadecanoyloxy)-4-thiaheptanoylamino]benzoyl}-L-glutamic Acid (*R*-**30d**): Yield, 92%. Colorless powder. IR (KBr): 3450, 1735, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.25 (48H, s), 1.50—1.70 (4H, m), 2.05—3.00 (14H, m), 4.16 (1H, dd, J=6.4, 12.0), 4.42 (1H, dd, J=2.6, 12.0), 4.70 (1H, m), 5.17 (1H, m), 7.51 (1H, d, J=7.2), 7.63 (2H, d, J=8.8), 7.78 (2H, d, J=8.8), 9.00 (1H, s).

N-{3-[6,7-Bis(hexadecanoyloxy)-4-thiaheptanoylamino]benzoyl}-L-glutamic Acid (**30e**): Yield, 100%. Colorless powder. IR (KBr): 3360, 1730, 1635 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (6H, t, J=6.8), 1.15—1.45 (48H, m), 1.50—1.70 (4H, m), 2.05—2.55 (4H, m), 2.62—3.05 (6H, m), 4.05—4.70 (3H, m), 5.10—5.25 (1H, m), 7.37—8.05 (4H, m).

N-{2-[6,7-Bis(hexadecanoyloxy)-4-thiaheptanoylamino]benzoyl}-L-glutamic Acid (**30f**): Yield, 100%. Colorless powder. IR (KBr): 1730, 1690, 1670 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.81 (6H, t, J=6.8), 1.02—1.47 (48H, m), 1.47—1.70 (4H, m), 2.10—2.40 (4H, m), 2.50—3.00 (6H, m), 3.90—4.40 (3H, m), 5.10—5.20 (1H, m), 6.99—8.77 (4H, m).

N-{4-[6,7-Bis(hexadecanoyloxy)-4-thiaheptanoylamino]-2-fluorobenzoyl}-L-glutamic Acid (**30g**): Yield, 100%. Colorless powder. IR (KBr): 3410, 1730, 1620 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (6H, t, J=7.0), 1.03—1.49 (48H, m), 1.53—1.72 (4H, m), 2.02—2.59 (4H, m), 2.33 (2H, t, J=7.8), 2.35 (2H, t, J=7.4), 2.69 (2H, t, J=7.2), 2.75 (2H, d, J=6.6), 2.94 (2H, t, J=7.2), 4.17 (1H, dd, J=6.4, 12.2), 4.42 (1H, dd, J=3.0, 12.2), 4.72—4.85 (1H, m), 5.10—5.25 (1H, m), 7.21 (1H, dd, J=2.0, 8.8), 7.55 (1H, dd, J=7.2, 12.0), 7.80 (1H, dd, J=2.0, 12.6), 7.93 (1H, t, J=8.8).

N-{4-[6,7-Bis(hexadecanoyloxy)-4-thiaheptanoylamino]benzenesulfonyl}-L-glutamic Acid (**30h**): Yield, 100%. Colorless powder. IR (KBr): 3340, 3260, 1730, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.03—1.49 (48H, m), 1.49 —1.72 (4H, m), 1.72—3.10 (14H, m), 3.88—4.55 (3H, m), 5.12—5.30 (1H, m), 5.95—6.10 (1H, m), 7.65—7.90 (4H, m), 8.78 (1H, br s).

N-{4-[6,7-Bis(hexadecanoyloxy)-2-methyl-4-thiaheptanoylamino]-benzoyl}-L-glutamic Acid (**30i**): Yield, 100%. Colorless powder. IR (KBr): 3350, 1750, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.03—1.50 (51H, m), 1.50—1.70 (4H, m), 2.02—3.15 (13H, m), 4.06—5.26 (4H, m), 7.57—7.90 (4H, m).

N-{4-[6,7-Bis(hexadecanoyloxy)-3-methyl-4-thiaheptanoylamino]-benzoyl}-L-glutamic Acid (**30j**): Yield, 99%. Colorless powder. IR (KBr): 3350, 1735, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.03—1.50 (48H, m), 1.39 (3H, t, J=6.8), 1.50—1.73 (4H, m), 2.03—2.88 (9H, m), 4.18 (1H, dd, J=6.2, 11.8), 4.32—4.45 (1H, m), 4.62—4.77 (1H, m), 5.05—5.23 (1H, m), 7.58—7.83 (4H, m).

N-{4-[6,7-Bis(hexadecanoyloxy)-6-methyl-4-thiaheptanoylaminomethyl]benzoyl}-L-glutamic Acid (**30k**): Yield, 96%. Colorless powder. IR (KBr): 3267, 1730, 1645, 1547, 1222 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (6H, t, J=6.6), 1.14—1.40 (51H, m), 1.50—1.75 (4H, m), 1.95—2.15 (2H, m), 2.18—2.60 (8H, m), 2.70 (2H, m), 2.88 (2H, m), 4.30—4.49 (4H, m), 4.65—4.74 (1H, m), 7.36 (2H, d, J=8.0), 7.79 (2H, d, J=8.0).

N-{4-[6,7-Bis(hexadecanoyloxy)-4-thiaoctanoylaminomethyl]benzoyl}-L-glutamic Acid (**30l**): Yield, 99%. Colorless powder. IR (KBr): 3320, 1734, 1637, 1543, 1265, 1245, 1222, 1198, 1178 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (6H, t, J=6.6), 1.20 (3H, d, J=6.6), 1.26 (48H, s), 1.61 (4H, m), 2.12—2.40 (6H, m), 2.46—2.57 (4H, m), 2.66—2.72 (2H, m), 2.82—2.91 (2H, m), 4.46 (2H, d, J=5.4), 4.71 (1H, m), 5.02 (1/2 × 1H, m), 5.13 (1/2 × 1H, m), 5.16 (1H, m), 7.36 (2H, d, J=8.2), 7.79 (2H, d, J=8.2).

N-{*N*-[5,6-Bis(hexadecanoyloxy)-3-thiahexanoyl]glycylglycylglycyl}-L-glutamic Acid (**30m**): Yield, 99%. Colorless powder. IR (KBr): 3350, 1735, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (6H, t, J=6.8), 1.03—1.49 (48H, m), 1.51—1.73 (4H, m), 1.95—2.53 (4H, m), 2.32 (2H, t, J=8.0), 2.33 (2H, t, J=7.4), 2.76—2.86 (2H, m), 3.31 (2H, s), 3.73—4.30 (7H, m), 4.37 (1H, dd, J=3.2, 12.2), 4.43—4.58 (1H, m), 5.15—5.30 (1H, m).

N-{*N*-[7,8-Bis(hexadecanoyloxy)-5-thiaoctanoyl]glycylglycylglycyl}-L-glutamic Acid (**30n**): Yield, 100%. Colorless powder. IR (KBr): 3275, 1735, 1635 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (6H, t, J=6.6), 1.12—1.50 (48H, m), 1.50—1.70 (4H, m), 1.75—2.50 (8H, m), 2.32 (2H, t, J=8.0), 2.33 (2H, t, J=7.6), 2.61 (2H, t, J=7.0), 2.70 (2H, d, J=6.6), 3.67—4.30 (6H, m), 4.16 (1H, dd, J=6.2, 11.6), 4.39 (1H, dd, J=3.2, 11.6), 4.48 (1H, ddd, J=3.2, 6.2, 6.6), 5.07—5.22 (1H, m).

