

Synthesis and Mitogenic Activity of Chiral Lipopeptide WS1279 and Its Derivatives

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Optically active lipopeptide derivatives have been synthesized by the use of chiral glycerol derivatives. Lipopeptide WS1279 derivatives with (*R*)-glycerol moieties showed a higher mitogenic activity than those with the (*S*)-configuration. Various *N*-protected lipopeptide and *N*-deprotected derivatives showed increased mitogenic activity.

Keywords peptide synthesis; lipopeptide; mitogenic activity; WS1279; chiral glycerol derivative

In 1990, lipopeptide WS1279 was isolated from *Streptomyces willmorei* No. 1279 as an immunoactive lipopeptide which was synthesized in *dl* form by Okada *et al.*¹⁾ It is composed of 6 amino acids with one amide-linked and two ester-linked fatty acids attached to *S*-(2,3-dihydroxypropyl) cysteine at the *N*-terminus.

Lipopeptides from various bacteria and their cell components are potent polyclonal activators for B-lymphocytes and have various other biological activities.¹⁻⁸⁾ Many kinds of lipopeptide have been synthesized.^{1,5,9-13)} To determine the molecular structure responsible for the biological activity of WS1279, we have synthesized *S*-[2,3-bis(palmitoyloxy)-(2*R* and 2*S*)-propyl]-*N*-palmitoyl-(*R*)-cysteinyl-(*S*)-asparaginyl-(*S*)-seryl-glycyl-glycyl-(*S*)-serine (**1** and **4**), their Troc derivatives (**2** and **5**) and Troc-deprotected lipopeptides (**3** and **6**) by using the *N*-(2,2,2-trichloroethoxycarbonyl) cysteinyl intermediate, which can prevent the racemization of their cysteinyl moieties in the condensation steps as we have previously reported.¹⁰⁾

The compounds (**1**—**6**) were synthesized according to the reaction sequence shown in Chart 1. The starting chiral materials *R*-**17** and *S*-**17** were prepared according to our method.¹⁰⁾ Deprotection of the *tert*-butyl group of *R*-**17** was carried out by treatment with TFA (trifluoroacetic acid)

to give **16**. Compound **16** was condensed with pentapeptide **18**¹⁾ in the presence of DEPC-TEA (diethyl phosphorocyanidate-triethylamine) in DMF (dimethylformamide) to give *R*-**19**. Deprotection of all *tert*-butyl groups of *R*-**19** was carried out by treatment with TFA to give **2**. The trichloroethoxy-carbonyl group of *R*-**19** was removed by treatment with zinc powder in acetic acid to give *R*-**20**. Deprotection of all *tert*-butyl groups of *R*-**20** was carried out by treatment with TFA to give **3**, then the compound *R*-**20** was acylated with palmitoyl chloride and *N,N*-diisopropylethylamine in CH₂Cl₂ in the presence of a catalytic amount of 4-dimethylaminopyridine to afford *R*-**21**. The final deprotection of all *tert*-butyl groups of *R*-**21** was carried out by treatment with TFA to give **1**. In the same way, the unnatural lipopeptide WS1279 **4**, its Troc derivative **5** and Troc-deprotected derivative **6** were synthesized from *S*-**17** in place of *R*-**17**. The structures of **1**—**6** were confirmed by elemental analysis and analysis of their IR spectra and FAB-MS. The mitogenic activities of **1**—**6** indicated that the natural [(2*R*)-propyl] type **1** has a higher activity than that of the unnatural [(2*S*)-propyl] type, and that Troc-deprotected lipopeptide and Troc derivatives showed increased mitogenic activity.

