Synthesis of Optically Active Lipopeptide Analogs from the Outer Membrane of *Escherichia coli*

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The synthesis of optically active lipopeptide derivatives has been accomplished by the use of chiral glycerol derivatives. Lipopeptide derivatives with (*R*)-glycerol moieties showed higher mitogenic activities than those with the (*S*)-configuration. *N*-2,2,2-Trichloroethoxycarbonyl lipopeptide derivatives increased mitogenic activity.

Keywords peptide synthesis; lipoprotein; mitogenic activity; chiral glycerol derivative; *S*-[2,3-bis(palmitoyloxy)propyl]-*N*-trichloroethoxycarbonyl pentapeptide

Lipoprotein[1] from the outer membrane of *Escherichia coli* and other Enterobacteriaceae is a potent polyclonal activator for *B* lymphocytes. It is composed of 58 amino acids with one amidelinked- and two ester linked-fatty acids attached to *S*-(2,3-dihydroxypropyl)cysteine at the N-terminus, which contains a mixture of different fatty acids, palmitic acid being the main component (Chart 1).

To determine the molecular structure responsible for the biological activities of lipoprotein, a series of oligopeptide analogs of its *N*-terminal part containing only palmitoyl residues were synthesized.[2,3] *S*-[2,3-Bis(palmitoyloxy)-(2-*RS*)-propyl]-*N*-palmitoyl-(*R*)-cysteinyl-(*S*)-seryl-(*S*)-seryl-(*S*)-asparaginyl-(*S*)-alanine was an active mitogen and polyclonal B lymphocyte activator *in vitro* and *in vivo*.[4-6] It also supplements *Salmonella vaccines*.[7]

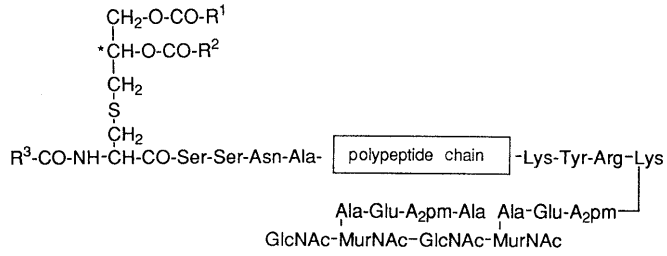


Chart 1. Lipoprotein

In the preceding paper,[8] we have reported a new synthesis of *S*-[2,3-bis(palmitoyloxy)-(2*R* and 2*S*)-propyl]-*N*-palmitoyl-(*R*)-cysteinyl-(*S*)-seryl-(*S*)-seryl-(*S*)-asparaginyl-(*S*)-alanine (**1** and **3**) and their *N*-2,2,2-trichloroethoxycarbonyl (Troc) derivatives (**2** and **4**) by using *N*-(2,2,2-trichloroethoxycarbonyl)cysteinyl intermediates, which prevent a racemization of their cysteinyl parts in the condensation steps. The biological assay results of these compounds indicated that the natural [(2*R*)-propyl] type **1** has a higher activity than the unnatural [(2*S*)-propyl] type **3**, and that their Troc derivatives increase mitogenic activities.[9] Comparison of the activities among compounds **1**, **2**, **3** and **4** showed that the most active was compound **2**. Therefore, we focused our attention on compound **2**. In order to discover more highly active derivatives and structure–activity relationships, we synthesized from the lipopenta- to the lipomonopeptide trichloroethoxycarbonyl derivatives **2**, **8**, **7**, **6** and **5**. Likewise to find out the effect of a trichloroethoxycarbonyl group on mitogenic activity, we synthesized derivatives **9** and **10**. We wish to report

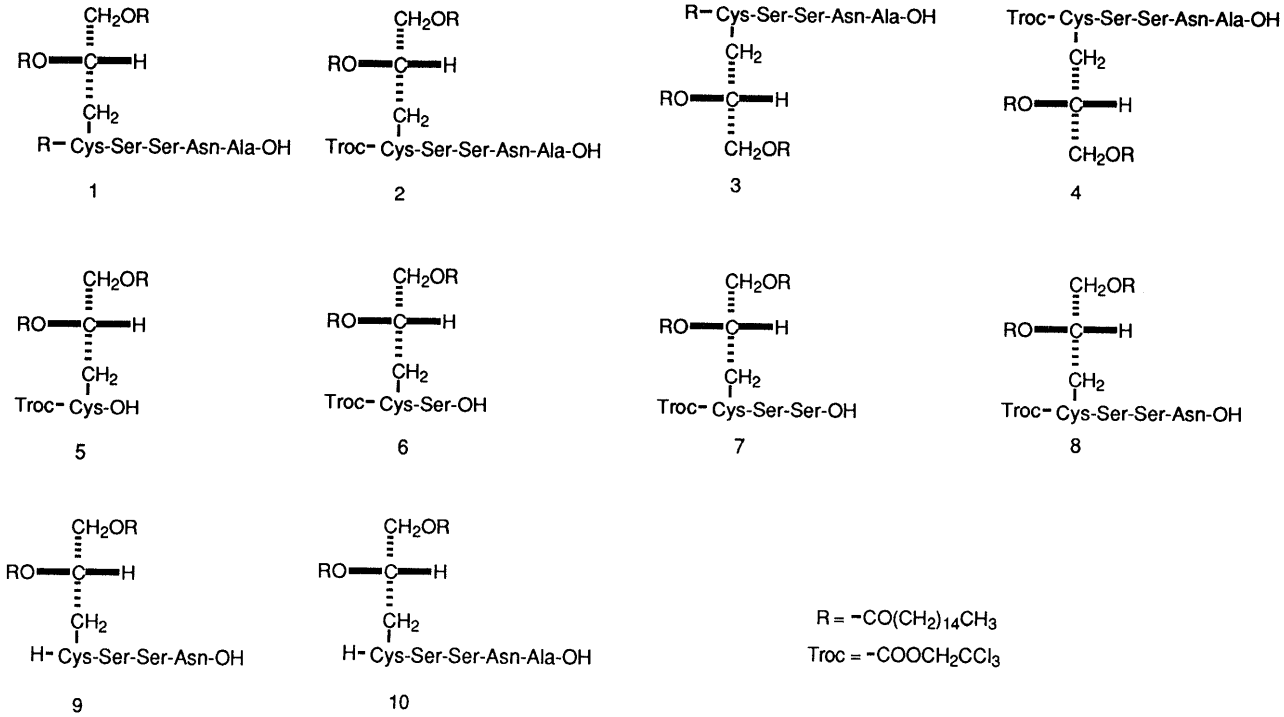


Chart 2

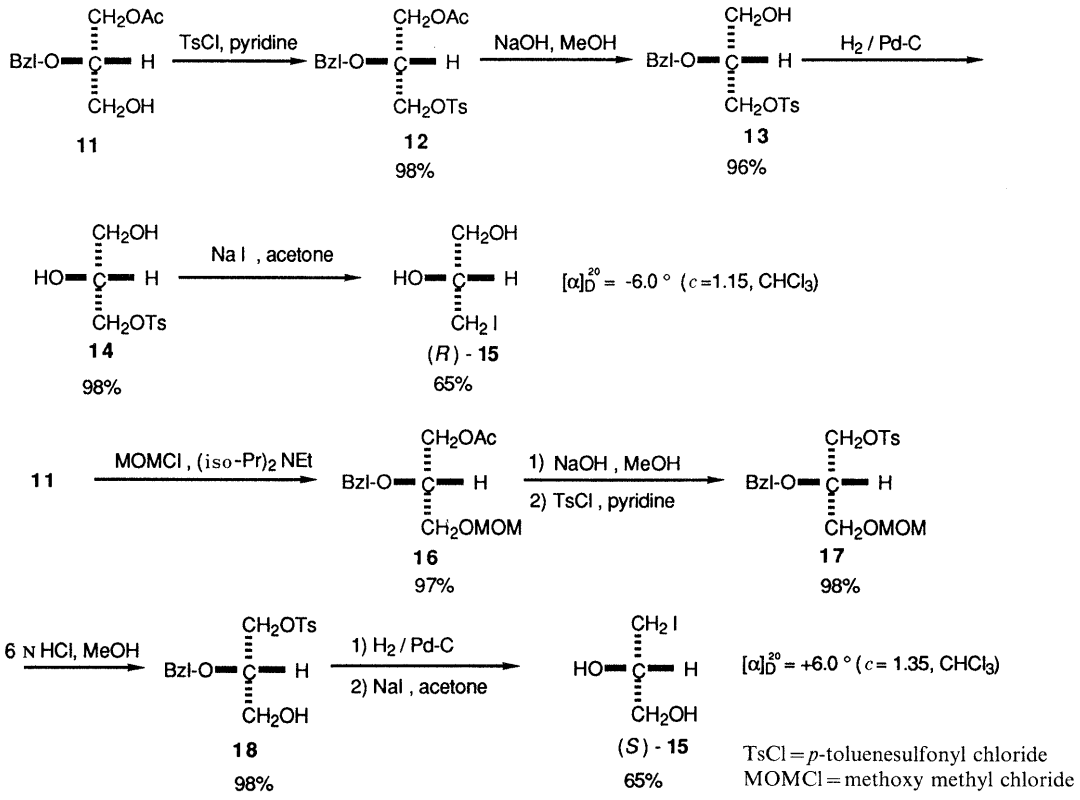


Chart 3

here the synthetic details and mitogenic activity of these derivatives (**1**—**10**) (Chart 2).