N-{4-[6,7-Bis(decanoyloxy)-4-thiaheptanoylamino]benzoyl}-L-glutamic Acid (**30o**): Yield, 93%. Colorless powder. IR (KBr): 3400, 1740, 1640, 1600 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, m), 1.27 (24H, m), 1.62 (4H, m), 2.10—2.40 (7H, m), 2.49 (1H, m), 2.60—2.90 (3H, m), 2.94 (2H, m), 4.17 (1H, dd, J=6.4, 11.8), 4.42 (1H, dd, J=3.2, 11.8), 4.70 (1H, m), 5.19 (1H, m), 7.64 (2H, d, J=8.4), 7.79 (2H, d, J=8.4).

N-{4-[6,7-Bis(dodecanoyloxy)-4-thiaheptanoylamino]benzoyl}-L-glutamic Acid (**30p**): Yield, 96%. Colorless powder. IR (KBr): 3300, 1740, 1710, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (6H, t, J=6.8), 1.03—1.50 (32H, m), 1.50—1.72 (4H, m), 2.03—2.62 (8H, m), 2.69 (2H, t, J=7.4), 2.76 (2H, d, J=6.8), 2.95 (2H, t, J=7.4), 4.17 (1H, dd, J=6.4, 12.0), 4.42 (1H, dd, J=3.2, 12.0), 4.63—4.77 (1H, m), 5.10—5.26 (1H, m), 7.64 (2H, d, J=8.8), 7.81 (2H, d, J=8.8).

N-{4-[6,7-Bis(tetradecanoyloxy)-4-thiaheptanoylamino]benzoyl}-L-glutamic Acid (**30q**): Yield, 100%. Colorless powder. IR (KBr): 3350, 1730, 1700, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (6H, t, J=6.6), 1.03—1.48 (40H, m), 1.51—1.72 (4H, m), 2.03—2.62 (8H, m), 2.69 (2H, t, J=7.4), 2.76 (2H, d, J=6.8), 2.95 (2H, t, J=7.4), 4.07—4.80 (3H, m), 5.10—5.26 (1H, m), 7.65 (2H, d, J=8.8), 7.80 (2H, d, J=8.8).

N-{4-[6,7-Bis(octadecanoyloxy)-4-thiaheptanoylamino]benzoyl}-L-glutamic Acid (**30r**): Yield, 94%. Colorless powder. IR (KBr): 1730, 1700 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (6H, t, J=6.4), 1.10—1.48 (56H, m), 1.52—1.72 (6H, m), 2.03—2.62 (8H, m), 2.69 (2H, t, J=7.2), 2.76 (2H, d, J=6.6), 2.94 (2H, t, J=7.2), 4.07—4.80 (3H, m), 5.10—5.25 (1H, m), 7.65 (2H, d, J=8.8), 7.80 (2H, d, J=8.8).

N-{4-[6,7-Bis(12-cyclohexyldodecanoyloxy)-4-thiaheptanoylamino]-benzoyl}-L-glutamic Acid (**30s**): Yield, 100%. Colorless powder. IR (KBr): 3320, 2920, 2850, 1730, 1640, 1600, 1535, 1500, 1440, 1400, 1305, 1250, 1170 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.86 (4H, m), 1.17 (4H, m), 1.24 (42H, s), 1.64 (16H, m), 1.90—2.20 (2H, m), 2.31 (2H, t, J=6.8), 2.33 (2H, t, J=6.8), 2.44 (2H, br s), 2.70 (4H, m), 2.89 (2H, br s), 4.16 (1H, dd, J=6.4, 12.4), 4.41 (1H, dd, J=2.2, 12.4), 4.64 (1H, m), 5.17 (1H, m), 7.44 (2H, d, J=9.2), 7.61 (2H, d, J=9.2), 7.75 (1H, m), 8.88 (1H, s).

N-{4-[6,7-Bis(12-phenyldodecanoyloxy)-4-thiaheptanoylamino]benzoyl}-L-glutamic Acid (**30t**): Yield, 84%. Colorless syrup. IR (neat): 3300, 1730, 1710, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.24 (28H, s), 1.50—1.70

(8H, m), 1.90—2.80 (12H, m), 2.57 (4H, t, $J=8.0$), 2.80—3.00 (2H, m), 4.11 (1H, dd, $J=6.2$, 12.0), 4.39 (1H, dd, $J=2.4$, 12.0), 4.62 (1H, m), 5.16 (1H, m), 7.10—7.30 (10H, m), 7.43 (2H, d, $J=8.2$), 7.66 (2H, d, $J=8.2$), 7.70—7.85 (1H, m), 8.88 (1H, s).

N-[4-(6-Hexadecanoyloxy-7-octadecylcarbamoyloxy-4-thiaheptanoylamino)benzoyl]-L-glutamic Acid (**30u**): Yield, 81%. Colorless powder. IR (KBr): 1730, 1710, 1700, 1680, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.8$), 1.25 (54H, s), 1.40—1.70 (4H, m), 2.10—2.50 (4H, m), 2.60—2.85 (6H, m), 2.90—3.05 (2H, m), 3.05—3.20 (2H, m), 4.23 (1H, m), 4.44 (1H, m), 4.88 (1H, m), 5.21 (1H, m), 7.45 (1H, d, $J=6.8$), 7.63 (2H, d, $J=8.8$), 7.74 (2H, d, $J=8.8$), 8.71 (1H, br s).

N-{4-[6,7-Bis(octadecylcarbamoyloxy)-4-thiaheptanoylamino]benzoyl}-L-glutamic Acid (**30v**): Yield, 100%. Colorless powder. IR (KBr): 1715, 1700, 1680, 1650, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.6$), 1.25 (60H, s), 1.40—1.60 (4H, m), 2.10—2.55 (4H, m), 2.65—2.85 (6H, m), 2.90—3.05 (2H, m), 3.15 (2H, t, $J=6.6$), 4.20—4.55 (2H, m), 4.95 (1H, m), 5.09 (1H, m), 7.35 (1H, d, $J=7.2$), 7.66 (2H, d, $J=8.2$), 7.79 (2H, d, $J=8.2$), 8.70 (1H, br s).

Method G, *N*-{4-[6,7-Bis(hexadecanoyloxy)-4-thiaheptanoylamino]benzoyl}-L-glutamic Acid 4-Oxide (34a) and *N*-{4-[6,7-Bis(hexadecanoyloxy)-4-thiaheptanoylamino]benzoyl}-L-glutamic Acid 4,4-Dioxide (34b) A solution of *m*-chloroperbenzoic acid (*m*CPBA, 67 mg) in $CHCl_3$ (2 ml) was added dropwise to a stirred solution of **29d** (230 mg) in $CHCl_3$ (6 ml) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h, diluted with $CHCl_3$, washed with aqueous solution of $NaHCO_3$, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane : AcOEt = 1 : 1) to give the sulfone **33b** (119 mg, 50%) as an amorphous solid, then eluted with a solution of hexane : AcOEt = 1 : 4 to give the sulfoxide **33a** (116 mg, 50%) as an amorphous solid.