Therefore, to investigate the structure-activity relation-

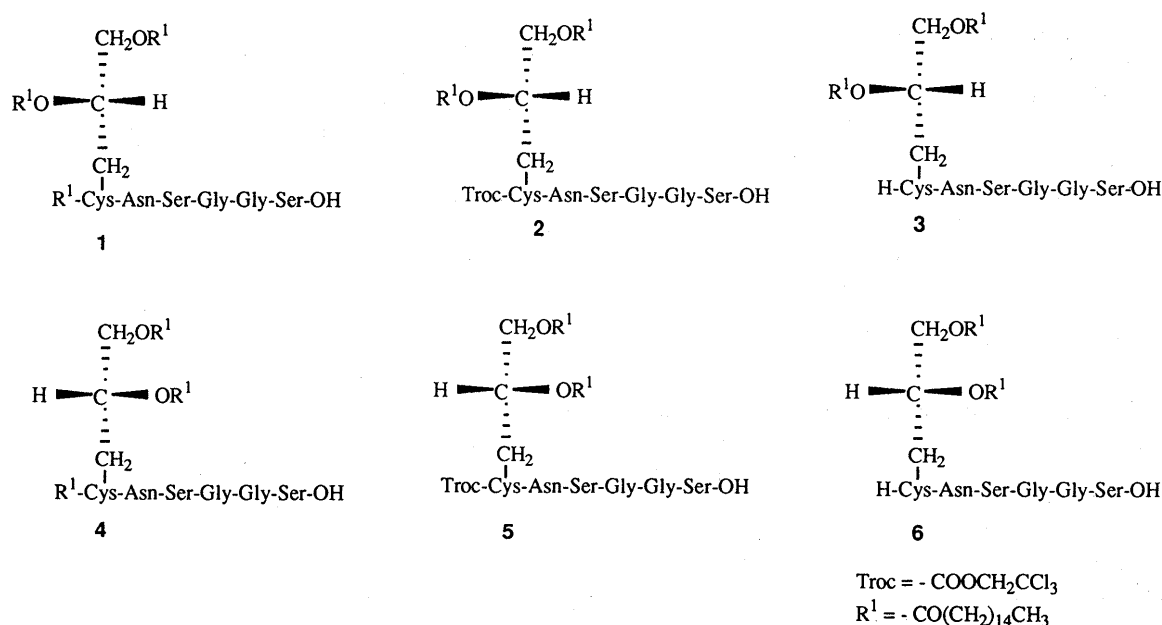


Fig. 1. Structures of Lipopeptide WS1279 and Its Derivatives

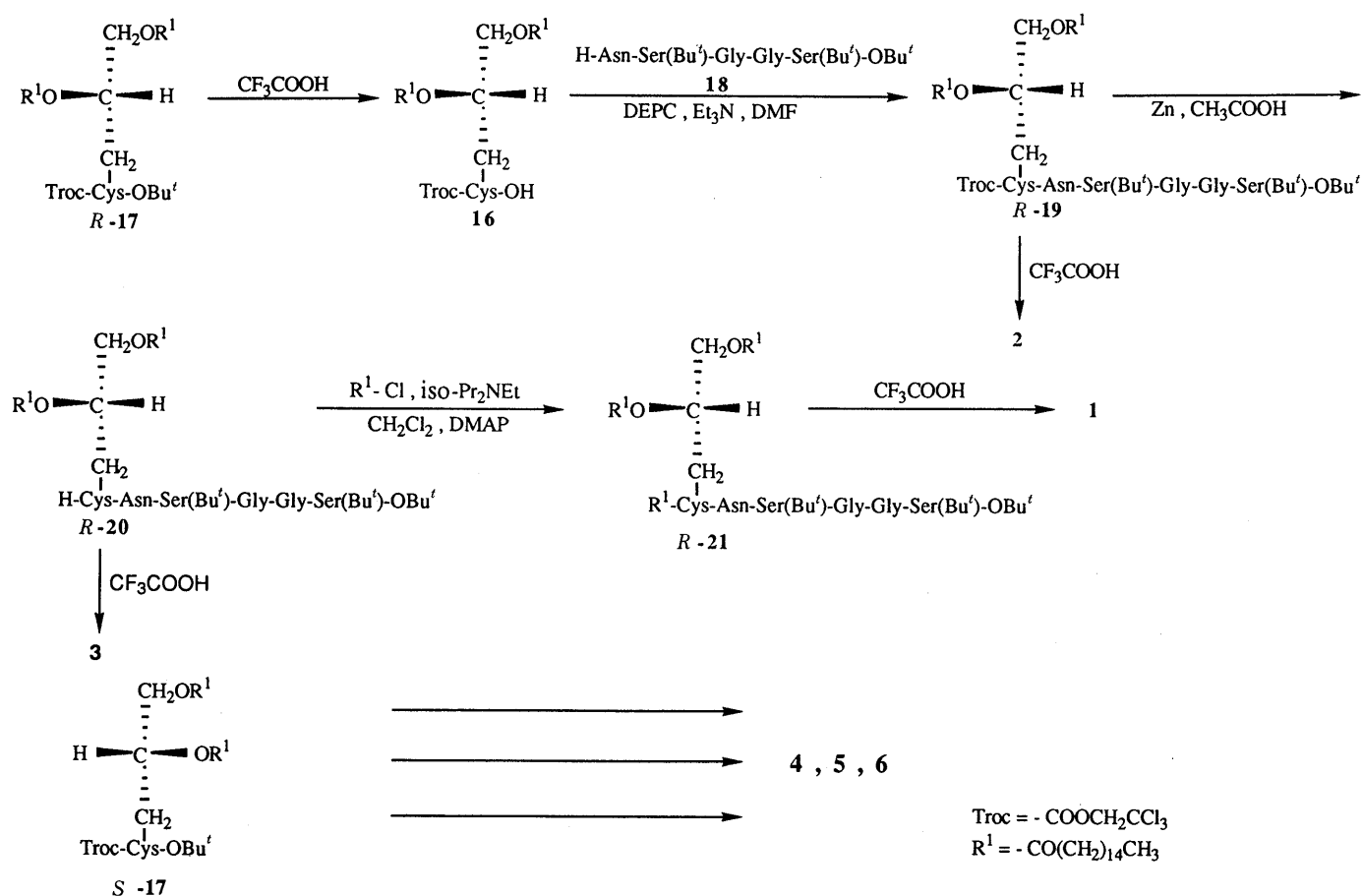


Chart 1. Synthesis of Chiral Lipopeptide WS1279 and Its Derivatives

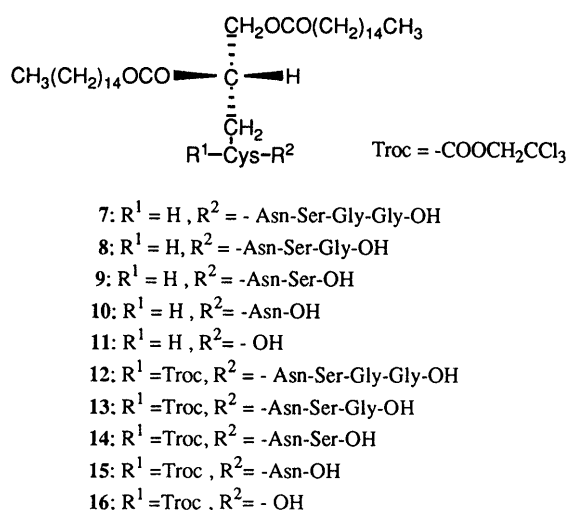


Fig. 2. Structures of Lipopeptide WS1279 Derivatives

ship of the lipopeptide, we focused our attention on the peptide length of the Troc derivative **2** and Troc-deprotected derivatives **3**. Thus we synthesized the lipopeptide derivatives **7**–**16** shown in Fig. 2.

The synthesis of peptide sequences is illustrated in Chart 2. Compound **16** was employed for coupling with the tetrapeptide H-Asn-Ser(Bu')-Gly-Gly-OBu' **25**, which was prepared by stepwise chain elongation by the DEPC-TEA method as shown in Chart 3. H-Gly-OBu'·HCl was

coupled to Z (carbobenzyloxy)-Gly-OH to give **22**. The Z group of **22** was removed by hydrogenation, and the free base was coupled to Z-Ser(Bu')-OH to give **23**. The Z group of **23** was removed and the free base was coupled to Z-Asn-OH to give **24**, which was hydrogenated to afford **25**. In the same way, the tripeptide **28** and dipeptide **30** were obtained from H-Gly-OBu'·HCl and H-Ser(Bu')-OBu'·HCl. Compounds **32**, **34**, **36** and **38** were obtained by the coupling of **16** with **25**, **28**, **30** and H-Asn-OBu'·HCl as shown in Chart 3. Deprotection of all *tert*-butyl groups of **32**, **34**, **36** and **38** was carried out by treatment with TFA to give **12**, **13**, **14** and **15**. The trichloroethoxycarbonyl group of the compounds **32**, **34**, **36** and **38** was removed by treatment with zinc powder in acetic acid to give **33**, **35**, **37** and **39**. The final deprotection of all *tert*-butyl groups of **33**, **35**, **37** and **39** was carried out by treatment with TFA to give **7**, **8**, **9** and **10**, respectively.