K. H. Wiesmuller *et al.*[2] have reported the synthesis of lipopeptides from racemic 3-bromo-1,2-propanediol. Therefore, we have synthesized lipopeptides from optically active glycerol derivatives. Natural lipopeptide **1** can be obtained from (*R*)-1-iodoglycerol (*R*)-**15**. On the other hand, unnatural lipopeptide **3** can be obtained from (*S*)-1-iodoglycerol (*S*)-**15**.

Compounds (*R*)-**15** and (*S*)-**15** were synthesized according to the reaction sequence shown in Chart 3. K. Achiwa *et al.*[10] reported that a chiral glycerol derivative (*S*)-1-*O*-acetyl-2-*O*-benzyl glycerol **11** is prepared by lipase-catalyzed asymmetric transesterification. Thus compound **11** was prepared according to Achiwa's method. Treatment of **11** with *p*-toluenesulfonyl chloride in pyridine followed by hydrolysis with sodium hydroxide in ethanol gave **13** in 94% yield from **11**. Hydrogenolysis of **13** over 5% Pd–C in ethanol gave **14** in 98% yield. Treatment of **14** with NaI in a pressure bottle afforded (*R*)-**15** in 65% yield. (*S*)-**15** was synthesized in a following step. Treatment of **11** with methoxymethyl chloride in CH_2Cl_2 gave **16** in 97% yield. Hydrolysis of **16** with sodium hydroxide in ethanol followed by treatment with *p*-toluenesulfonyl chloride in CH_2Cl_2 afforded **17** in 98% yield. After demethoxymethylation of **17** with 6 N HCl in MeOH, the resulting **18** was hydrogenated over 5% Pd–C in ethanol, and treated with NaI in a pressure bottle to afford (*S*)-**15** in 65% yield. These compounds (*R*)-**15** and (*S*)-**15** showed $[\alpha]_D^{22}$ $-6.0°$ ($c=1.35$, $CHCl_3$) and $[\alpha]_D^{22}$ $+6.0°$ ($c=1.23$, $CHCl_3$), respectively.

Compounds **1**, **2**, **3**, **4** and **5** were synthesized according to the reaction sequence shown in Chart 4. The starting material, **19**, was prepared according to the method reported by K. H. Wiesmuller *et al.*[2] N-protection of **19** with 2,2,2-trichloroethoxychloroformate (3 eq) in pyridine, followed by reduction with dithioerythritol (4 eq) in $CHCl_3$ in the presence of triethylamine (3 eq) afforded **21**, which was used without further purification. In coupling **21** with glycerol moieties, the natural lipopeptide **1** could be obtained by (*R*)-**15** and the unnatural **3** by (*S*)-**15**. Compounds **1**, **2** and **5** were synthesized from (*R*)-**15** *via* the following steps. Reaction of **21** with (*R*)-**15** in dimethylformamide (DMF) in the presence of *N*,*N*-diisopropylethylamine (4 eq) gave **22** (55% from **20**). Esterification of **22** with palmitoyl chloride (2 eq) and *N*,*N*-diisopropylethylamine (4 eq) in CH_2Cl_2 in the presence of a catalytic amount of 4-dimethylaminopyridine (DMAP), followed by deprotection of the *tert*-butyl group of **23** with trifluoroacetic acid, afforded **5** in 69% yield from (*R*)-**15**. Compound **5** was employed for coupling with tetrapeptide H–Ser(Bu^t)–Ser(Bu^t)–Asn–Ala–OBu^t **30**, which was prepared by stepwise chain elongation using the DCC–HOBt method[11] as shown in Chart 4. **24** was condensed with Z (carbobenzoxy)–Asn–OH by the DCC–HOBt method in DMF in the presence of *N*-methylmorpholine to give **25**[11] in 68% yield. The Z group of **25** was removed by hydrogenation, and the free base **26** was coupled to Z–Ser(Bu^t)–OH to afford **27** in 76% yield. In the same way, **27** was hydrogenated to give **28**, which was coupled to Z–Ser(Bu^t)–OH to afford **29** in 47% yield. The Z group of **29** was removed by hydrogenation to afford **30**[2], which is the same partially protected tetrapeptide as reported by K. H. Wiesmuller *et al.*[2] Compound **31** was obtained by coupling **5** with **30**[2] in DMF by the DCC–HOBt method in 50% yield. Deprotection of all

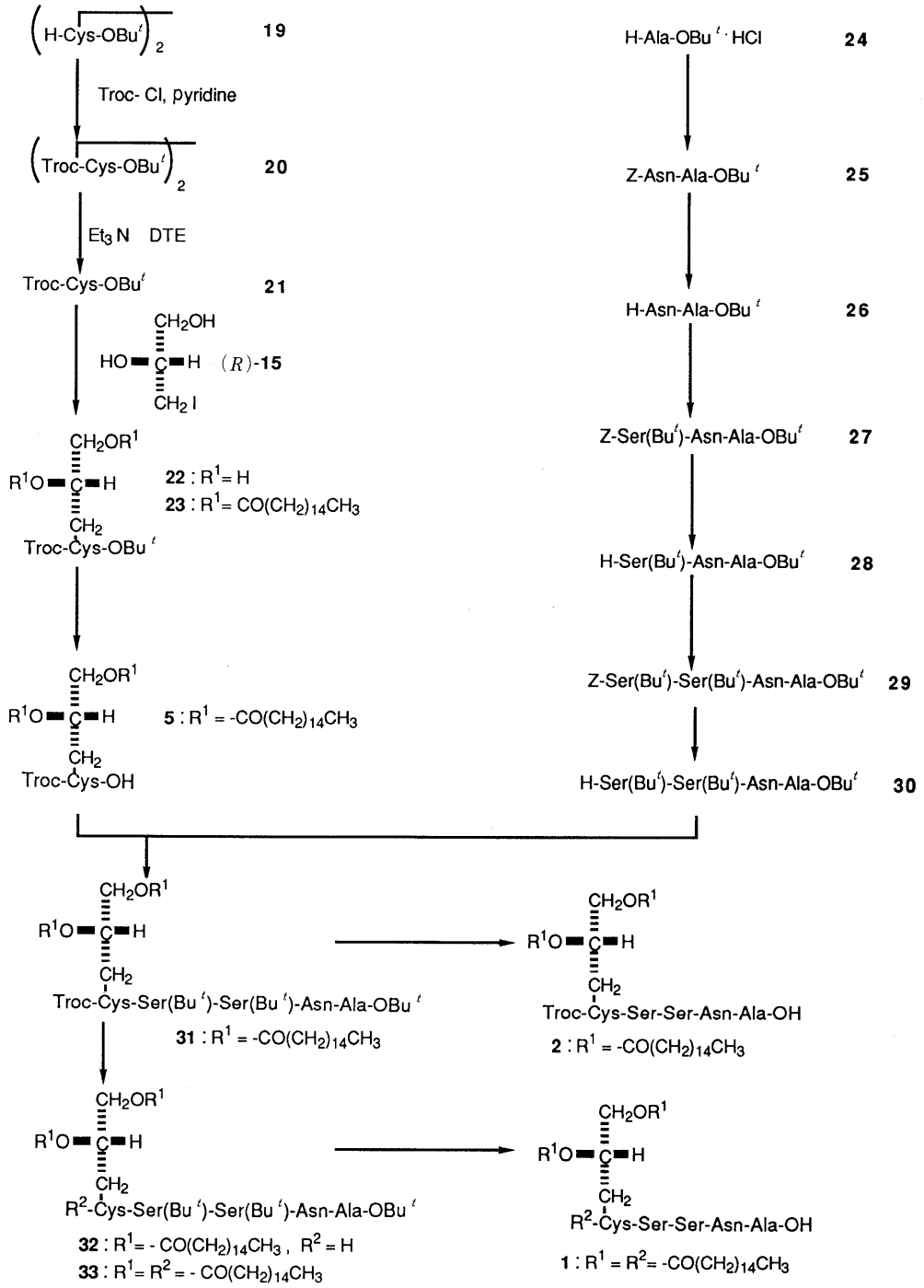
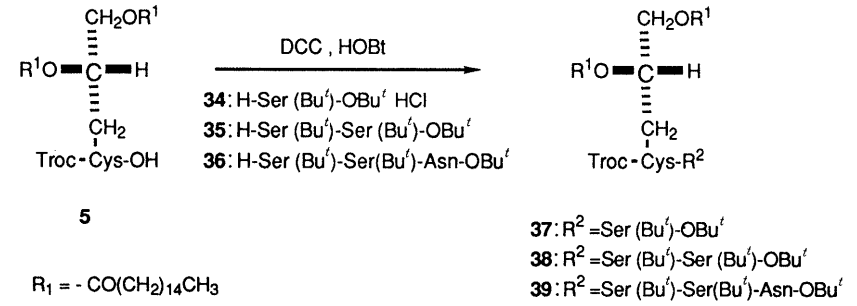