33a: IR (KBr): 1730, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.8$), 1.25 (48H, s), 1.42 (9H, s), 1.49 (9H, s), 1.50—1.70 (4H, m), 1.90—2.50 (8H, m), 2.90—3.40 (6H, m), 4.10—4.30 (1H, m), 4.35—4.50 (1H, m), 4.60—4.75 (1H, m), 5.50 (1H, m), 7.01 (1H, d, $J=7.2$), 7.62 (2H, d, $J=8.8$), 7.79 (2H, d, $J=8.8$), 8.88 (1/2 × 1H, br s), 8.94 (1/2 × 1H, br s).

33b: IR (KBr): 3330, 1730, 1695, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.8$), 1.25 (48H, s), 1.42 (9H, s), 1.49 (9H, s), 1.50—1.70 (4H, m), 2.00—2.50 (8H, m), 2.95 (2H, t, $J=7.0$), 3.33 (1H, dd, $J=4.8$, 14.8), 3.46 (1H, dd, $J=7.2$, 14.8), 3.53 (2H, t, $J=7.0$), 4.19 (1H, dd, $J=5.2$, 12.0), 4.43 (1H, $J=3.8$, 12.0), 4.65 (1H, m), 5.58 (1H, m), 7.05 (1H, d, $J=7.8$), 7.57 (2H, d, $J=8.8$), 7.78 (2H, d, $J=8.8$), 8.23 (1H, br s).

Each of **34a** and **34b** was prepared by a similar method to that described for **30a**.

34a: Yield, 94%. Colorless powder. IR (KBr): 2919, 2852, 1737, 1639, 1531, 1307, 1253, 1178 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD–TFA) δ: 0.88 (6H, t, $J=6.6$), 1.25 (48H, s), 1.50—1.70 (4H, m), 2.00—2.40 (8H, m), 2.80—3.40 (6H, m), 4.10—4.30 (1H, m), 4.35—4.80 (2H, m), 5.49 (1H, m), 7.50—7.80 (4H, m).

34b: Yield, 97%. Colorless powder. IR (KBr): 3336, 2919, 2850, 1741, 1641, 1604, 1533, 1463, 1407, 1311, 1255, 1178 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (6H, t, $J=6.6$), 1.25 (48H, s), 1.50—1.70 (4H, m), 1.90—2.60 (8H, m), 2.92 (2H, t, $J=7.0$), 3.35—3.60 (4H, m), 4.18 (1H, dd, $J=5.0$, 12.0), 4.42 (1H, dd, $J=3.2$, 12.0), 4.69 (1H, m), 5.60 (1H, m), 7.50 (2H, d, $J=8.8$), 7.67 (2H, d, $J=8.8$).

Method H, *tert*-Butyl 6,7-Dihydroxy-4-thia-2(*E*)-heptenoate (*E*-36) and *tert*-Butyl 6,7-Dihydroxy-4-thia-2(*Z*)-heptenoate (*Z*-36) One drop of Et_3N was added to a stirred mixture of *tert*-butyl propiolate (**35**, 1.17 g) and thioglycerol (**20**, 1.0 g) in CH_2Cl_2 (5 ml). The whole was stirred at room temperature for 30 min, and then concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane : AcOEt = 1 : 1) to give *E*-**36** (1.50 g, 70%) and *Z*-**36** (645 mg, 30%), each as a colorless oil. IR (neat): 3400, 1700, 1680, 1575, 1365, 1310, 1250, 1140 cm^{-1}. *E*-form: ^{1}H-NMR ($CDCl_3$) δ: 1.48 (9H, s), 2.39 (1H, br s), 2.80—3.10 (3H, m), 3.55—3.85 (2H, m), 3.93 (1H, m), 5.76 (1H, d, $J=15.2$), 7.54 (1H, d, $J=15.2$). *Z*-form: ^{1}H-NMR ($CDCl_3$) δ: 1.50 (9H, s), 2.58 (1H, br s), 2.80—3.10 (3H, m), 3.55—3.85 (2H, m), 3.92 (1H, m), 5.81 (1H, d, $J=10.2$), 7.02 (1H, d, $J=10.2$).

N-{*N*-[6,7-Bis(hexadecanoyloxy)-4-thia-2(*E*)-heptenoyl]glycylglycylglycyl}-L-glutamic Acid (*E*-**40a**): Palmitoyl chloride (1.55 g) was added to a mixture of *E*-**36** (600 mg) and DMAP (735 mg) in CH_2Cl_2 (10 ml). The reaction mixture was stirred at room temperature for 1 h, and concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane : AcOEt = 10 : 1) to give *tert*-butyl 6,7-bis(hexadecanoyloxy)-4-thia-2(*E*)-heptenoate (*E*-**37**, 1.48 g, 81%) as a colorless solid. IR (KBr): 1740, 1690, 1570 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.8$), 1.25 (48H, s), 1.48 (9H, s), 1.50—1.70 (4H, m), 2.32 (2H, t, $J=7.6$), 2.33 (2H, t, $J=7.6$), 3.03 (2H, d, $J=6.4$), 4.17 (1H, dd, $J=5.4$, 12.0), 4.32 (1H, dd, $J=4.0$, 12.0), 5.21 (1H, m), 5.78 (1H, d, $J=15.2$), 7.49 (1H, d, $J=15.2$).

A solution of *E*-**37** (1.4 g) in CH_2Cl_2 (2 ml) was treated with TFA (4 ml). The reaction mixture was stirred at room temperature for 1 h, then concentrated under reduced pressure to give 6,7-bis(hexadecanoyloxy)-4-thia-2(*E*)-heptenoic acid (*E*-**38**, 1.26 g, 98%) as a colorless solid. IR (KBr): 1735, 1664, 1579 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.8$), 1.25 (48H, s), 1.50—1.70 (4H, m), 2.25—2.40 (4H, m), 3.07 (2H, d, $J=6.6$), 4.18 (1H, dd, $J=5.4$, 12.0), 4.33 (1H, dd, $J=4.2$, 12.0), 5.22 (1H, m), 5.89 (1H, d, $J=15.2$), 7.74 (1H, d, $J=15.2$).