The structures of **7**–**16** were confirmed by elemental analysis and analysis of their IR spectra and FAB-MS. Among the Troc derivatives **2**, **12**, **13**, **14**, **15** and **16**, the lipopeptides with more than two amino acids (**2**, **12**, **13**, **14**, **15**) showed high mitogenic activity. On the other hand, in a series of Troc-deprotected lipopeptides **3**, **7**, **8**, **9**, **10** and **11**, the lipopeptides with more than four amino acids (**3**, **7**, **8**) showed high activity.¹⁴⁾

Experimental

Melting points were determined on a micro melting point apparatus BY-1 (Yazawa) and are uncorrected. Optical rotations were measured on

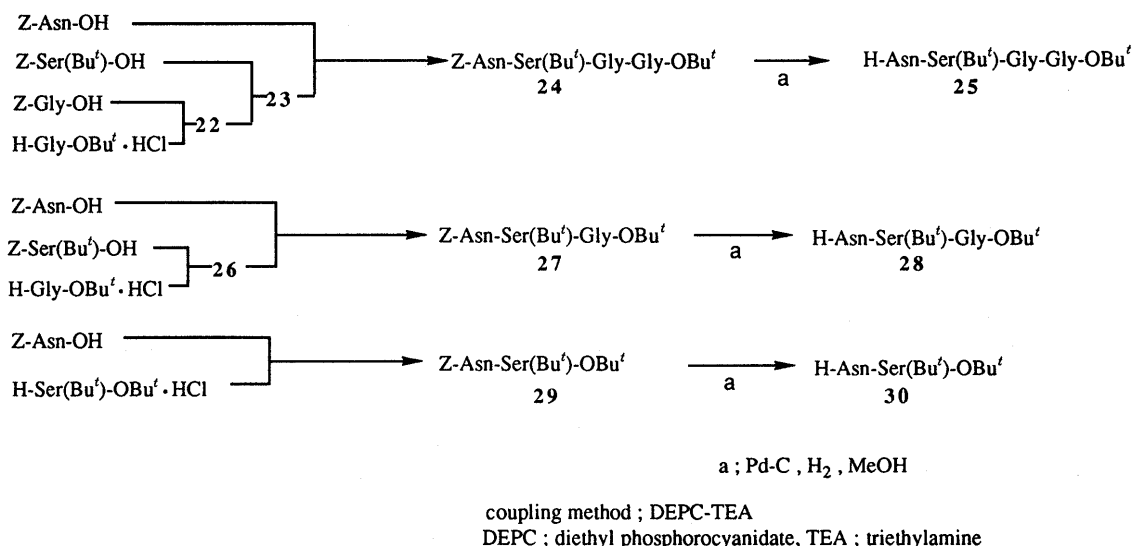


Chart 2. Synthesis of Peptides

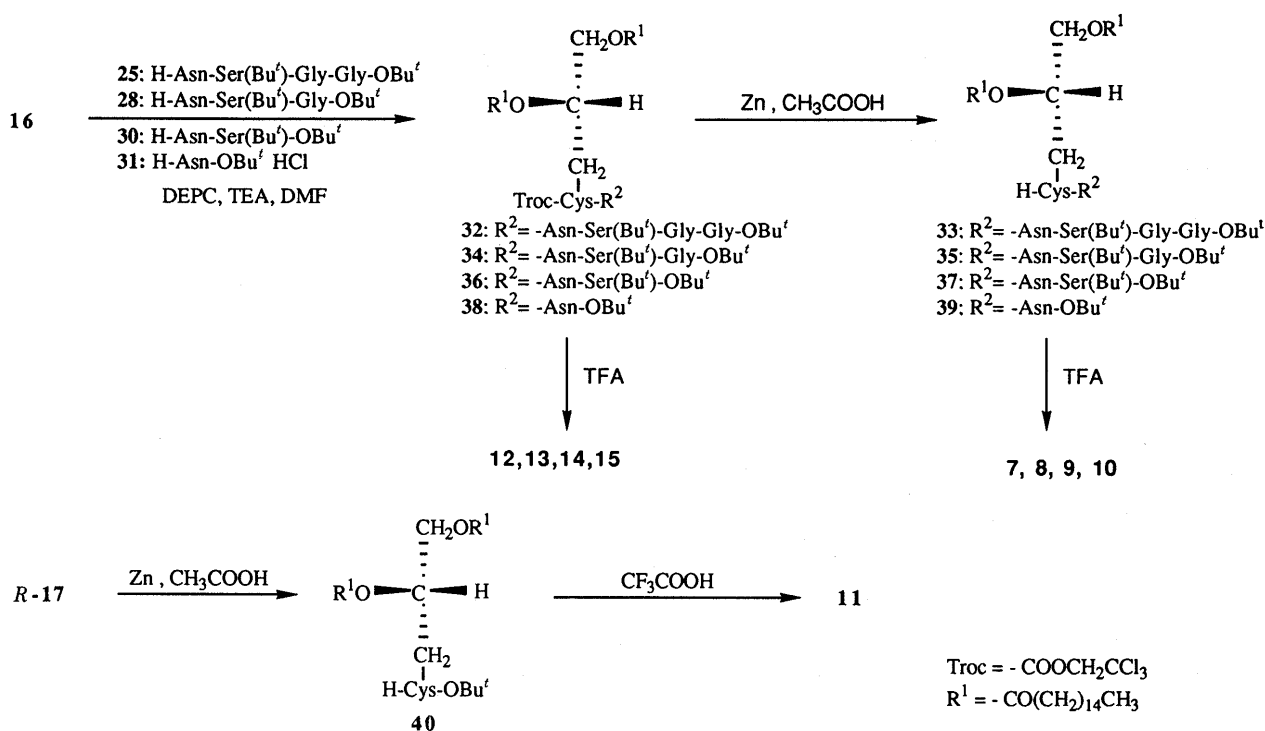


Chart 3. Synthesis of Lipopeptide WS1279 Derivatives

a JASCO DIP-140 digital polarimeter. IR spectra were taken on JASCO IR-180 IR spectrophotometers and absorptions are given in cm⁻¹. Thin layer chromatography (TLC) was performed on silica gel (Kiesel 60F₂₅₄ on aluminum 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 60F₂₅₄ 2 and 0.5 mm, Merck). Column chromatography was performed on silica gel (Kieselgel 60, 70—230 mesh, Merck).