Chart 4

tert-butyl groups of **31** was carried out by treatment with trifluoroacetic acid to give **2** in 45% yield. In the same route, the unnatural Troc derivative **4** was synthesized by using (*S*)-**15** in place of (*R*)-**15**. The trichloroethoxycarbonyl group of **31** was removed by treatment with zinc in acetic acid to give **32**, which was then acylated in the presence of a catalytic amount of DMAP with palmitoyl chloride and *N*,*N*-diisopropylethylamine in CH_2Cl_2 to afford **33**. The final deprotection of all *tert*-butyl groups of **33** was carried out by treatment with trifluoroacetic acid to give **1** (53% yield from **31**). In the same way, unnatural compound **3** was synthesized by using (*S*)-**15** in place of (*R*)-**15**.

Compounds **6**, **7** and **8** were synthesized according to the reaction sequence shown in Chart 5. Compound **5** was condensed with the peptides **34**, **35** and **36** by the DCC–HOBt method in DMF to give **37** (57%), **38** (67%) and **39** (52%), respectively. The final deprotection of all *tert*-butyl groups of **37**, **38** and **39** was carried out by treatment with trifluoroacetic acid to give **6** (50%), **7** (53%) and **8** (67%), respectively.

Compounds **9** and **10** were synthesized according to the reaction sequence shown in Chart 6. The trichloroethoxycarbonyl groups of **36** and **31** were removed by treatment



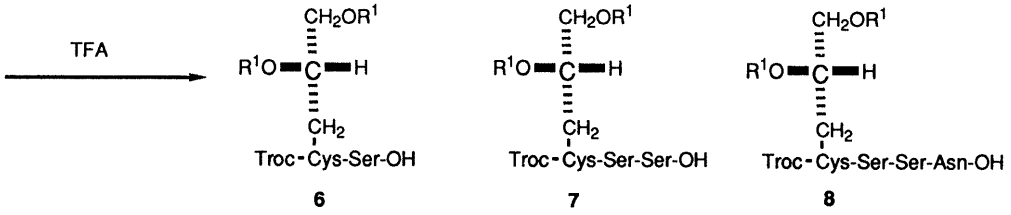
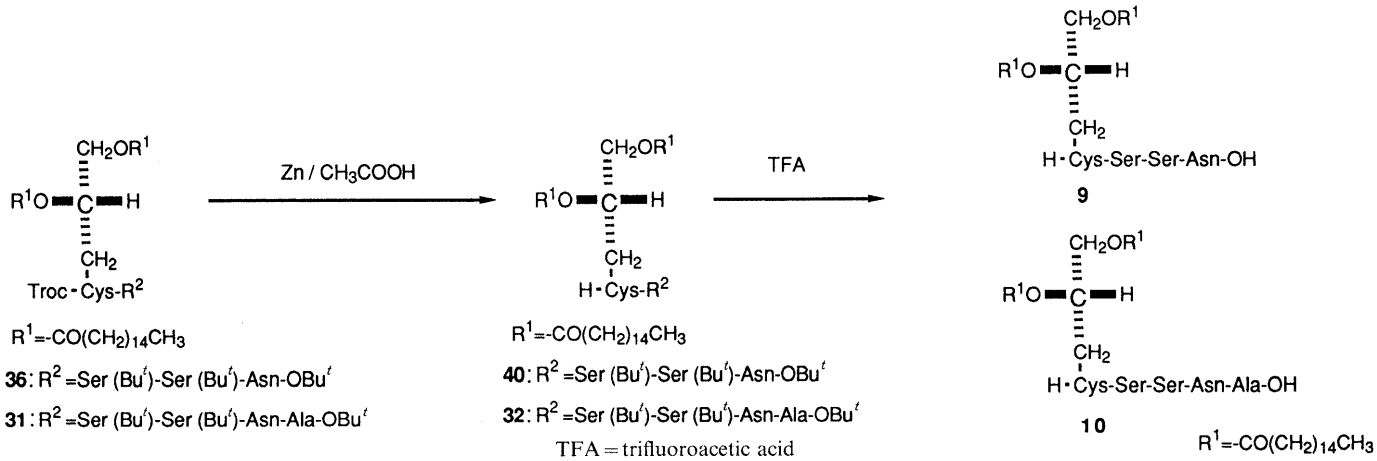


Chart 5



TFA = trifluoroacetic acid

Chart 6

with zinc in acetic acid to give **40** (84%) and **32** (85%), respectively. The final deprotection of all *tert*-butyl groups of **40** and **32** was carried out by treatment with trifluoroacetic acid to give **9** (58%) and **10** (64%).

The structures of **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **9** and **10** were supported by elemental analysis and confirmed by analysis of the infrared (IR), proton nuclear magnetic resonance (^{1}H-NMR) and fast atom bombardment mass spectrum (FAB-MS) spectra. The mitogenic activities of all compounds (**1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **9** and **10**) were measured. Of compounds **1**, **2**, **3** and **4**, **1** and **4** maintained the same degree of activity. The activity of **2** was greatly enhanced, while the activity of compound **3** was weak. These results indicated that the natural [(2*R*)-propyl] type **1** has higher activity than the unnatural [(2*S*)-propyl] type **3**, and that the Troc derivative increases mitogenic activity. Comparison of compounds **2**, **8**, **7**, **6** and **5** indicated that after shortening the peptide chain from the lipopenta- to the lipomonopeptide, mitogenic activity is still maintained in a high concentration. However, in a low concentration, the activity of **2** and **8** was still maintained, but **6**, **7** and **5** exhibited weak activity. In compounds **2**, **9** and **10**, the mitogenic activities of **2**, **9** and **10** were maintained in a high concentration; however, in a low concentration, only the activity of **2** and **10** was still maintained.

Experimental

Melting points were determined on a micro melting point BY-1 (Yazawa) and are uncorrected. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. IR spectra were taken on JASCO IR-810 IR spectrophotometers and are given in cm^{-1}. ^{1}H-NMR spectra were recorded on a JEOL JNM-FX90Q (90 MHz) spectrophotometer in $CDCl_3$. Chemical shifts are given in δ (ppm) downfield from tetramethylsilane, and the abbreviations of signal patterns are as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. Thin layer chromatography (TLC) was performed on silica gel (Kiesel $60F_{254}$ on aluminium sheets, Merck). All compounds were located by spraying the TLC plate with 10% phosphomolybdic acid in ethanol and heating it on a hot plate. Preparative TLC was performed on a preparative layer chromatography plate (Kieselgel $60F_{254}$ 2 and 0.5 mm, Merck). Column chromatography was performed on silica gel (Kieselgel 60, 70—230 mesh, Merck).

(*R*)-1-*O*-Acetyl-2-*O*-benzyl-3-tosylglycerol (12) *p*-Toluenesulfonyl chloride (19 g, 0.10 mol) was added to a stirred solution of (*S*)-1-*O*-acetyl-2-*O*-benzylglycerol[10] (13 g, 0.06 mol) in pyridine (60 ml) at 0 °C and the mixture was stirred for 15 h at room temperature. The reaction mixture was poured onto ice-H_2O (100 ml) and extracted with CH_2Cl_2 (100 ml). The organic layer was washed with 1 N HCl (150 ml × 1) and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel with isopropylether (IPE)–$CHCl_3$ (1 : 10) as an eluent to give **12** (22 g 98%) as a colorless oil. **12**: $[\alpha]_D^{22}$ +13.5° (*c* = 1.22, $CHCl_3$). IR (neat): 1742 (ester), 1365, 1178 (SO_2) cm^{-1}. ^{1}H-NMR: 1.99 (3H, s), 2.44 (3H, s), 3.64—3.96 (1H, m), 4.12, (4H, d, *J* = 4.9 Hz), 4.57 (2H, s), 7.30 (5H, s), 7.31 (2H, d, *J* = 8.0 Hz).