A stirred solution of *E*-**38** (200 mg) and **6a** (171 mg) in DMF (5 ml) was treated with DEPC (75 mg) at 0 °C, then Et_3N (64 mg) was added. The reaction mixture was stirred at room temperature for 1 h, then concentrated under reduced pressure, and the residue was chromatographed on silica gel ($CHCl_3$: MeOH = 99 : 1) to give di-*tert*-butyl *N*-{*N*-[6,7-bis(hexadecanoyloxy)-4-thia-2(*E*)-heptenoyl]glycylglycylglycyl}-L-glutamate (*E*-**39a**, 306 mg, 94%) as a colorless wax. IR (neat): 3300, 1730, 1695, 1630, 1590 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.8$), 1.25 (48H, s), 1.44 (9H, s), 1.47 (9H, s), 1.50—1.70 (4H, m), 1.80—2.40 (8H, m), 3.05 (2H, t, $J=4.6$), 4.00—4.15 (6H, m), 4.18 (1H, dd, $J=6.0$, 12.4), 4.36 (1H, dd, $J=3.2$, 12.4), 4.47 (1H, m), 5.21 (1H, m), 6.07 (1H, d, $J=15.0$), 6.75 (1H, br s), 7.19 (1H, br s), 7.35 (1H, d, $J=7.4$), 7.53 (1H, br s), 7.60 (1H, d, $J=15.0$).

A solution of *E*-**39a** (295 mg) in CH_2Cl_2 (0.5 ml) was treated with TFA (2 ml) and the mixture was stirred at room temperature for 1 h, then concentrated under reduced pressure. The residue was crystallized from $CHCl_3$–MeOH–H_2O to give *E*-**40a** (234 mg, 89%) as a colorless powder. IR (KBr): 3282, 1735, 1631, 1565 cm^{-1}. ^{1}H-NMR ($CDCl_3$–TFA) δ: 0.88 (6H, t, $J=6.6$), 1.26 (48H, s), 1.50—1.70 (4H, m), 2.05—2.45 (6H, m), 2.55 (2H, t, $J=6.6$), 2.97 (1H, dd, $J=7.2$, 14.4), 3.14 (1H, dd, $J=6.4$, 14.4), 4.00—4.35 (7H, m), 4.45 (1H, dd, $J=2.6$, 12.0), 4.68 (1H, m), 5.25 (1H, m), 6.08 (1H, d, $J=15.2$), 7.32 (1H, br s), 7.63 (1H, br s), 7.65 (1H, d, $J=15.2$), 7.75—7.85 (2H, m).

Compounds *E*-**40b** and *Z*-**40a** were prepared by a similar method to that described for *E*-**40a**.

***N*-{*N*-[6,7-Bis(hexadecanoyloxy)-4-thia-2(*Z*)-heptenoyl]glycylglycylglycyl}-L-glutamic Acid (*Z*-40a)** *Z*-**37**: Yield, 49%. Colorless solid. IR (KBr): 1740, 1690, 1570 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.8$), 1.26 (48H, s), 1.50 (9H, s), 1.50—1.70 (4H, m), 2.32 (4H, t, $J=7.4$), 2.95 (2H, d, $J=6.4$), 4.20 (1H, dd, $J=5.4$, 12.0), 4.38 (1H, dd, $J=3.6$, 12.0), 5.14 (1H, m), 5.80 (1H, d, $J=10.2$), 7.00 (1H, d, $J=10.2$).

Z-**38**: Yield, 98%. Colorless solid. IR (KBr): 1740, 1690, 1570 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.8$), 1.26 (48H, s), 1.50 (9H, s), 1.50—1.70 (4H, m), 2.32 (4H, t, $J=7.4$), 2.95 (2H, d, $J=6.4$), 4.20 (1H, dd, $J=5.4$, 12.0), 4.38 (1H, dd, $J=3.6$, 12.0), 5.14 (1H, m), 5.80 (1H, d, $J=10.2$), 7.00 (1H, d, $J=10.2$).

Z-**39a**: Yield, 95%. Colorless wax. IR (neat): 3300, 1730, 1630, 1520 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.8$), 1.25 (48H, s), 1.44 (9H, s), 1.45 (9H, s), 1.50—1.70 (4H,m), 1.80—2.20 (2H, m), 2.31 (6H, t, $J=7.6$), 2.92 (2H, d, $J=6.2$), 3.90—4.10 (6H, m), 4.18 (1H, dd, $J=5.4$, 12.1), 4.30—4.50 (2H, m), 5.12 (1H, m), 5.99 (1H, d, $J=10.0$), 6.93 (1H, d, $J=10.0$), 7.12 (1H, br s), 7.20 (1H, d, $J=7.8$), 7.44 (1H, br s), 7.55 (1H, br s).

Z-**40a**: Yield, 96%. Colorless powder. IR (KBr): 3290, 1735, 1635, 1560 cm^{-1}. ^{1}H-NMR ($CDCl_3$–TFA) δ: 0.88 (6H, t, $J=6.6$), 1.26 (48H, s), 1.50—1.70 (4H, m), 2.00—2.45 (6H, m), 2.50—2.65 (2H, m), 2.90—3.00 (2H, m), 4.00—4.50 (8H, m), 4.67 (1H, m), 5.20 (1H, m), 5.93 (1H, d, $J=9.8$), 7.05—7.20 (2H, m), 7.63 (1H, d, $J=9.8$), 7.75—7.85 (1H, m), 8.02 (1H, br s).

***N*-{4-[6,7-Bis(hexadecanoyloxy)-4-thia-2(*E*)-heptenoylaminomethyl]benzoyl}-L-glutamic Acid (*E*-40b)** *E*-**39b**: Yield, 99%. Colorless amorphous solid. IR (KBr): 3300, 1735, 1640, 1580 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.6$), 1.25 (48H, s), 1.42 (9H, s), 1.49 (9H, s), 1.50—1.70 (4H, m), 1.95—2.50 (8H, m), 2.96 (1H, dd, $J=7.2$, 14.4), 3.08 (1H, dd, $J=6.4$, 14.4), 4.20 (1H, dd, $J=5.4$, 12.0), 4.35 (1H, dd, $J=3.6$, 12.0), 4.55 (2H, d, $J=5.8$), 4.65 (1H, m), 5.19 (1H, m), 5.94 (1H, br s), 6.00 (1H, d, $J=18.6$), 7.02 (1H, d, $J=7.6$), 7.34 (2H, d, $J=8.2$), 7.57 (1H, d, $J=18.6$), 7.77 (2H, d, $J=8.2$).

E-**40b**: Yield, 92%. Colorless powder. IR (KBr): 3296, 1739, 1633, 1575 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, $J=6.6$), 1.25 (48H, s),

1.50—1.70 (4H, m), 1.95—2.50 (8H, m), 2.90—3.10 (2H, m), 4.16 (1H, dd, J=5.4, 12.0), 4.30—4.45 (3H, m), 4.60 (1H, m), 5.20 (1H, m), 6.06 (1H, d, J=15.2), 7.10 (2H, d, J=8.2), 7.10—7.20 (1H, m), 7.50—7.65 (3H, m), 7.82 (1H, m).