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-N-2,2,2-trichloroethoxycarbonyl-(R)-cysteinyl-(S)-asparaginyl-O-tert-butyl-(S)-seryl-glycyl-glycyl-O-tert-butyl-(S)-serine tert-Butyl Ester (R-19) DEPC (33 mg, 2.0 × 10⁻⁴ mol) was added to a stirred solution of 16 (0.17 g, 2.0 × 10⁻⁴ mol) and the protected pentapeptide 18¹¹ (0.12 g, 2.0 × 10⁻⁴ mol) in DMF (2 ml) at 0°C, followed by the addition of TEA (20 mg, 2.0 × 10⁻⁴ mol) in DMF (1 ml) at 0°C. The mixture was stirred at 0°C for 4 h, then at room temperature overnight. After dilution with CH₂Cl₂ (20 ml), the mixture was successively washed with 5% aqueous citric acid (× 2), water (× 2), 5% aqueous

NaHCO₃ (× 2), and saturated aqueous NaCl (× 1), then dried over MgSO₄. Removal of the solvent by concentration *in vacuo* gave a white powder, which was subjected to column chromatography on silica gel with CHCl₃-MeOH (20:1) as an eluent to give **R-19** (0.22 g, 78%). **R-19**: mp 158—160°C, [α]_D²² + 3.3° (c = 1.0, CHCl₃). FAB-MS *m/z*: 1417 (M + H)⁺. IR (KBr): 3310 (NH), 1740 (ester), 1663, 1538 (amide). Anal. Calcd for C₆₇H₁₂₀Cl₃N₇O₁₆S·H₂O: C, 56.03; H, 8.56; N, 6.82. Found: C, 55.96; H, 8.40; N, 6.58.

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-(R)-cysteinyl-(S)-asparaginyl-O-tert-butyl-(S)-seryl-glycyl-glycyl-O-tert-butyl-(S)-serine tert-Butyl Ester (R-20) Zinc powder (0.5 g) was added to a stirred solution of **R-19** (97 mg, 6.8 × 10⁻⁵ mol) in CH₃COOH (2 ml). The mixture was stirred for 15 h at room temperature, then CH₂Cl₂ (50 ml) was added and zinc powder was filtered off. The filtrate was washed with saturated aqueous NaHCO₃ (50 ml × 3) and brine. The CH₂Cl₂ layer was dried over MgSO₄ and concentrated *in vacuo* to give **R-20** (68 mg, 80%) as a white powder, which was used without further purification. **R-20**: mp 135—137°C, [α]_D²² - 12.6° (c = 1.16, CHCl₃). FAB-MS *m/z*: 1243 (M + H)⁺. IR (KBr): 3290 (NH₂),

1740 (ester), 1640, 1538 (amide).

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-N-palmitoyl-(R)-cysteinyl-(S)-asparaginyl-O-tert-butyl-(S)-seryl-glycyl-glycyl-O-tert-butyl-(S)-serine tert-Butyl Ester (R-21) Palmitoyl chloride (14 mg, 5.1×10^{-5} mol) in CH_2Cl_2 (2 ml) was added to a stirred solution of **R-20** (58 mg, 4.7×10^{-5} mol), 4-dimethylaminopyridine (1.4 mg, 1.2×10^{-5} mol) and *N,N*-diisopropylethylamine (24 mg, 1.9×10^{-4} mol) in CH_2Cl_2 (10 ml) at 0°C . After being stirred for 5 h at room temperature, the mixture was diluted with CH_2Cl_2 and washed with 5% aqueous citric acid ($\times 2$), 5% aqueous NaHCO_3 ($\times 2$) and saturated aqueous NaCl ($\times 1$), dried over MgSO_4 and concentrated *in vacuo*. The residue was subjected to column chromatography on silica gel with CHCl_3 -MeOH (20:1) as an eluent to give **R-21** (50 mg, 72%). **R-21**: mp 183 – 185°C , $[\alpha]_D^{25} + 7.38^\circ$ ($c = 2.68$, CHCl_3). FAB-MS m/z : 1481 ($\text{M} + \text{H}$) $^+$. IR (KBr): 3296 (NH), 1730 (ester), 1628, 1540 (amide).

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-N-palmitoyl-(R)-cysteinyl-(S)-asparaginyl-(S)-seryl-glycyl-glycyl-(S)-serine (1) CF_3COOH (2 ml) was added to **R-21** (85 mg, 5.7×10^{-3} mol). After being stirred for 1 h at room temperature, the mixture was concentrated *in vacuo* and a precipitate was obtained from MeOH- CHCl_3 (3:1) by cooling at -20°C to give **1** (73 mg, 75%) as a white powder. **1**: mp 186 – 188°C , FAB-MS m/z : 1313 ($\text{M} + \text{H}$) $^+$. IR (KBr): 3286 (OH, NH), 1737 (ester), 1662, 1540 (amide). Anal. Calcd for $\text{C}_{68}\text{H}_{125}\text{N}_7\text{O}_{15} \cdot \text{H}_2\text{O}$: C, 61.37; H, 9.62; N, 7.06. Found: C, 60.83; H, 9.52; N, 7.06.

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-N-2,2,2-trichloro-ethoxycarbonyl-(R)-cysteinyl-(S)-asparaginyl-(S)-seryl-glycyl-glycyl-(S)-serine (2) Compound **2** was obtained from **R-19** by a procedure similar to that described for **1**, in 75% yield. **2**: mp 173 – 175°C , $[\alpha]_D^{25} - 14.8^\circ$ ($c = 0.21$, CHCl_3). FAB-MS m/z : 1249 ($\text{M} + \text{H}$) $^+$. IR (KBr): 3284 (OH, NH), 1737 (ester), 1662, 1538 (amide). Anal. Calcd for $\text{C}_{55}\text{H}_{96}\text{Cl}_3\text{N}_7\text{O}_{16} \cdot \text{H}_2\text{O}$: C, 52.10; H, 7.79; N, 7.73. Found: C, 52.32; H, 7.76; N, 7.26.