(*R*)-1-*O*-Tosyl-2-*O*-benzylglycerol (13) 25% ammonium hydroxide (10 ml) was added to a stirred solution of **12** (22 g, 0.06 mol) in methanol at room temperature and the mixture was stirred for 15 h at the same temperature. The reaction mixture was concentrated *in vacuo* and extracted with CH_2Cl_2 (100 ml). The organic layer was washed with water (50 ml × 3) and brine, and concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel with $CHCl_3$–EtOH (20 : 1) as an eluent to give **13** (18 g, 96%) as a colorless oil. **13**: $[\alpha]_D^{22}$ +29.5° (*c* = 1.01, $CHCl_3$). IR (neat): 1360, 1380 (SO_2) cm^{-1}. ^{1}H-NMR: 2.41 (3H, s), 3.49—3.73 (4H, m), 4.12 (2H, d, *J* = 4.6 Hz), 7.27 (5H, s), 7.30 (2H, d, *J* = 8.3 Hz), 7.76 (2H, d, *J* = 8.3 Hz).

(*R*)-1-*O*-Tosylglycerol (14) **13** (18.4 g, 0.06 mol) was hydrogenated over 5% Pd–C as a catalyst in ethanol (100 ml). After removal of Pd–C, the filtrate was concentrated *in vacuo* to give **14** (13.7 g, 98%) as a colorless crystal. The product was used without further purification. **14**: mp 54—56 °C, $[\alpha]_D^{22}$ −8.36° (*c* = 1.02, MeOH). IR (KBr): 3400 (OH), 1365, 1180 (SO_2) cm^{-1}. ^{1}H-NMR: 2.30 (3H, s), 2.91 (2H, br s), 3.55—3.97 (5H, m), 7.28 (2H, d, *J* = 8.0 Hz), 7.53 (2H, d, *J* = 8.0 Hz).

(*R*)-1-Iodoglycerol (*R*-15) A mixture of **14** (1.9 g, 9.4 mmol) and NaI (3.63 g, 24 mmol) in acetone (5 ml) was stirred for 9 h at 90 °C in a pressure bottle. The reaction mixture was filtrated and the filtrate was concentrated *in vacuo*. Ether (150 ml) was added to the residue and the mixture was stirred for a while and extracted with ether. The organic layer was filtered off and 0.1 N sodium thiosulfate was added to the filtrate to make it colorless. After washing with brine (100 ml) and drying over $MgSO_4$, the solvent was concentrated *in vacuo*. The residue was washed with *n*-hexane (100 ml × 3) and collected by suction to give (*R*)-**15** (1.1 g, 65%) as yellow needles. (*R*)-**15**: mp 33—35 °C, $[\alpha]_D^{22}$ −6.0° (*c* = 1.35, $CHCl_3$). IR (KBr): 3330 (OH) cm^{-1}. ^{1}H-NMR: 2.60—3.20 (2H, br s), 3.23 (2H, d, *J* = 6.0 Hz), 3.40—3.90 (3H, br s).

(*S*)-1-*O*-Acetyl-2-*O*-benzyl-3-*O*-methoxymethylglycerol (16) Methoxymethyl chloride (6.5 g, 81 mmol) in CH_2Cl_2 (20 ml) was added to a stirred mixture of **11** (15 g, 67 mmol) and *N*,*N*-diisopropylethylamine (13 g, 0.10 mol) in CH_2Cl_2 (80 ml) at 0 °C. After being stirred for 15 h at room temperature, the reaction mixture was washed with water (50 ml × 3) and brine (50 ml), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel with *n*-hexane–AcOEt (15 : 1) as an eluent to give **16** (33 g, 97%) as a colorless oil.

(*S*)-1-*O*-Tosyl-2-*O*-benzyl-3-*O*-methoxymethylglycerol (17) A solution of NaOH (2.7 g, 67 mmol) in EtOH (50 ml) was added to **16** (18 g, 67 mmol) in EtOH (80 ml) at 0 °C. After being stirred for 1 h at the same temperature, the mixture was neutralized with 6 N HCl and removed off EtOH *in vacuo*. The residue was extracted with CH_2Cl_2 (100 ml) and the organic layer was washed with brine (50 ml), dried over $MgSO_4$ and concentrated *in vacuo* to give (*R*)-1-*O*-methoxymethyl-2-*O*-benzylglycerol (15 g, 100%). *p*-Toluenesulfonyl chloride (20 g, 0.10 mol) in CH_2Cl_2 (30 ml) was added to a stirred solution of (*R*)-1-*O*-methoxymethyl-2-*O*-benzylglycerol (15 g, 0.67 mol) and triethylamine (10 g, 0.10 mol) in CH_2Cl_2 (80 ml) at 0 °C. After being stirred for 15 h, the reaction mixture was washed with water (50 ml × 3) and brine (50 ml × 1), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel with *n*-hexane–AcOEt (5 : 1) as an eluent to give **17** (25 g, 98%) as a colorless oil.

(*S*)-1-*O*-Tosyl-2-*O*-benzylglycerol (18) 6 N HCl (15 ml) in MeOH (30 ml) was added to **17** (25 g, 65 mmol) in MeOH (80 ml). After being stirred for 8 h at 60 °C, the reaction mixture was neutralized with 2 N NaOH aq., and MeOH was removed *in vacuo*. The residue was extracted with CH_2Cl_2 (100 ml × 3) and the organic layer was washed with brine (100 ml × 1), dried over $MgSO_4$ and concentrated *in vacuo* to give **18** (21 g, 98%) as a colorless oil.

(*S*)-1-Iodoglycerol (*S*-15) **18** was hydrogenated over Pd–C as a catalyst in EtOH (50 ml). The mixture was treated by the same procedure described in the preparation of compound **14** to give (*S*)-1-*O*-tosylglycerol (6.5 g, 99%) as a colorless oil. NaI (3.6 g, 24 mmol) was added to (*S*)-1-*O*-tosylglycerol (1.9 g, 8.1 mmol) in acetone (5 ml). The mixture was treated by the same procedure described in the preparation of the compound **15** to give (*S*)-**15** (1.1 g, 65%) as yellow needles. (*S*)-**15**: mp 32—34 °C, $[\alpha]_D^{22}$ +6.0° (*c* = 1.23, $CHCl_3$). IR (KBr): 3340 (OH) cm^{-1}. ^{1}H-NMR: 2.60—3.20 (2H, br s), 3.23 (2H, d, *J* = 6.0 Hz), 3.40—3.90 (3H, br s).

***N*,*N*-Di-2,2,2-trichloroethoxycarbonylcystine Di-*tert*-butyl Ester (20)** *N*-2,2,2-Trichloroethoxycarbonyl chloride (1.0 g, 4.8 mmol) in CH_2Cl_2 (5 ml) was added dropwise under stirring to the solution of **19** (0.53 g, 1.5 mmol) and pyridine (0.50 g, 6.3 mmol) in CH_2Cl_2 (30 ml). After being stirred for 3 h at room temperature, CH_2Cl_2 (50 ml) was added and washed with 5% citric acid, 5% $NaHCO_3$ aq. and water (50 ml × 3 each). The CH_2Cl_2 layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel with *n*-hexane–AcOET (10 : 1) as an eluent to give **20** (0.47 g, 60%) as a yellow oil. **20**: IR (neat): 3330 (NH), 1728 (ester) cm^{-1}. ^{1}H-NMR: 1.60 (18H, s), 3.26 (4H, d, *J* = 5.6 Hz), 3.80—4.3 (2H, m), 4.85 (4H, s), 5.8 (2H, br s).

***N*-2,2,2-Trichloroethoxycarbonylcysteine *tert*-Butyl Ester (21)** **20** (0.85 g, 1.6 mmol) in $CHCl_3$ (50 ml) was reduced with dithioerythritol (1.0 g, 6.5 mmol) in the presence of triethylamine (0.48 g, 0.48 mmol). After being stirred for 2 h under argon, the solution was washed with 5% citric acid and brine (30 ml × 3 each). After drying over $MgSO_4$, the solvent was removed *in vacuo* to give **21** as a yellow oil. **21** was used without further purification because **21** was easily oxidized in air. **21**: ^{1}H-NMR: 1.60 (9H, s), 3.12 (2H, dd, *J* = 9.0 Hz, *J* = 4.2 Hz), 4.40—4.87 (1H, m), 4.70 (2H, s), 5.83 (1H, br s).