Method I, *N*-{4-[*O*-[2,3-Bis(hexadecanoyloxy)propyl]-*N*-carboxybenzyl-L-serylamino]benzoyl}-L-glutamic Acid (46a) A solution of *N*-carboxybenzyl-L-serine (5.0 g) in DMF (10 ml) was added to a suspension of 60% NaH (1.84 g) in DMF (100 ml) at 0 °C, and then allyl bromide (2.53 g) was added. The mxture was stirred at room temperature for 3 h, and concentrated under reduced pressure. The residue was dissolved in water and the solution was washed with ether. The aqueous solution was adjusted to pH 2 with 4 N HCl, then extracted with AcOEt. The organic layer was washed with water, dried over Na_2SO_4 and concentrated under reduced pressure to give *O*-allyl-*N*-carboxybenzyl-L-serine (**42a**, 5.0 g, 86%) as a pale yellow oil. IR (neat): 3320, 1720, 1700, 1515 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.69 (1H, dd, J=3.6, 9.4), 3.93 (1H, dd, J=3.0, 9.4), 3.99 (2H, d, J=5.6), 4.53 (1H, m), 5.13 (2H, s), 5.10—5.30 (2H, m), 5.69 (1H, d, J=8.4), 5.84 (1H, m), 7.35 (5H, s).

A stirred solution of **42a** (380 mg) and **8a** (190 mg) in pyridine (10 ml) was treated dropwise with PCl_3 (104 mg). The mixture was stirred at room temperature for 30 min, and then concentrated under reduced pressure. The residue was chromatographed on silica gel ($CHCl_3$: MeOH = 50 : 1) to give di-*tert*-butyl *N*-[4-(*O*-allyl-*N*-carboxybenzyl-L-serylamino)benzoyl]-L-glutamate (**43a**, 650 mg, 76%) as a colorless oil. IR (neat): 3330, 1725, 1700, 1630, 1600, 1520 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (9H, s), 1.49 (9H, s), 1.95—2.50 (4H, m), 3.61 (1H, dd, J=7.0, 9.2), 3.97 (1H, dd, J=3.8, 9.2), 4.00—4.15 (2H, m), 4.47 (1H, m), 4.65 (1H, m), 5.16 (2H, s), 5.20—5.35 (2H, m), 5.75—6.00 (2H, m), 7.02 (1H, d, J=7.6), 7.37 (5H, s), 7.55 (2H, d, J=8.6), 7.79 (2H, d, J=8.6), 8.63 (1H, br s).

A mixture of **43a** (620 mg) and *N*-methylmorpholine-*N*-oxide (200 mg) in acetone (10 ml)–tetrahydrofuran (THF, 4 ml)–H_2O (1 ml) was stirred in the presence of OsO_4 (5 mg) at room temperature for 10 h, and then concentrated under reduced pressure. The residue was chromatographed on silica gel ($CHCl_3$: MeOH = 25 : 1) to give di-*tert*-butyl *N*-{4-[*N*-carboxybenzyl-*O*-(2,3-dihydroxypropyl)-L-serylamino]benzoyl}-L-glutamate (**44a**, 467 mg, 72%) as an amorphous solid. IR (neat): 3330, 1720, 1695, 1685, 1650, 1630, 1600, 1525 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (9H, s), 1.49 (9H, s), 1.90—2.50 (4H, m), 3.50—3.75 (7H, m), 3.80—3.95 (2H, m), 4.49 (1H, m), 4.63 (1H, m), 5.13 (2H, s), 6.18 (1H, br s), 7.23 (1H, d, J=7.4), 7.26 (5H, s), 7.52 (1/2 × 2H, d, J=8.6), 7.53 (1/2 × 2H, d, J=8.6), 7.70 (2H, d, J=8.6), 9.06 (1H, br s).

Palmitoyl chloride (270 mg) was added dropwise to a stirred solution of **44a** (273 mg) and DMAP (132 mg) in CH_2Cl_2 (8 ml). The mixture was stirred at room temperature for 1 h, and then concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane : AcOEt = 3 : 1) to give di-*tert*-butyl *N*-{4-[*O*-[2,3-bis(hexadecanoyloxy)propyl]-*N*-carboxybenzyl-L-serylamino]benzoyl}-L-glutamate (**45a**, 400 mg, 86%) as a colorless solid. mp: 69—70 °C (MeOH). IR (KBr): 3320, 1730, 1695, 1680, 1670, 1650, 1600, 1530 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.6), 1.25 (48H, s), 1.42 (9H, s), 1.49 (9H, s), 1.50—1.70 (4H, m), 2.00—2.50 (8H, m), 3.55—3.75 (3H, m), 3.96 (1H, dd, J=3.8, 9.0), 4.05—4.35 (2H, m), 4.43 (1H, m), 4.66 (1H, m), 5.16 (2H, s), 5.24 (1H, m), 5.81 (1/2 × 1H, d, J=6.4), 5.87 (1/2 × 1H, d, J=6.6), 6.99 (1H, d, J=7.4), 7.36 (5H, m), 7.60 (1/2 × 2H, d, J=8.8), 7.64 (1/2 × 2H, d, J=8.8), 7.80 (2H, d, J=8.8), 8.48 (1H, br s).

A solution of **45a** (150 mg) in TFA (10 ml) was stirred at room temperature for 1 h, and then concentrated under reduced pressure. The residue was crystallized from MeOH–H_2O, washed with water and dried *in vacuo* to give **46a** (124 mg, 92%) as a colorless powder. IR (KBr): 3315, 1727, 1670, 1643 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (6H, t, J=6.6), 1.25 (48H, s), 1.45—1.65 (4H, m), 2.10—2.60 (8H, m), 3.55—3.75 (3H, m), 3.89 (1H, dd, J=4.4, 9.2), 4.14 (1H, m), 4.26 (1H, m), 4.46 (1H, m), 4.70 (1H, m), 5.15 (2H, s), 5.20 (1H, m), 6.17 (1H, br), 7.36 (5H, m), 7.50 (1H, d, J=7.4), 7.60 (1/2 × 2H, d, J=8.8), 7.62 (1/2 × 2H, d, J=8.8), 7.79 (2H, d, J=8.8), 9.11 (1H, br s).

Compounds **46b, c** were prepared by a similar method to that described for **46a**.

***N*-[4-[6,7-Bis(hexadecanoyloxy)-4-oxaheptanoylamino]benzoyl]-L-glutamic Acid (46b)** **43b**: Yield, 83%. Colorless oil. IR (neat): 3330, 1725, 1630, 1595, 1530 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (9H, s), 1.49 (9H, s), 1.90—2.55 (4H, m), 2.67 (2H, t, J=5.4), 3.80 (2H, t, J=5.4), 4.10 (2H, dt, J=1.4, 5.6), 4.66 (1H, m), 5.20—5.40 (2H, m), 5.96 (1H, ddt, J=5.6, 10.4, 17.2), 6.99 (1H, d, J=7.4), 7.58 (2H, d, J=8.8), 7.79 (2H, d, J=8.8), 8.64 (1H, br s).