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-N-2,2,2-trichloro-ethoxycarbonyl-(R)-cysteinyl-(S)-asparaginyl-(S)-seryl-glycyl-glycyl-(S)-serine (3) Compound **3** was obtained from **R-20** by a procedure similar to that described for **1**, in 45% yield. **3**: mp 167 – 169°C . FAB-MS m/z : 1075 ($\text{M} + \text{H}$) $^+$. IR (KBr): 3296 (OH, NH), 1737 (ester), 1668, 1537 (amide).

S-[2,3-Bis(palmitoyloxy)-(2S)-propyl]-N-2,2,2-trichloro-ethoxycarbonyl-(R)-cysteinyl-(S)-asparaginyl-O-tert-butyl-(S)-seryl-glycyl-glycyl-O-tert-butyl-(S)-serine tert-Butyl Ester (S-19) DEPC (21 mg, 1.3×10^{-4} mol) was added to a stirred solution of **S-16** (0.11 g, 1.3×10^{-4} mol) and the protected pentapeptide **18** (75 mg, 1.3×10^{-4} mol) in DMF (2 ml) at 0°C , followed by the addition of TEA (13 mg, 2.0×10^{-4} mol) in DMF (1 ml) at 0°C . After the usual work-up, the product was subjected to column chromatography on silica gel with CHCl_3 -MeOH (20:1) as an eluent to give **S-19** (0.11 g, 62%). **S-19**: mp 144 – 146°C , $[\alpha]_D^{25} + 4.9^\circ$ ($c = 2.2$, CHCl_3). FAB-MS m/z : 1417 ($\text{M} + \text{H}$) $^+$. IR (KBr): 3288 (NH), 1742 (ester), 1663, 1537 (amide). Anal. Calcd for $\text{C}_{67}\text{H}_{120}\text{Cl}_3\text{N}_7\text{O}_{16} \cdot \text{H}_2\text{O}$: C, 56.03; H, 8.56; N, 6.82. Found: C, 55.96; H, 8.40; N, 6.58.

S-[2,3-Bis(palmitoyloxy)-(2S)-propyl]-N-2,2,2-trichloro-ethoxycarbonyl-(R)-cysteinyl-(S)-asparaginyl-O-tert-butyl-(S)-serine tert-Butyl Ester (S-20) Compound **S-20** was obtained from **S-19** by a procedure similar to that described for **R-20**, in 83% yield. **S-20**: mp 126 – 128°C , $[\alpha]_D^{25} + 1.3^\circ$ ($c = 1.6$, CHCl_3). FAB-MS m/z : 1243 ($\text{M} + \text{H}$) $^+$. IR (KBr): 3296 (NH_2), 1739 (ester), 1640, 1538 (amide).

S-[2,3-Bis(palmitoyloxy)-(2S)-propyl]-N-palmitoyl-(R)-cysteinyl-(S)-asparaginyl-O-tert-butyl-(S)-seryl-glycyl-glycyl-O-tert-butyl-(S)-serine tert-Butyl Ester (S-21) Compound **S-21** was obtained from **S-20** by a procedure similar to that described for **R-21**, and was chromatographed on silica gel with CHCl_3 -MeOH (20:1) as an eluent to give **S-21**, in 80% yield. **S-21**: mp 173 – 175°C , $[\alpha]_D^{25} + 2.2^\circ$ ($c = 0.84$, CHCl_3). FAB-MS m/z : 1481 ($\text{M} + \text{H}$) $^+$. IR (KBr): 3294 (NH), 1734 (ester), 1628, 1543 (amide).

S-[2,3-Bis(palmitoyloxy)-(2S)-propyl]-N-palmitoyl-(R)-cysteinyl-(S)-asparaginyl-(S)-seryl-glycyl-glycyl-(S)-serine (4) Compound **4** was obtained from **S-21** by a procedure similar to that described for **1**, in 72% yield. **4**: mp 186 – 189°C , FAB-MS m/z : 1313 ($\text{M} + \text{H}$) $^+$. IR (KBr): 3288 (OH, NH), 1737 (ester), 1661, 1536 (amide). Anal. Calcd for $\text{C}_{68}\text{H}_{125}\text{N}_7\text{O}_{15} \cdot 3\text{H}_2\text{O}$: C, 59.75; H, 9.66; N, 7.17. Found: C, 59.78; H, 9.32; N, 7.15.

S-[2,3-Bis(palmitoyloxy)-(2S)-propyl]-N-2,2,2-trichloro-ethoxycarbonyl-(R)-cysteinyl-(S)-asparaginyl-(S)-seryl-glycyl-glycyl-(S)-serine (5) Compound **5** was obtained from **S-19** by a procedure similar to that described for **2**, in 61% yield. **5**: mp 171 – 173°C , $[\alpha]_D^{25} - 20.5^\circ$ ($c = 0.21$, CHCl_3). FAB-MS m/z : 1249 ($\text{M} + \text{H}$) $^+$. IR (KBr): 3282 (OH, NH), 1738 (ester), 1660, 1538 (amide). Anal. Calcd for $\text{C}_{55}\text{H}_{96}\text{Cl}_3\text{N}_7\text{O}_{16} \cdot \text{H}_2\text{O}$: C, 52.10; H, 7.79; N, 7.73. Found: C, 52.23; H, 7.75; N, 7.41.

S-[2,3-Bis(palmitoyloxy)-(2S)-propyl]-N-2,2,2-trichloro-ethoxycarbonyl-(R)-cysteinyl-(S)-asparaginyl-(S)-seryl-glycyl-glycyl-(S)-serine (6) Compound **6** was obtained from **S-20** by a procedure similar to that described for **3**, in 46% yield. **6**: mp 163 – 165°C . FAB-MS m/z : 1075 ($\text{M} + \text{H}$) $^+$. IR (KBr): 3290 (OH, NH), 1738 (ester), 1661, 1537 (amide).