***S*-{2,3-Dihydroxy-(2*R*)-propyl}-*N*-2,2,2-trichloroethoxycarbonylcysteine *tert*-Butyl Ester (*R*-22)** (*R*)-**15** (0.43 g, 2.1 mmol) was added to **21** (0.68 g, 1.9 mmol) in DMF (5 ml) in the presence of *N*,*N*-diisopropylethylamine (1.0 g, 7.7 mmol). After being stirred for 15 h at room temperature, CH_2Cl_2 (50 ml) was added to the reaction mixture and the mixture was washed with 1 N HCl (40 ml × 2) and brine (50 ml × 3). After drying over $MgSO_4$, the solvent was concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel with $CHCl_3$–MeOH (15 : 1) as an eluent to give (*R*)-**22** (0.49 g, 59%) as a yellow oil. (*R*)-**22**: FAB-MS *m*/*z*: 426 $(M+H)^+$. IR (neat): 3330 (OH, NH), 1739 (ester) cm^{-1}. ^{1}H-NMR: 1.47 (9H, s), 2.80—3.17 (6H, m), 3.60 (2H, br s), 4.27—4.57 (2H, m), 4.75 (2H, s), 6.00 (1H, d, *J* = 7.0 Hz).

***S*-{2,3-Bis(palmitoyloxy)-(2*R*)-propyl}-*N*-2,2,2-trichloroethoxycarbonyl-(*R*)-cysteine *tert*-Butyl Ester (*R*-23)** Palmitoyl chloride (0.83 g, 3.0 mmol) in CH_2Cl_2 (5 ml) was added to a stirred solution of (*R*)-**22** (0.64 g, 15 mmol), 4-dimethylaminopyridine (46 mg, 0.38 mmol) and *N*,*N*-diisopropylethylamine (0.78 g, 6.0 mmol) in CH_2Cl_2 (30 ml) at 0 °C. After being stirred for 5 h at room temperature, CH_2Cl_2 (30 ml) was added to the reaction mixture. The CH_2Cl_2 solution was washed with 5% citric acid, 4% $NaHCO_3$ aq. (50 ml × 3 each) and brine (50 ml × 1), dried over $MgSO_4$ and concentrated *in vacuo*, the residue was precipitated as a solid by cooling at −20 °C from MeOH–$CHCl_3$ (3 : 1) to give (*R*)-**23** (1.0 g, 73%) as a white powder. (*R*)-**23**: mp 43 °C, $[\alpha]_D^{22}$ +2.1° (*c* = 1.04, $CHCl_3$). FAB-MS *m*/*z*: 902 $(M+H)^+$. IR (KBr): 3298 (NH), 1739 (ester) cm^{-1}. ^{1}H-NMR: 0.89 (6H, t, *J* = 5.8 Hz), 1.23 (28H, s), 1.49 (9H, s), 2.10—3.17 (6H, m), 4.10—4.33 (2H, m), 4.71 (2H, s), 5.93 (1H, br s).

***S*-{2,3-Bis(palmitoyloxy)-(2*R*)-propyl}-*N*-2,2,2-trichloroethoxycarbonyl-(*R*)-cysteine (5)** CF_3COOH (2 ml) was added to (*R*)-**23** (0.41 g, 0.46 mmol) at room temperature. After being stirred for 1 h, the mixture was evaporated *in vacuo* and CH_2Cl_2 (50 ml) was added to the residue. After washing with water (30 ml × 3), the solution was dried over $MgSO_4$ and evaporated to dryness. The residue was precipitated to a solid by cooling at −20 °C from MeOH–$CHCl_3$ (3 : 1) to give **5** as a colorless powder. **5**: mp 31—33 °C, $[\alpha]_D^{22}$ +13.7° (*c* = 1.21, $CHCl_3$). FAB-MS *m*/*z*: 868 $(M+Na)^+$. IR (KBr): 3334 (OH, NH), 1740 (ester) cm^{-1}. ^{1}H-NMR: 0.93 (6H, t, *J* = 5.8 Hz), 1.33 (28H, s), 2.20—3.27 (6H, m), 3.76—4.33 (2H, m), 4.80 (2H, s), 6.03 (1H, d, *J* = 7.0 Hz). *Anal.* Calcd for $C_{41}H_{74}Cl_3NO_8S \cdot H_2O$: C, 56.90; H, 8.85; N, 1.62. Found: C, 57.42; H, 8.79; N, 1.67.

L-Alanine *tert*-Butyl Ester Hydrochloride (24) Z–Ala–OBu^t (3.8 g, 13 mmol) was hydrogenated over 5% Pd–C as a catalyst in EtOH (80 ml) for 3 h at room temperature. After removal of the catalyst, HCl (0.47 g, 13 mmol) in EtOH was added to the filtrate. The filtrate was evaporated *in vacuo* to give **24** (2.1 g, 87%) as a white powder.

***N*-Carbobenzoxy-L-asparaginyl-L-alanine *tert*-Butyl Ester (25)** Z–Asn–OH (1.1 g, 4.0 mmol), dicyclohexylcarbodiimide (0.91 g, 4.4 mmol) and 1-hydroxybenzotriazole (0.61 g, 4.0 mmol) were added under stirring to solution of **24** (0.72 g, 4.0 mmol) and *N*-methylmorphorine (0.41 g, 4.0 mmol) in dimethylformamide (3 ml). After being stirred for 15 h,

N,*N*-dicyclohexylurea (DCUrea) was filtered off and the filtrate was concentrated *in vacuo*. The residue was dissolved in AcOEt (10 ml) and DCUrea was filtered off again. After evaporation of the solvent, the residue was dissolved in CH_2Cl_2 (100 ml) and washed with 5% citric acid, 4% $NaHCO_3$ aq. (50 ml × 3, each) and brine (50 ml × 1). After drying over $MgSO_4$, the solvent was evaporated *in vacuo*. The residue was dissolved in a small amount of $CHCl_3$ and precipitated by the addition of *n*-hexane and dried *in vacuo* to give **25** (1.1 g, 68%) as a white powder. **25**: mp 144—147 °C, $[\alpha]_D^{22}$ +10.5° (*c* = 1.0, $CHCl_3$). *Anal.* Calcd for $C_{19}H_{27}N_3O_6$: C, 57.84; H, 7.16; N, 10.64. Found: C, 57.45; H, 7.07; N, 10.62.

L-Asparaginyl-L-alanine *tert*-Butyl Ester (26) **25** (0.20 g, 0.5 mmol) was hydrogenated over 5% Pd–C as a catalyst in EtOH (30 ml) for 3 h at room temperature. After removal of Pd–C, the filtrate was concentrated *in vacuo* to give **26** (0.13 g, 97%) as a white powder.

***N*-Carbobenzoxy-*O-tert*-butyl-L-seryl-L-asparaginyl-L-alanine *tert*-Butyl Ester (27)** HOBt (0.12 g, 0.77 mmol) and DCC (0.16 g, 0.77 mmol) were added to a solution of Z–Ser(Bu^t)–OH (0.23 g, 0.77 mmol) and **26** (0.20 g, 0.77 mmol) in DMF (3 ml). After being stirred for 15 h at room temperature, DCUrea was filtered off and the filtrate was concentrated *in vacuo*. The residue was dissolved in AcOEt (10 ml) and DCUrea was filtered off again. The filtrate was concentrated *in vacuo* and CH_2Cl_2 was added to the residue. The CH_2Cl_2 layer was washed with 4% $NaHCO_3$ aq. (50 ml × 3) and brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was dissolved in a small amount of $CHCl_3$ and precipitated to a solid by the addition of *n*-hexane. The colorless product was filtrated, washed with *n*-hexane and dried *in vacuo* to give **27** (0.31 g, 76%) as a white powder. **27**: mp 139—142 °C, $[\alpha]_D^{22}$ +14.8° (*c* = 0.4, $CHCl_3$). *Anal.* Calcd for $C_{26}H_{40}N_4O_8 \cdot 1/2H_2O$: C, 57.21; H, 7.58; N, 10.27. Found: C, 57.56; H, 7.37; N, 10.14.

***O-tert*-Butyl-L-seryl-L-asparaginyl-L-alanine *tert*-Butyl Ester (28)** **27** (0.80 g, 1.5×10^{-3} mol) was hydrogenated over 5% Pd–C as a catalyst in EtOH (50 ml) for 3 h at room temperature. The mixture was treated by the same procedure described in the preparation of compound **26** to give **28** (0.13 g, 97%) as a white powder.

***N*-Carbobenzoxy-*O-tert*-butyl-L-seryl-*O-tert*-butyl-L-seryl-L-asparaginyl-L-alanine *tert*-Butyl Ester (29)** HOBt (0.50 g, 3.3×10^{-3} mol) and DCC (0.74 g, 3.6×10^{-3} mol) was added to a solution of Z–Ser(Bu^t)–OH (0.97 g, 3.3×10^{-3} mol) and **28** (1.3 g, 3.3×10^{-3} mol) in DMF (5 ml). After being stirred for 15 h at room temperature, the mixture was treated by the same procedure described in the preparation of compound **27** to give **29** (1.1 g, 47%) as a white powder. **29**: mp 127—131 °C, $[\alpha]_D^{22}$ +19.8° (*c* = 1.02, $CHCl_3$). FAB-MS *m*/*z*: 680 $(M+H)^+$. *Anal.* Calcd for $C_{33}H_{53}N_5O_{10}$: C, 58.30; H, 7.86; N, 10.30. Found: C, 58.45; H, 7.75; N, 10.32.