44b: Yield, 100%. Colorless oil. IR (neat): 3320, 1725, 1640, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (9H, s), 1.49 (9H, s), 1.90—2.45 (4H, m), 2.66 (2H, t, J=5.6), 3.55—4.00 (7H, m), 4.63 (1H, m), 7.12 (1H, d, J=7.8), 7.61 (2H, d, J=8.8), 7.74 (2H, d, J=8.8), 8.67 (1H, br s).

45b: Yield, 83%. Colorless amorphous solid. IR (KBr): 1730, 1635 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.25 (48H, s), 1.42 (9H, s), 1.49 (9H, s), 1.40—1.70 (4H, m), 1.90—2.50 (8H, m), 2.64 (2H, t, J=5.6), 3.67 (2H, d, J=5.4), 3.81 (2H, t, J=5.6), 4.19 (1H, dd, J=6.2, 11.8), 4.34 (1H, dd, J=4.0, 11.8), 4.66 (1H, m), 5.29 (1H, m), 6.97 (1H, d, J=7.2), 7.64 (2H, d, J=8.8), 7.80 (2H, d, J=8.8), 8.29 (1H, br s).

46b: Yield, 98%. Colorless powder. IR (KBr): 1730, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.6), 1.25 (48H, s), 1.50—1.70 (4H, m), 2.10—2.48 (6H, m), 2.53 (2H, m), 2.64 (2H, m), 3.64 (2H, d, J=4.8), 3.81 (2H, m), 4.17 (1H, dd, J=5.4, 12.4), 4.33 (1H, dd, J=3.0, 12.4), 4.72 (1H, m), 5.26 (1H, m), 7.53 (2H, d, J=8.2), 7.60 (1H, d, J=7.2), 7.71 (2H, d, J=8.2), 8.67 (1H, br s).

***N*-{4-[6,7-Bis(hexadecanoyloxy)heptanoylamino]benzoyl}-L-glutamic Acid (46c)** **43c**: Yield, 100%. Colorless oil. IR (neat): 1730, 1635, 1525 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (9H, s), 1.49 (9H, s), 1.30—1.60 (2H, m), 1.65—1.85 (2H, m), 1.95—2.50 (8H, m), 4.65 (1H, m), 4.90—5.10 (2H, m), 5.80 (1H, m), 7.01 (1H, d, J=7.6), 7.49 (1H, br s), 7.60 (2H, d, J=8.6), 7.79 (2H, d, J=8.6).

44c: Yield, 90%. Colorless oil. IR (neat): 3320, 1725, 1635 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.41 (9H, s), 1.47 (9H, s), 1.30—1.70 (4H, m), 1.95—2.50 (8H, m), 3.30—3.90 (5H, m), 4.63 (1H, m), 7.32 (1H, d, J=7.4), 7.63 (2H, d, J=8.8), 7.75 (2H, d, J=8.8), 8.86 (1H, br s).

45c: Yield, 87%. Colorless amorphous solid. IR (KBr): 1730, 1635 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.6), 1.25 (48H, s), 1.42 (9H, s), 1.49 (9H, s), 1.30—1.85 (10H, m), 1.95—2.50 (10H, m), 4.04 (1H, dd, J=6.6, 12.0), 4.23 (1H, dd, J=3.6, 12.0), 4.65 (1H, m), 5.10 (1H, m), 7.01 (1H, d, J=7.4), 7.54 (1H, br s), 7.61 (2H, d, J=8.6), 7.80 (2H, d, J=8.6).

46c: Yield, 82%. Colorless powder. IR (KBr): 1730, 1670, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (6H, t, J=6.6), 1.25 (48H, s), 1.15—1.80 (10H, m), 2.00—2.55 (10H, m), 4.10 (1H, m), 4.21 (1H, m), 4.65 (1H, m), 5.08 (1H, m), 7.47 (2H, d, J=8.2), 7.64 (2H, d, J=8.2), 7.71 (1H, m), 8.58 (1H, br s).

Method J, *N*-{4-[*O*-[2,3-Bis(hexadecanoyloxy)propyl]-L-serylamino]-benzoyl}-L-glutamic Acid Hydrochloride (48) A mixture of **45a** (240 mg) in AcOEt (8 ml) was hydrogenated over 10% Pd–C (120 mg) at room temperature. The catalyst was filtered off and the filtrate was concentrated under reduced pressure. The residue was chromatographed on silica gel ($CHCl_3$: MeOH = 40 : 1) to give di-*tert*-butyl *N*-{4-[*O*-[2,3-bis(hexadecanoyloxy)propyl]-L-serylamino]benzoyl}-L-glutamate (**47**, 176 mg; 93%) as a brown wax. IR (neat): 3330, 1730, 1650, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.6), 1.25 (48H, s), 1.42 (9H, s), 1.49 (9H, s), 1.50—1.70 (4H, m), 1.95—2.50 (10H, m), 3.55—3.75 (3H, m), 3.80 (2H, d, J=4.6), 4.14 (1H, m), 4.31 (1H, m), 4.66 (1H, m), 5.23 (1H, m), 6.99 (1H, d, J=7.6), 7.68 (2H, d, J=8.8), 7.82 (2H, d, J=8.8), 9.75 (1H, br s).

A solution of **47** (176 mg) in 4 N HCl in ethyl acetate (6 ml) was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure and the residual solid was washed with isopropyl ether and dried *in vacuo* to give **48** (150 mg, 93%) as a colorless powder. IR (KBr): 3600—2600, 1730, 1720, 1700, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (6H, t, J=6.6), 1.25 (48H, s), 1.40—1.65 (4H, m), 1.90—2.60 (8H, m), 3.60—3.75 (2H, m), 3.90—4.40 (4H, m), 4.50—4.65 (2H, m), 5.23 (1H, m), 7.25—7.50 (2H, m), 7.49 (2H, d, J=8.8).

Method K, *N*-[4-(6-Hexadecanoylamino-7-hexadecanoyloxy-4-thiaheptanoylaminomethyl)benzoyl]-L-glutamic Acid (56) A solution of trityl chloride (1.17 g) in CH_2Cl_2 (5 ml) was added to a stirred mixture of **24a** (944 mg) and Et_3N (606 mg) in CH_2Cl_2 (20 ml) and the whole was stirred at room temperature for 30 min, then concentrated under reduced pressure. The residue was chromatographed on silica gel ($CHCl_3$) to give *tert*-butyl 6-hydroxy-7-trityloxy-4-thiaheptanoate (**49**, 1.659 g, 87%) as a colorless oil. IR (neat): 3250, 1730 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (9H, s), 2.40—2.80 (7H, m), 3.18 (1H, dd, J=5.2, 9.6), 3.24 (1H, dd, J=5.6, 9.6), 3.85 (1H, m), 7.15—7.50 (15H, m).