N-Carbobenzoyloxy-glycyl-glycine tert-Butyl Ester (22) DEPC (0.36 g, 2.2×10^{-3} mol) was added to a stirred solution of **Z-Gly-OH** (0.38 g, 1.8×10^{-3} mol) and $\text{H-Gly-OBu}^t \cdot \text{HCl}$ (0.31 g, 1.8×10^{-3} mol) in DMF (2 ml) at 0°C , followed by the addition of TEA (0.37 g, 3.6×10^{-3} mol) in DMF (1 ml) at 0°C . After the usual work-up, the product was subjected to column chromatography on silica gel with *n*-hexane-AcOEt (3:1) as an eluent to give **22** (0.42 g, 71%) as a colorless oil. **22**: FAB-MS m/z : 323 ($\text{M} + \text{H}$) $^+$. IR (neat): 3328 (NH), 1731 (ester), 1533 (amide).

N-Carbobenzoyloxy-O-tert-butyl-L-seryl-glycyl-glycine tert-Butyl Ester (23) Compound **22** was hydrogenated with Pd-C in MeOH and DEPC (0.23 g, 1.4×10^{-3} mol) was added to a stirred solution of the resulting free base (0.22 g, 1.2×10^{-3} mol) and **Z-Ser(Bu) t -OH** (0.35 g, 1.2×10^{-3} mol) in DMF (2 ml) at 0°C , followed by the addition of TEA (0.14 g, 1.4×10^{-3} mol) in DMF (1 ml) at 0°C . After the usual work-up, the product was purified by silica gel chromatography with *n*-hexane-AcOEt (5:1) as an eluent to give **23** (0.48 g, 86%) as a colorless oil. **23**: $[\alpha]_D^{25} + 14.1^\circ$ ($c = 0.61$, CHCl_3). FAB-MS m/z : 466 ($\text{M} + \text{H}$) $^+$.

N-Carbobenzoyloxy-L-asparaginyl-O-tert-butyl-L-seryl-glycyl-glycine tert-Butyl Ester (24) Compound **23** was hydrogenated with Pd-C in MeOH, and DEPC (0.15 g, 8.9×10^{-4} mol) was added to a stirred solution of the resulting free base (0.24 g, 7.4×10^{-4} mol) and **Z-Asn-OH** (0.20 g, 7.4×10^{-4} mol) in DMF (2 ml) at 0°C , followed by the addition of TEA (89 mg, 8.9×10^{-4} mol) in DMF (1 ml) at 0°C . The mixture was stirred at 0°C for 4 h, then at room temperature overnight. The mixture was evaporated *in vacuo* and the residue was washed with 5% aqueous NaHCO_3 and water many times, then dried *in vacuo* to give **24** (0.29 g, 67%) as a green powder. **24**: mp 149 – 151°C , $[\alpha]_D^{25} - 9.51^\circ$ ($c = 1.0$, CHCl_3). FAB-MS m/z : 580 ($\text{M} + \text{H}$) $^+$. IR (KBr): 3300 (NH), 1743 (ester), 1640, 1537 (amide).

L-Asparaginyl-O-tert-butyl-L-seryl-glycyl-glycine tert-Butyl Ester (25) Compound **24** (0.15 g, 2.6×10^{-4} mol) was hydrogenated over 5% Pd-C as a catalyst in MeOH for 3 h at room temperature. After removal of Pd-C, the filtrate was concentrated *in vacuo* to give **25** (0.11 g, 96%) as a white powder.

N-Carbobenzoyloxy-O-tert-butyl-L-seryl-glycyl-glycine tert-Butyl Ester (26) DEPC (0.23 g, 1.4×10^{-3} mol) was added to a stirred solution of **Z-Ser(Bu) t -OH** (0.35 g, 1.2×10^{-3} mol) and $\text{HCl} \cdot \text{H-Gly-OBu}^t$ (0.20 g, 1.2×10^{-3} mol) in DMF (2 ml) at 0°C , followed by the addition of TEA (0.24 g, 2.4×10^{-3} mol) in DMF (1 ml) at 0°C . After the usual work-up, the product was purified by silica gel chromatography with *n*-hexane-AcOEt (5:1) as an eluent to give **26** (0.49 g, 79%) as a colorless oil. **26**: $[\alpha]_D^{25} + 21.6^\circ$ ($c = 0.64$, CHCl_3). FAB-MS m/z : 409 ($\text{M} + \text{H}$) $^+$.

N-Carbobenzoyloxy-L-asparaginyl-O-tert-butyl-L-seryl-glycyl-glycine tert-Butyl Ester (27) Compound **26** was hydrogenated with Pd-C in MeOH, and DEPC (53 mg, 3.2×10^{-4} mol) was added to a stirred solution of the resulting free base (0.11 g, 2.7×10^{-4} mol) and **Z-Asn-OH** (72 mg, 2.7×10^{-4} mol) in DMF (2 ml) at 0°C , followed by the addition of TEA (32 mg, 3.2×10^{-4} mol) in DMF (1 ml) at 0°C . After the usual work-up, the product was purified by silica gel chromatography with CHCl_3 -MeOH (15:1) as an eluent to give **27** (0.11 g, 67%) as a white powder. **27**: mp 156 – 159°C , $[\alpha]_D^{25} + 22.6^\circ$ ($c = 1.0$, CHCl_3). FAB-MS m/z : 523 ($\text{M} + \text{H}$) $^+$. IR (KBr): 3292 (NH), 1742 (ester), 1642, 1540 (amide). Anal. Calcd for $\text{C}_{25}\text{H}_{38}\text{N}_4\text{O}_8 \cdot 1/2\text{H}_2\text{O}$: C, 56.48; H, 7.39; N, 10.54. Found: C, 56.63; H, 7.45; N, 10.34.

L-Asparaginyl-O-tert-butyl-L-seryl-glycyl-glycine tert-Butyl Ester (28) Compound **27** (0.11 g, 2.1×10^{-4} mol) was hydrogenated over 5% Pd-C as a catalyst in MeOH for 3 h at room temperature. The mixture was treated by the same procedure described for **25** to give **28** (78 mg, 95%) as a white powder.