***O-tert*-Butyl-L-seryl-*O-tert*-butyl-L-seryl-L-asparaginyl-L-alanine *tert*-Butyl Ester (30)** **29** (0.78 g, 1.2×10^{-3} mol) was hydrogenated over 5% Pd–C as a catalyst in EtOH (50 ml) for 3 h at room temperature. The mixture was treated by the same procedure described in the compound **26** to give **30** (0.55 g, 88%) as a white powder.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-*N*-2,2,2-trichloroethoxycarbonyl-(*R*)-cysteinyl-*O-tert*-butyl-(*S*)-seryl-*O-tert*-butyl-(*S*)-seryl-(*S*)-asparaginyl-(*S*)-alanine *tert*-Butyl Ester (*R*-31)** HOBt (60 mg, 3.8×10^{-4} mol) and DCC (90 mg, 4.1×10^{-4} mol) were added to a solution of **5** (0.32 g, 3.8×10^{-3} mol) and **30** (0.21 g, 3.8×10^{-3} mol) in DMF (3 ml). After being stirred for 15 h, the mixture was treated by the same procedure described in the preparation of compound **27**. The residue was precipitated to a solid by cooling at −20 °C from $CHCl_3$–MeOH (1 : 3) to give (*R*)-**31** (0.25 g, 50%) as a white powder. (*R*)-**31**: mp 132—134 °C, $[\alpha]_D^{22}$ +3.8° (*c* = 1.0, $CHCl_3$). FAB-MS *m*/*z*: 1374 $(M+H)^+$. *Anal.* Calcd for $C_{66}H_{119}Cl_3N_6O_{15}S$: C, 57.65; H, 8.72; N, 6.11. Found: C, 57.63; H, 8.63; N, 5.92.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-(*R*)-cysteinyl-*O-tert*-butyl-(*S*)-seryl-*O-tert*-butyl-(*S*)-seryl-(*S*)-asparaginyl-(*S*)-alanine *tert*-Butyl Ester (*R*-32)** Zinc powder (0.75 g) was added to a stirred solution of (*R*)-**31** (0.15 g, 1.1×10^{-4} mol) in CH_3COOH (2 ml). After being stirred for 15 h at room temperature, CH_2Cl_2 (50 ml) was added to the filtrate and zinc powder was filtered off. The filtrate was washed with sat. $NaHCO_3$ aq. (50 ml × 3) and brine. The CH_2Cl_2 layer was dried over $MgSO_4$ and concentrated *in vacuo* to give (*R*)-**32** (0.13 g, 96%), which was used without further purification. (*R*)-**32**: mp 118—121 °C, $[\alpha]_D^{22}$ −3.6° (*c* = 1.2, $CHCl_3$). FAB-MS *m*/*z*: 1200 $(M+H)^+$.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-*N*-palmitoyl-(*R*)-cysteinyl-*O-tert*-butyl-L-seryl-*O-tert*-butyl-L-seryl-L-asparaginyl-L-alanine *tert*-Butyl Ester (*R*-33)** Palmitoyl chloride (14 mg, 5.0×10^{-5} mol) in CH_2Cl_2 (2 ml) was added to a stirred solution of (*R*)-**32** (60 mg, 5.0×10^{-5} mol), 4-dimethylaminopyridine (2.0 mg, 1.3×10^{-5} mol) and *N*,*N*-diisopropylethylamine (26 mg, 2.0×10^{-4} mol) in CH_2Cl_2 (10 ml) at 0 °C. After being stirred for 5 h at room temperature, the mixture was treated by the same procedure for the preparation of (*R*)-**23** to give (*R*)-**33** (54 mg, 76%) as a white powder. (*R*)-**33**: mp 187—189 °C, $[\alpha]_D^{22}$ −1.9° (*c* = 0.86, $CHCl_3$). FAB-MS *m*/*z*: 1438 $(M+H^+)$. *Anal.* Calcd for $C_{79}H_{148}SN_6O_{14}$: C, 65.98; H, 10.37; N, 5.84. Found: C, 65.60; H, 10.40; N, 5.44.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-*N*-palmitoyl-(*R*)-cysteinyl-(*S*)-seryl-(*S*)-seryl-(*S*)-asparaginyl-(*S*)-alanine (1)** CF_3COOH (2 ml) was added to **33** (70 mg, 4.9×10^{-3} mol). After being stirred for 1 h at room temperature, the mixture was concentrated *in vacuo* and the residue was precipitated to a solid by cooling at −20 °C from MeOH–$CHCl_3$ (3 : 1) to give **1** (33 mg, 53%) as a white powder. **1**: mp 211—213 °C, $[\alpha]_D^{22}$ +56.5° (*c* = 1.02, $CHCl_3$). FAB-MS *m*/*z*: 1270 $(M+H)^+$. IR (KBr): 3324 (OH, NH), 1732 (ester), 1627, 1537 (amide) cm^{-1}. *Anal.* Calcd for $C_{67}H_{124}N_6O_{14} \cdot 2H_2O$: C, 61.57; H, 9.87; N, 6.43. Found: C, 61.83; H, 9.60; N, 6.05.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-*N*-2,2,2-trichloroethoxycarbonyl-(*R*)-cysteinyl-(*S*)-seryl-(*S*)-seryl-(*S*)-asparaginyl-(*S*)-alanine (2)** CF_3COOH (2 ml) was added to (*R*)-**31** (90 mg, 6.6×10^{-5} mol). After being stirred for 1 h at room temperature, the mixture was concentrated *in vacuo* and the residue was precipitated to a solid by cooling at −20 °C from MeOH–$CHCl_3$ (3 : 1) to give **2** (32 mg, 45%) as a white powder. **2**: mp 205—207 °C, $[\alpha]_D^{22}$ +9.20° (*c* = 1.00, $CHCl_3$). FAB-MS *m*/*z*: 1205 $(M+H)^+$. IR (KBr): 3300 (OH, NH), 1736 (ester), 1662, 1537 (amide) cm^{-1}. *Anal.* Calcd for $C_{54}H_{95}Cl_3N_6O_{15}$: C, 53.75; H, 7.93; N, 6.96. Found: C, 53.56; H, 8.17; N, 6.54.

***S*-{2,3-Dihydroxy-(2*S*)-propyl}-*N*-2,2,2-trichloroethoxycarbonyl-(*R*)-cysteine *tert*-Butyl Ester (*S*-22)** (*S*)-**15** (0.98 g, 4.8×10^{-3} mol) was added to **21** (1.6 g, 4.4×10^{-3} mol) in DMF (5 ml) in the presence of *N*,*N*-diisopropylethylamine (2.3 g, 1.8×10^{-2} mol). After being stirred for 15 h at room temperature, CH_2Cl_2 (50 ml) was added to the reaction mixture and the mixture was washed with 1 N HCl (40 ml × 2) and brine (50 ml × 3). After drying over $MgSO_4$, the solvent was concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel with $CHCl_3$–MeOH (15 : 1) as an eluent to give (*S*)-**22** (1.2 g, 62%) as an yellow oil. (*S*)-**22**: FAB-MS *m*/*z*: 426 $(M+H)^+$. IR (neat): 3330 (OH, NH), 1739 (ester) cm^{-1}.

***S*-{2,3-Bis(palmitoyloxy)-(2*S*)-propyl}-*N*-2,2,2-trichloroethoxycarbonyl-(*R*)-cysteine *tert*-Butyl Ester (*S*-23)** Palmitoyl chloride (0.46 g, 1.7×10^{-3} mol) in CH_2Cl_2 (5 ml) was added to a stirred solution of (*S*)-**22** (0.34 g, 8.0×10^{-4} mmol), 4-dimethylaminopyridine (25 mg, 2.0×10^{-4} mmol) and *N*,*N*-diisopropylethylamine (0.41 g, 3.2×10^{-3} mol) in CH_2Cl_2 (30 ml) at 0 °C. After being stirred for 5 h at room temperature, CH_2Cl_2 (30 ml) was added to a reaction mixture. The CH_2Cl_2 solution was washed with 5% citric acid, 4% $NaHCO_3$ aq. (50 ml × 3 each) and brine (50 ml × 1), dried over $MgSO_4$ and concentrated *in vacuo*. The residue was precipitated to a solid by cooling at −20 °C from MeOH–$CHCl_3$ (3 : 1) to give (*S*)-**23** (0.48 g, 70%) as a white powder. (*S*)-**23**: mp 45—46 °C, $[\alpha]_D^{22}$ −2.1° (*c* = 1.04, $CHCl_3$). FAB-MS *m*/*z*: 902 $(M+H)^+$. IR (KBr): 3366 (NH), 1732 (ester) cm^{-1}.