A solution of diethyl azodicarboxylate (582 mg) in THF (1 ml) was added dropwise to a stirred mixture of **49** (800 mg), triphenylphosphine (877 mg) and phthalimide (370 mg) in THF (16 ml), and the mixture was stirred at room temperature for 5 h, then concentrated under reduced

pressure. The residue was chromatographed on silica gel ($CHCl_3$) to give *tert*-butyl 6-phthaloylamino-7-trityloxy-4-thiaheptanoate (**50**, 831 mg, 82%) as a colorless oil. IR (neat): 1770, 1710 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (9H, s), 2.36 (2H, t, J=7.5), 2.55—2.75 (2H, m), 3.15—3.50 (3H, m), 3.78 (1H, dd, J=7.8, 14.0), 4.00 (1H, dd, J=6.5, 14.0), 7.00—7.45 (15H, m), 7.65—7.85 (4H, m).

Hydrazine (88 mg) was added to a stirred solution of **50** (239 mg) in THF (2 ml)–EtOH (2 ml) and the mixture was stirred at room temperature for 2 d. The resulting precipitate was filtered off and the filtrate was concentrated under reduced pressure. The residue **51** was dissolved in THF (5 ml), and Et_3N (277 mg) was added, followed by palmitoyl chloride (375 mg). The reaction mixture was stirred at room temperature for 30 min, and then concentrated under reduced pressure. The residue was chromatographed on silica gel ($CHCl_3$: MeOH = 100 : 1) to give *tert*-butyl 6-hexadecanoylamino-7-trityloxy-4-thiaheptanoate (**52**, 959 mg, 98%) as a colorless oil. IR (neat): 1730, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.8), 1.25 (24H, s), 1.45 (9H, s), 1.40—4.65 (2H, m), 2.04 (2H, t, J=7.4), 2.44 (2H, t, J=7.0), 2.65—2.90 (3H, m), 3.25—3.45 (3H, m), 3.50—3.65 (1H, m), 5.92 (1H, br t, J=5.4), 7.15—7.50 (15H, m).

A stirred solution of **52** (239 mg) in CH_2Cl_2 (0.5 ml) was treated with TFA (1 ml) and the mixture was stirred at room temperature for 30 min, then concentrated under reduced pressure. The residue **53** was dissolved in DMF (5 ml) and **6e** (145 mg) and DEPC (83 mg) were added to the mixture, followed by Et_3N (130 mg). The reaction mixture was stirred at room temperature for 30 min, and then concentrated under reduced pressure. The residue was chromatographed on silica gel ($CHCl_3$: MeOH = 10 : 1) to give di-*tert*-butyl *N*-[4-(6-hexadecanoylamino-7-hydroxy-4-thiaheptanoylaminomethyl)benzoyl]-L-glutamate (**54**, 228 mg, 86%) as a colorless wax. IR (neat): 3300, 1730, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.25 (24H, s), 1.42 (9H, s), 1.49 (9H, s), 1.45—1.70 (2H, m), 1.80—3.00 (10H, m), 3.20—3.50 (2H, m), 3.60—3.80 (1H, m), 4.00—4.30 (2H, m), 4.49 (2H, d, J=5.6), 4.64 (1H, m), 6.60 (1H, br t, J=6.6), 6.90 (1H, br t, J=5.6), 7.15 (1H, d, J=7.6), 7.34 (2H, d, J=8.2), 7.76 (2H, d, J=8.2).

Palmitoyl chloride (87 mg) was added to a mixture of **54** (228 mg) and DMAP (42 mg) in CH_2Cl_2 (5 ml) and the mixture was stirred at room temperature for 30 min, then concentrated under reduced pressure. The residue was chromatographed on silica gel ($CHCl_3$: MeOH = 100 : 1) to give di-*tert*-butyl *N*-[4-(6-hexadecanoylamino-7-hexadecanoyloxy-4-thiaheptanoylaminomethyl)benzoyl]-L-glutamate (**55**, 253 mg, 85%) as a colorless wax. IR (neat): 3300, 1730, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.8), 1.25 (48H, s), 1.42 (9H, s), 1.49 (9H, s), 1.40—1.70 (4H, m), 1.90—2.70 (10H, m), 2.80—3.05 (2H, m), 3.20—3.60 (2H, m), 4.00—4.40 (3H, m), 4.50 (2H, d, J=5.8), 4.64 (1H, m), 6.39 (1H, br t, J=5.8), 6.82 (1H, br t, J=5.6), 7.09 (1H, d, J=7.4), 7.35 (2H, d, J=8.2), 7.76 (2H, d, J=8.2).

56 (181 mg; 83%) was prepared from **55** (245 mg) by a similar method to that described for **30a**. Colorless powder. IR (KBr): 3305, 1731, 1643 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (6H, t, J=6.8), 1.25 (48H, s), 1.50—1.70 (4H, m), 2.05—2.70 (10H, m), 2.80—3.10 (3H, m), 3.30—3.40 (2H, m), 4.06 (1H, dd, J=6.0, 11.0), 4.32 (1H, dd, J=4.8, 11.0), 4.47 (2H, m), 4.68 (1H, m), 7.36 (2H, d, J=8.2), 7.77 (2H, d, J=8.2).

Method L, *N*-[4-(7-Hexadecanoylamino-6-hexadecanoyloxy-4-thiaheptanoylaminomethyl)benzoyl]-L-glutamic Acid (63) A solution of *m*CPBA (3.75 g) in $CHCl_3$ (25 ml) was added to a solution of **57** (2.25 g) in $CHCl_3$ (25 ml) at 0 °C and the mixture was stirred at room temperature for 5 h, then concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane : AcOEt = 1 : 1) to give **58** as a colorless solid. The prepared **58** was dissolved in THF (20 ml), and **21a** (5 ml) was added to the solution, followed by 28% sodium methylate (1 ml). The reaction mixture was stirred at room temperature for 2 h, and then concentrated under reduced pressure. The residue was chromatographed on silica gel ($CHCl_3$: MeOH = 20 : 1) to give methyl 7-hexadecanoylamino-6-hydroxy-4-thiaheptanoate (**59**, 1.225 g, 30%) as a colorless powder. IR (KBr): 3300, 1730, 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.8), 1.25 (24H, s), 1.50—1.70 (2H, m), 2.21 (2H, t, J=7.4), 2.50—2.95 (6H, m), 3.24 (1H, m), 3.50—3.90 (3H, m), 3.71 (3H, s), 6.12 (1H, br s).

A stirred solution of **59** (1.225 g) in THF (30 ml)–MeOH (5 ml) was treated with 1 N NaOH (3 ml). The reaction mixture was stirred at room temperature for 5 h, concentrated under reduced pressure and adjusted to pH 1 with 1 N HCl. The resulting precipitate was collected by filtration, washed with water and dried *in vacuo* to give 7-hexadecanoylamino-6-hydroxy-4-thiaheptanoic acid (**60**, 1.06 g, 90%) as a colorless powder. IR (KBr): 3300, 1700, 1635 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=6.6), 1.25 (24H, s), 1.60—1.70 (2H, m), 2.22 (2H, t, J=7.4), 2.45—2.90 (5H, m), 3.15—3.95 (4H, m), 6.10 (1/2 × 1H, br s), 6.28 (1/2 × 1H, br s).