N-Carbobenzoyloxy-L-asparaginyl-O-tert-butyl-L-serine tert-Butyl Ester (29) DEPC (0.21 g, 1.3×10^{-3} mol) was added to a stirred solution of $\text{HCl} \cdot \text{H-Ser(Bu) t -OBu}^t$ (0.30 g, 1.2×10^{-3} mol) and **Z-Asn-OH** (0.32 g, 1.2×10^{-3} mol) in DMF (2 ml) at 0°C , followed by the addition of TEA (0.26 g, 2.6×10^{-3} mol) in DMF (1 ml) at 0°C . After the usual work-up, the product was purified by silica gel chromatography with CHCl_3 -MeOH (20:1) as an eluent to give **29** (0.42 g, 75%) as a white powder. **29**: mp 142 – 144°C , $[\alpha]_D^{25} + 22.6^\circ$ ($c = 1.0$, CHCl_3). FAB-MS m/z : 466 ($\text{M} + \text{H}$) $^+$. IR (KBr): 3312 (NH), 1741 (ester), 1675, 1538 (amide). Anal. Calcd for $\text{C}_{23}\text{H}_{35}\text{N}_3\text{O}_7 \cdot 1/2\text{H}_2\text{O}$: C, 58.20; H, 7.64; N, 8.85. Found: C, 58.44; H, 7.67; N, 8.79.

L-Asparaginyl-O-tert-butyl-L-serine tert-Butyl Ester (30) Compound 29 (0.19 g, 3.6×10^{-4} mol) was hydrogenated over 5% Pd-C as a catalyst in MeOH for 3 h at room temperature. The mixture was treated by the same procedure described for 25 to give 30 (0.11 g, 94%) as a white powder.

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-N-2,2,2-trichloro-ethoxycarbonyl-(R)-cysteinyl-(S)-asparaginyl-O-tert-butyl-(S)-seryl-glycyl-glycine tert-Butyl Ester (32) DEPC (53 mg, 3.2×10^{-4} mol) was added to a stirred solution of 16 (0.23 g, 2.7×10^{-4} mol) and 25 (0.12 g, 2.7×10^{-4} mol) in DMF (2 ml) at 0°C, followed by the addition of TEA (33 mg, 3.2×10^{-4} mol) in DMF (1 ml) at 0°C. After the usual work-up, the product was precipitated from MeOH-CHCl₃ (3:1) as a solid by cooling at -20°C to give 32 (0.21 g, 60%) as a white powder. 32: mp 169–170°C, $[\alpha]_D^{22} -9.0^\circ$ ($c=1.0$, CHCl₃). FAB-MS m/z : 1274 (M+H)⁺. IR (KBr): 3296 (NH), 1743 (ester), 1664, 1532 (amide). *Anal.* Calcd for C₆₀H₁₀₇Cl₃N₆O₁₄S·H₂O: C, 56.53; H, 8.46; N, 6.59. Found: C, 56.36; H, 8.39; N, 6.33.

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl-(R)-cysteinyl-(S)-asparaginyl-O-tert-butyl-(S)-seryl-glycyl-glycine tert-Butyl Ester (33) Compound 33 was obtained from 32 by a procedure similar to that described for R-20, in 90% yield. 33: mp 144–146°C, $[\alpha]_D^{22} -16.3^\circ$ ($c=1.34$, CHCl₃). FAB-MS m/z : 1200 (M+H)⁺.

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-(R)-cysteinyl-(S)-asparaginyl-(S)-seryl-glycyl-glycine (7) Compound 7 was obtained from 33 by a procedure similar to that described for 1, in 51% yield. 7: mp 186–188°C. $[\alpha]_D^{22} -4.4^\circ$ ($c=0.20$, CHCl₃). FAB-MS m/z : 988 (M+H)⁺. IR (KBr): 3308 (OH, NH), 1737 (ester), 1669, 1557 (amide).

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-N-2,2,2-trichloro-ethoxycarbonyl-(R)-cysteinyl-(S)-asparaginyl-(S)-seryl-glycyl-glycine (12) Compound 12 was obtained from 32 by a procedure similar to that described for 1, in 52% yield. 12: mp 195–198°C. $[\alpha]_D^{22} -48.4^\circ$ ($c=0.21$, CHCl₃). FAB-MS m/z : 1161 (M+H)⁺. IR (KBr): 3304 (OH, NH), 1736 (ester), 1639, 1541 (amide). *Anal.* Calcd for C₅₂H₉₁Cl₃N₆O₁₄S·2H₂O: C, 52.10; H, 7.99; N, 7.01. Found: C, 52.27; H, 8.07; N, 6.75.

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-N-2,2,2-trichloro-ethoxycarbonyl-(R)-cysteinyl-(S)-asparaginyl-O-tert-butyl-(S)-seryl-glycyl-glycine tert-Butyl Ester (34) DEPC (50 mg, 3.1×10^{-4} mol) was added to a stirred solution of 16 (0.22 g, 2.6×10^{-4} mol) and 28 (0.10 g, 2.6×10^{-4} mol) in DMF (2 ml) at 0°C, followed by the addition of TEA (31 mg, 3.1×10^{-4} mol) in DMF (1 ml) at 0°C. After the usual work-up, the product was precipitated as a solid from MeOH-CHCl₃ (3:1) by cooling at -20°C to give 34 (0.21 g, 66%) as a white powder. 34: mp 147–150°C, $[\alpha]_D^{22} +4.51^\circ$ ($c=1.2$, CHCl₃). FAB-MS m/z : 1217 (M+H)⁺. IR (KBr): 3292 (OH, NH), 1742 (ester), 1639, 1540 (amide). *Anal.* Calcd for C₅₈H₁₀₄Cl₃N₆O₁₃S: C, 57.20; H, 8.61; N, 5.75. Found: C, 57.49; H, 8.66; N, 5.80.

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-(R)-cysteinyl-(S)-asparaginyl-O-tert-butyl-(S)-seryl-glycyl-glycine tert-Butyl Ester (35) Compound 35 was obtained from 34 by a procedure similar to that described for R-20, in 90% yield. 35: mp 123–125°C, $[\alpha]_D^{22} -6.7^\circ$ ($c=0.6$, CHCl₃). FAB-MS m/z : 1043 (M+H)⁺.

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-(R)-cysteinyl-(S)-asparaginyl-(S)-seryl-glycyl-glycine (8) Compound 8 was obtained from 35 by a procedure similar to that described for 1, in 52% yield. 8: mp 170–172°C, $[\alpha]_D^{22} +32.0^\circ$ ($c=0.24$, CHCl₃). FAB-MS m/z : 930 (M+H)⁺. IR (KBr): 3308 (OH, NH), 1741 (ester), 1639, 1539 (amide).