***S*-{2,3-Bis(palmitoyloxy)-(2*S*)-propyl}-*N*-2,2,2-trichloroethoxycarbonyl-(*R*)-cysteine (*S*-5)** CF_3COOH (2 ml) was added to (*S*)-**23** (0.15 g, 1.7×10^{-4} mol). After being stirred for 1 h at room temperature, the mixture was concentrated *in vacuo* and added to CH_2Cl_2 (50 ml). After washing with water (30 ml × 3), the solution was dried over $MgSO_4$ and evaporated to dryness. The residue was precipitated to a solid by cooling at −20 °C from MeOH–$CHCl_3$ (3 : 1) to give (*S*)-**5** (0.11 g, 80%) as a colorless powder. (*S*)-**5**: mp 31—33 °C, $[\alpha]_D^{22}$ +9.7° (*c* = 1.42, $CHCl_3$). FAB-MS *m*/*z*: 868 $(M+Na)^+$. IR (KBr): 3298 (OH, NH), 1739 (ester) cm^{-1}.

***S*-[2,3-Bis(palmitoyloxy)-(2*S*)-propyl]-*N*-2,2,2-trichloroethoxycarbonyl-(*R*)-cysteinyl-*O-tert*-butyl-(*S*)-seryl-*O-tert*-butyl-(*S*)-seryl-(*S*)-asparaginyl-(*S*)-alanine *tert*-Butyl Ester (*S*-31)** HOBt (27 mg, 1.7×10^{-4} mol) and DCC (40 mg, 2.0×10^{-4} mol) were added to the solution of **5** (0.14 g, 1.7×10^{-4} mol) and **30** (95 mg, 1.7×10^{-4} mol) in DMF (3 ml). After being stirred for 15 h, the mixture was treated by the same procedure described in the preparation of compound **27**. The residue was precipitated to a solid by cooling at −20 °C from $CHCl_3$–MeOH (1 : 3) to give (*S*)-**31** (0.11 g, 46%) as white powder. (*S*)-**31**: mp 146—148 °C, $[\alpha]_D^{22}$ −6.6° (*c* = 0.80, $CHCl_3$). FAB-MS *m*/*z*: 1374 $(M+H)^+$. IR (KBr): 3288 (NH), 1739 (ester), 1639, 1541 (amide) cm^{-1}.

***S*-[2,3-Bis(palmitoyloxy)-(2*S*)-propyl]-(*R*)-cysteinyl-*O-tert*-butyl-(*S*)-seryl-*O-tert*-butyl-(*S*)-seryl-(*S*)-asparaginyl-(*S*)-alanine *tert*-Butyl Ester (*S*-32)** Zinc powder (0.75 g) was added to a stirred solution of (*S*)-**31** (0.15 g, 1.1×10^{-4} mol) in CH_3COOH (2 ml). After being stirred for 15 h,

CH_2Cl_2 (50 ml) was added to the filtrate and zinc powder was filtered off. The filtrate was washed with sat. $NaHCO_3$ aq. (50 ml × 3) and brine. The CH_2Cl_2 layer was dried over $MgSO_4$ and concentrated *in vacuo* to give (*S*)-**32** (0.13 g, 96%), which was used without further purification. (*S*)-**32**: mp 122—124 °C, $[\alpha]_D^{22}$ +3.4° (*c*=1.2, $CHCl_3$). FAB-MS *m/z*: 1200 $(M+H)^+$. IR (KBr): 3296 (NH_2), 1740 (ester), 1642, 1537 (amide) cm^{-1}.

***S*-[2,3-Bis(palmitoyloxy)-(2*S*)-propyl]-*N*-palmitoyl-(*R*)-cysteinyl-*O*-*tert*-butyl-(*S*)-seryl-*O*-*tert*-butyl-(*S*)-seryl-L-asparaginyl-(*S*)-alanine *tert*-Butyl Ester (*S*-33)** Palmitoyl chloride (28 mg, 1.0×10^{-4} mol) in CH_2Cl_2 (2 ml) was added to a stirred solution of (*S*)-**32** (0.12 g, 1.0×10^{-4} mol), 4-dimethylaminopyridine (3.0 mg, 2.5×10^{-5} mol) and *N*,*N*-diisopropylethylamine (26 mg, 4.1×10^{-4} mol) in CH_2Cl_2 (10 ml) at 0 °C. After being stirred for 5 h at room temperature, the mixture was treated by the same preparation procedure as (*R*)-**23** to give (*S*)-**33** (0.10 g, 75%) as a white powder. (*S*)-**33**: mp 194 °C, $[\alpha]_D^{22}$ +4.3° (*c*=1.02, $CHCl_3$). FAB-MS *m/z*: 1438 $(M+H)^+$. IR (KBr): 3295 (NH), 1728 (ester), 1628, 1538 (amide) cm^{-1}.

***S*-[2,3-Bis(palmitoyloxy)-(2*S*)-propyl]-*N*-palmitoyl-(*R*)-cysteinyl-(*S*)-seryl-(*S*)-seryl-(*S*)-asparaginyl-(*S*)-alanine (3)** CF_3COOH (2 ml) was added to (*S*)-**33** (84 mg, 5.9×10^{-5} mol). After being stirred for 1 h at room temperature, the mixture was evaporated *in vacuo* and the residue was precipitated to a solid by cooling at −20 °C from MeOH–$CHCl_3$ (3:1) to give **3** (40 mg, 53%) as a white powder. **3**: mp 210—212 °C, $[\alpha]_D^{22}$ −28.3° (*c*=0.86, $CHCl_3$). FAB-MS *m/z*: 1270 $(M+H)^+$. IR (KBr): 3296 (OH, NH), 1736 (ester), 1639, 1538 (amide) cm^{-1}. *Anal.* Calcd for $C_{67}H_{124}N_6O_{14}\cdot3H_2O$: C, 60.79; H, 9.90; N, 6.35. Found: C, 61.00; H, 9.88; N, 6.14.