A stirred mixture of **60** (150 mg) and **6d** (190 mg) in DMF (6 ml) was treated with DEPC (88 mg) at 0 °C, then Et_3N (110 mg) was added. The reaction mixture was stirred at room temperature for 1 h, then concentrated under reduced pressure, and the residue was chromatographed on silica gel (AcOEt) to give di-*tert*-butyl *N*-[4-(7-hexadecanoylamino-6-hydroxy-4-thiaheptanoylaminomethyl)benzoyl]-L-glutamate (**61**, 170 mg, 60%) as a colorless wax. IR (neat): 3300, 1730, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.6), 1.25 (24H, s), 1.42 (9H, s), 1.49 (9H, s), 1.50—1.70 (2H, m), 1.90—2.70 (9H, m), 2.86 (2H, t, J=6.6), 3.16 (1H, m), 3.50 (1H, m), 3.77 (1H, m), 4.09 (1H, m), 4.15 (1H, br s), 4.47 (2H, d, J=5.8), 4.62 (1H, m), 6.38 (1H, br s), 6.99 (1H, br s), 7.23 (1H, d, J=7.6), 7.32 (2H, d, J=8.2), 7.73 (2H, d, J=8.2).

Palmitoyl chloride (60 mg) was added to a mixture of **61** (165 mg) and DMAP (38 mg) in CH_2Cl_2 (5 ml) and the whole was stirred at room temperature for 30 min, then concentrated under reduced pressure. The residue was chromatographed on silica gel ($CHCl_3$: MeOH = 20 : 1) to give di-*tert*-butyl *N*-[4-(7-hexadecanoylamino-6-hexadecanoyloxy-4-thiaheptanoylaminomethyl)benzoyl]-L-glutamate (**62**, 214 mg, 99%) as a colorless wax. IR (neat): 3300, 1730, 1645 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.6), 1.25 (48H, s), 1.42 (9H, s), 1.49 (9H, s), 1.45—1.65 (4H, m), 1.90—3.05 (14H, m), 3.29 (1H, m), 3.70 (1H, m), 4.35—4.55 (2H, m), 4.65 (1H, m), 4.99 (1H, m), 5.88 (1H, br s), 7.06 (1H, br s), 7.08 (1H, d, J=7.0), 7.37 (2H, d, J=8.2), 7.76 (2H, d, J=8.2).

63 (134 mg; 72%) was prepared from **62** (210 mg) by a similar method to that described for **30a**. Colorless powder. IR (KBr): 3307, 1731, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.88 (6H, t, J=6.2), 1.25 (48H, s), 1.45—1.65 (4H, m), 2.00—2.70 (12H, m), 2.80—2.95 (2H, m), 3.33 (1H, m), 3.59 (1H, m), 4.40—4.50 (2H, m), 4.67 (1H, m), 4.99 (1H, m), 6.50 (1H, br s), 7.34 (2H, d, J=8.2), 7.50—7.70 (2H, m), 7.74 (2H, d, J=8.2).

Method M, *N*-{4-[6,7-Bis(hexadecaoxy)-4-thiaheptanoylamino]-benzoyl}-L-glutamic Acid (68) A mixture of 2,3-dihexadecanoxy-1-mercaptopropane (**64**,[7] 933 mg) and **19a** (5.0 ml) was treated with Et_3N (0.5 ml) and the whole was stirred at 60 °C overnight under an argon atomosphere. The mixture was concentrated under reduced pressure and the residue was chromatographed on silica gel (hexane : AcOEt = 20 : 1) to give tert-butyl 6,7-bis(hexadecanyloxy)-4-thiaheptanoate (**65**, 811 mg, 73%) as a colorless oil. IR (neat): 2925, 2850, 1740, 1140 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.0), 1.26 (52H, m), 1.45 (9H, s), 1.55 (4H, t, J=7.0), 2.52 (2H, t, J=7.6), 2.70 (2H, m), 2.79 (2H, t, J=7.2), 3.40—3.57 (7H, m).

A solution of **65** (810 mg) in CH_2Cl_2 (5 ml) was treated with TFA (0.5 ml). The mixture was stirred at room temperature for 2.5 h, then concentrated under reduced pressure to give 6,7-bis(hexadecanyloxy)-4-thiaheptanoic acid (**66**, 800 mg, 100%) as a colorless wax. IR (KBr): 2925, 2850, 1720, 1160 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.0), 1.26 (52H, m), 1.55 (4H, t, J=6.2), 2.52 (2H, t, J=6.8), 2.70 (2H, t, J=5.2), 2.80 (2H, t, J=7.0), 3.40—3.58 (7H, m).

Di-*tert*-butyl *N*-{4-[6,7-bis(hexadecanyloxy)-4-thiaheptanoylamino]-benzoyl}-L-glutamate (**67**, 183 mg, 65%) was prepared from **66** (180 mg) and **8a** (237 mg) by a similar method to that described for **29d**. Colorless syrup. IR (neat): 3360, 3260, 1715, 1690, 1590 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.6), 1.02—2.20 (58H, m), 1.46 (9H, s), 1.59 (9H, s), 2.32—2.46 (2H, m), 2.62—3.00 (6H, m), 3.40—3.70 (6H, m), 3.70—3.89 (1H, m), 7.73 (2H, d, J=8.6), 7.78 (2H, d, J=8.6), 8.40 (1H, br s).

68 (105 mg; 100%) was prepared from **67** (118 mg) by a similar method to that described for **30a**. Colorless powder. IR (KBr): 3410, 1730, 1710, 1640, 1525 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t, J=6.6), 1.02—3.00 (64H, m), 3.31—3.70 (7H, m), 4.45—4.70 (1H, m), 7.30—7.63 (4H, m), 7.75—7.95 (1H, m), 9.02—9.15 (1H, m).

Preparation of BMCs BMCs were obtained from the femora of female BALB/c mice aged 8—10 weeks (Japan Charles River, Japan). The femora were removed aseptically, and the marrow was flushed and suspended in RPMI-1640 containing 10% FCS and 50 μM 2-mercaptoethanol. The suspension was passed through cotton gauze and centrifuged. The cells then were resuspended in the same medium at an appropriate concentration.

Proliferation of BMCs BMCs in 0.1 ml of culture medium were incubated with samples at 37 °C for 3 d in an atmosphere of 5% CO_2

in air. The proliferation of the cells was measured by means of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) colorimetry.[11]

Recovery of the Number of White Blood Cells in an Experimental Leukopenia Model 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 administration of CY according to Hattori *et al.* with a slight modification.[12] Female CDF1/Crj mice aged 6 weeks ($n=5$) were orally given 150 mg/kg of CY in physiological saline on day 0. Solutions of compounds were administered subcutaneously to the mice once a day from day 1 to day 5. Physiological saline and 5% glucose (0.2 ml/20 g body weight) were administered to vehicle control groups instead of CY and test compound, 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 (% of control) was calculated based on that of the vehicle control group as 100%. The % of control in the CY control group was 41 ± 11% (mean $\pm$ SD) throughout the study.

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