***S*-[2,3-Bis(palmitoyloxy)-(2*S*)-propyl]-*N*-2,2,2-trichloroethoxycarbonyl-(*R*)-cysteinyl-(*S*)-seryl-(*S*)-seryl-(*S*)-asparaginyl-(*S*)-alanine (4)** CF_3COOH (2 ml) was added to (*S*)-**31** (75 mg, 5.5×10^{-5} mol). After being stirred for 1 h, the mixture was evaporated *in vacuo* and the residue was precipitated to a solid by cooling at −20 °C from MeOH–$CHCl_3$ (3:1) to give **4** (27 mg, 45%) as a white powder. **4**: mp 204—207 °C, $[\alpha]_D^{22}$ +16.6° (*c*=1.00, $CHCl_3$). FAB-MS *m/z*: 1205 $(M+H)^+$. IR (KBr): 3302 (OH, NH), 1737 (ester), 1629, 1538 (amide) cm^{-1}. *Anal.* Calcd for $C_{54}H_{95}Cl_3N_6O_{15}$: C, 53.75; H, 7.93; N, 6.96. Found: C, 53.56; H, 8.34; N, 6.47.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-*N*-2,2,2-trichloroethoxycarbonyl-(*R*)-cysteinyl-*O*-*tert*-butyl-(*S*)-serine *tert*-Butyl Ester (37)** **5** (0.13 g, 1.6×10^{-4} mol), DCC (36 mg, 1.8×10^{-4} mol), and HOBt (24 mg, 1.6×10^{-4} mol) were added under stirring to a solution of **34** (40 mg, 1.6×10^{-4} mol) and *N*-methylmorphorine (16 mg, 1.6×10^{-4} mol) in DMF (3 ml). After being stirred for 15 h at room temperature, the mixture was treated by the same procedure described in the preparation of compound **26**, and chromatographed on silica gel with *n*-hexane–AcOEt (7:1) as an eluent to give **37** (95 mg, 57%) as a colorless oil. **37**: $[\alpha]_D^{22}$ +9.7° (*c*=1.9, $CHCl_3$). FAB-MS *m/z*: 1046 $(M+H)^+$: IR (neat): 3300 (NH), 1738 (ester), 1658, 1528 (amide) cm^{-1}.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-*N*-2,2,2-trichloroethoxycarbonyl-(*R*)-cysteinyl-*O*-*tert*-butyl-(*S*)-seryl-*O*-*tert*-butyl-(*S*)-serine *tert*-Butyl Ester (38)** HOBt (80 mg, 5.2×10^{-4} mol) and DCC (36 mg, 1.8×10^{-4} mol) was added to the solution of **5** (0.20 g, 2.4×10^{-4} mol) and **35** (85 mg, 2.4×10^{-4} mol) in DMF (2 ml). After being stirred for 15 h at room temperature, the mixture was treated by the same procedure described in the preparation of compound **27**, and subjected to column chromatography on silica gel with *n*-hexane–AcOEt (7:1) as an eluent to give **38** (0.19 g, 67%) as a colorless oil. **38**: $[\alpha]_D^{22}$ +15.3° (*c*=2.1, $CHCl_3$). FAB-MS *m/z*: 1189 $(M+H)^+$. IR (neat): 3280 (NH), 1737 (ester), 1638, 1524 (amide) cm^{-1}.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-*N*-2,2,2-trichloroethoxycarbonyl-(*R*)-cysteinyl-*O*-*tert*-butyl-(*S*)-seryl-*O*-*tert*-butyl-(*S*)-seryl-(*S*)-asparagine *tert*-Butyl Ester (39)** HOBt (25 mg, 1.6×10^{-4} mol) and DCC (40 mg, 2.0×10^{-4} mol) were added to a solution of **5** (0.15 g, 1.6×10^{-4} mol) and **36** (78 mg, 1.6×10^{-4} mol) in DMF (2 ml). After being stirred for 15 h at room temperature, the mixture was treated by the same procedure described in the preparation of the compound **27** to give **39** as a white powder. **39**: mp 129—131 °C, $[\alpha]_D^{22}$ +12.7° (*c*=1.25, $CHCl_3$). FAB-MS *m/z*: 1305 $(M+H)^+$. IR (KBr): 3288 (NH), 1742 (ester), 1641, 1539 (amide) cm^{-1}.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-*N*-2,2,2-trichloroethoxycarbonyl-(*R*)-cysteinyl-(*S*)-serine (6)** CF_3COOH (2 ml) was added to **34** (0.13 g, 1.2×10^{-4} mol). After being stirred for 1 h at room temperature, the mixture was treated by the same procedure described in the preparation of compound **1** to give **6** (0.10 g, 86%) as a white powder. **6**: mp 50—52 °C, $[\alpha]_D^{22}$ +6.4° (*c*=0.52, $CHCl_3$). FAB-MS *m/z*: 933 $(M+H)^+$. IR (neat): 3324 (OH, NH), 1741 (ester), 1666, 1537 (CONH). *Anal.* Calcd for $C_{44}H_{79}Cl_3N_2O_{10}S\cdot H_2O$: C, 55.46; H, 8.57; N, 2.94. Found: C, 56.05; H, 8.46; N, 2.94.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-*N*-2,2,2-trichloroethoxycarbonyl-(*R*)-cysteinyl-(*S*)-seryl-(*S*)-serine (7)** CF_3COOH (2 ml) was added to **35** (0.19 g, 1.6×10^{-4} mol). After being stirred for 1 h at room temperature, the mixture was treated by the same procedure described in the preparation of the compound **1** to give **7** (0.10 g, 86%) as a white powder. **7**: mp 102—105 °C; $[\alpha]_D^{22}$ +3.99° (*c*=1.04, $CHCl_3$). FAB-MS *m/z*: 1020 $(M+H)^+$. IR (neat): 3314 (OH, NH), 1741 (ester), 1633, 1537 (CONH) cm^{-1}. *Anal.* Calcd for $C_{47}H_{84}Cl_3N_3O_{12}S\cdot H_2O$: C, 54.30; H, 8.34; N, 4.04. Found: C, 54.65; H, 8.20; N, 3.94.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-*N*-2,2,2-trichloroethoxycarbonyl-(*R*)-cysteinyl-(*S*)-seryl-(*S*)-serine-(*S*)-asparagine (8)** CF_3COOH (2 ml) was added to **36** (60 mg, 5.0×10^{-5} mol). After being stirred for 1 h at room temperature, the mixture was treated by the same procedure described in the preparation of compound **1** to give **8** (0.10 g, 86%) as a white powder. **8**: mp 183—185 °C, $[\alpha]_D^{22}$ −9.46° (*c*=1.13, $CHCl_3$: MeOH = 1:1). FAB-MS *m/z*: 1135 $(M+H)^+$. IR (neat): 3302 (OH, NH), 1731 (ester), 1632, 1537 (CONH) cm^{-1}. *Anal.* Calcd for $C_{51}H_{90}Cl_3N_5O_{14}S$: C, 53.94; H, 7.99; N, 6.16. Found: C, 53.90; H, 8.00; N, 5.74.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-(*R*)-cysteinyl-*O*-*tert*-butyl-(*S*)-seryl-*O*-*tert*-butyl-(*S*)-seryl-(*S*)-asparagine *tert*-Butyl Ester (40)** Zinc powder (0.65 g) was added to a stirred solution of **36** (0.13 g, 1.0×10^{-4} mol) in CH_3COOH (2 ml). After being stirred for 15 h at room temperature, CH_2Cl_2 (50 ml) was added to the mixture and zinc powder was filtered off. The filtrate was washed with sat. $NaHCO_3$ aq. (50 ml × 3) and brine. The CH_2Cl_2 layer was dried over $MgSO_4$ and concentrated *in vacuo* to give **37** (94 mg, 84%) as a white powder, which was used without further purification. **37**: mp 91—93 °C, $[\alpha]_D^{22}$ +3.3° (*c*=1.84, $CHCl_3$). FAB-MS *m/z*: 1129 $(M+H)^+$. IR (KBr): 3292 (OH, NH), 1741 (ester), 1641, 1542 (amide) cm^{-1}.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-(*R*)-cysteinyl-(*S*)-seryl-(*S*)-seryl-(*S*)-asparagine (9)** CF_3COOH (2 ml) was added to **40**. After being stirred for 1 h at room temperature, the mixture was treated by the same procedure described in the preparation of compound **1** to give **9** (45 mg, 58%) as a white powder. **9**: mp 142—145 °C, $[\alpha]_D^{22}$ +6.4° (*c*=0.38, $CHCl_3$). FAB-MS *m/z*: 961 $(M+H)^+$. IR (neat): 3322 (OH, NH), 1738 (ester), 1663, 1541 (amide) cm^{-1}.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-(*R*)-cysteinyl-(*S*)-seryl-(*S*)-seryl-(*S*)-asparaginyl-alanine (10)** CF_3COOH (2 ml) was added to **32** (75 mg, 6.3×10^{-5} mol). After being stirred for 1 h at room temperature, the mixture was treated by the same procedure described in the preparation of compound **1** to give **10** (45 mg, 58%) as a white powder. **10**: mp 198—200 °C, $[\alpha]_D^{22}$ +31.6° (*c*=0.22, $CHCl_3$). FAB-MS *m/z*: 1032 $(M+H)^+$. IR (neat): 3296 (OH, NH), 1738 (ester), 1666, 1537 (amide) cm^{-1}.

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References

1) V. Braun, *Biochim. Biophys. Acta*, **415**, 335 (1975).
2) K. H. Wiesmuller, W. G. Bessler, and G. Jung, *Hoppe Seyler Z. Physiol. Chem.*, **364**, 593 (1983).
3) W. Prass, H. Ringsdorf, W. Bessler, K. H. Wiesmuller, and G. Jung, *Biochim. Biophys. Acta*, **900**, 116 (1987).
4) W. G. Bessler, R. B. Johnson, K. H. Wiesmuller, and G. Jung, *Hoppe. Seyler Z. Physiol. Chem.*, **363**, 767 (1982).
5) R. B. Johnson, S. Kohl, K. Wiesmuller, G. Jung, and W. G. Bessler, *Immunobiology*, **165**, 27 (1983).
6) W. G. Bessler, M. Cox, A. Lex, B. Suhr, K. H. Wiesmuller, and G. Jung, *J. Immunology*, **135**, 1900 (1985).
7) S. Schlecht, K. H. Wiesmuller, G. Jung, and W. G. Bessler, *Zbl. Bakt.*, **271**, 493 (1989).
8) M. Kurimura, M. Takemoto, and K. Achiwa, *Chem. Pharm. Bull.*, **38**, 1110 (1990).
9) T. Shimizu, Y. Ohotsuka, Y. Yanagihara, M. Kurimura, M. Takemoto, and K. Achiwa, *Mol. Biother.*, **3**, 46 (1991).
10) Y. Terao, M. Murata, K. Achiwa, T. Nishio, M. Akamatsu, and M. Kamimura, *Tetrahedron Lett.*, **29**, 5173 (1988).
11) W. Konig and R. Geiger, *Chem. Ber.*, **103**, 788 (1970).