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-N-2,2,2-trichloro-ethoxycarbonyl-(R)-cysteinyl-(S)-asparaginyl-(S)-seryl-glycyl-glycine (13) Compound 13 was obtained from 34 by a procedure similar to that described for 1, in 85% yield. 13: mp 168–170°C, $[\alpha]_D^{22} -13.3^\circ$ ($c=0.20$, CHCl₃: MeOH=1:1). FAB-MS m/z : 1105 (M+H)⁺. IR (KBr): 3296 (OH, NH), 1734 (ester), 1655, 1540 (amide). *Anal.* Calcd for C₅₀H₈₈Cl₃N₅O₁₃S·H₂O: C, 53.44; H, 8.07; N, 6.23. Found: C, 53.50; H, 8.03; N, 6.30.

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-N-2,2,2-trichloro-ethoxycarbonyl-(R)-cysteinyl-(S)-asparaginyl-O-tert-butyl-(S)-serine tert-Butyl Ester (36) DEPC (65 mg, 4.0×10^{-4} mol) was added to a stirred solution of 16 (0.28 g, 3.3×10^{-4} mol) and 29 (0.11 g, 3.3×10^{-4} mol) in DMF (2 ml) at 0°C, followed by the addition of TEA (40 mg, 4.0×10^{-4} mol) in DMF (1 ml) at 0°C. After the usual work-up, the product was purified by silica gel chromatography with CHCl₃-MeOH (40:1) as an eluent to give 36 (0.31 g, 80%) as a white powder. 36: mp 95–97°C, $[\alpha]_D^{22} +4.97^\circ$ ($c=1.1$, CHCl₃). FAB-MS m/z : 1160 (M+H)⁺. IR (KBr): 3314 (OH, NH), 1741 (ester), 1641, 1538 (amide). *Anal.* Calcd for C₅₆H₁₀₁Cl₃N₄O₁₂S: C, 57.94; H, 8.77; N, 4.83. Found: C, 57.92; H, 8.74; N, 4.61.

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-(R)-cysteinyl-(S)-asparaginyl-O-tert-butyl-(S)-serine tert-Butyl Ester (37) Compound 37 was obtained from 36 by a procedure similar to that described for R-20, in 80% yield.

37: mp 47–48°C, $[\alpha]_D^{22} -5.6^\circ$ ($c=0.54$, CHCl₃). FAB-MS m/z : 986 (M+H)⁺.

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-(R)-cysteinyl-(S)-asparaginyl-(S)-serine (9) CF₃COOH (2 ml) was added to 37 (27 mg, 2.7×10^{-6} mol). After being stirred for 1 h at room temperature, the reaction mixture was evaporated *in vacuo*. Water was added to the residue and the whole was filtered to give 9 (16 mg, 67%) as a white powder. 9: mp 155–157°C, $[\alpha]_D^{22} -99.5^\circ$ ($c=0.22$, CHCl₃). FAB-MS m/z : 874 (M+H)⁺. IR (KBr): 3404 (OH, NH), 1742 (ester), 1664, 1538 (amide).

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-N-2,2,2-trichloro-ethoxycarbonyl-(R)-cysteinyl-(S)-asparaginyl-(S)-serine (14) Compound 14 was obtained from 36 by a procedure similar to that described for 1, in 84% yield. 14: mp 145–148°C, $[\alpha]_D^{22} +5.4^\circ$ ($c=0.28$, CHCl₃). FAB-MS m/z : 1047 (M+H)⁺. IR (KBr): 3302 (OH, NH), 1739 (ester), 1659, 1537 (amide). *Anal.* Calcd for C₄₈H₈₅Cl₃N₄O₁₂·S·H₂O: C, 54.98; H, 8.17; N, 5.34. Found: C, 55.02; H, 8.30; N, 5.08.

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-N-2,2,2-trichloro-ethoxycarbonyl-(R)-cysteinyl-(S)-asparagine tert-Butyl Ester (38) DEPC (33 mg, 2.0×10^{-4} mol) was added to a stirred solution of 16 (0.17 g, 2.0×10^{-4} mol) and H-Asn-OBu^t·HCl (42 mg, 2.0×10^{-4} mol) in DMF (2 ml) at 0°C, followed by the addition of TEA (40 g, 4.0×10^{-4} mol) in DMF (1 ml) at 0°C. After the usual work-up, the product was purified by silica gel chromatography with CHCl₃-MeOH (40:1) as an eluent to give 38 (0.13 g, 64%) as a white powder. 38: mp 79–80°C, $[\alpha]_D^{22} +6.23^\circ$ ($c=0.81$, CHCl₃). FAB-MS m/z : 1017 (M+H)⁺. IR (KBr): 3376 (OH, NH), 1727 (ester), 1680, 1665 (amide). *Anal.* Calcd for C₄₉H₈₈Cl₃N₃O₁₀S·1/2H₂O: C, 57.32; H, 8.74; N, 4.09. Found: C, 57.36; H, 8.62; N, 3.88.

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-(R)-cysteinyl-(S)-asparagine tert-Butyl Ester (39) Compound 39 was obtained from 38 by a procedure similar to that described for R-20, in 76% yield. 39: mp 52–53°C, $[\alpha]_D^{22} -2.1^\circ$ ($c=0.88$, CHCl₃). FAB-MS m/z : 843 (M+H)⁺.

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-(R)-cysteinyl-(S)-asparagine (10) Compound 10 was obtained from 39 by a procedure similar to that described for 9, in 46% yield. 10: mp 139–142°C, $[\alpha]_D^{22} -38.7^\circ$ ($c=0.20$, CHCl₃). FAB-MS m/z : 787 (M+H)⁺. IR (KBr): 3404 (OH, NH), 1741 (ester), 1630, 1536 (amide).

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-N-2,2,2-trichloro-ethoxycarbonyl-(R)-cysteinyl-(S)-asparagine (14) Compound 14 was obtained from 38 by a procedure similar to that described for 1, in 64% yield. 14: mp 107–109°C, $[\alpha]_D^{22} +18.3^\circ$ ($c=0.20$, CHCl₃). FAB-MS m/z : 960 (M+H)⁺. IR (KBr): 3348 (OH, NH), 1740 (ester), 1666 (amide). *Anal.* Calcd for C₄₅H₈₀Cl₃N₃O₁₀S: C, 56.21; H, 8.39; N, 4.37. Found: C, 56.04; H, 8.63; N, 4.22.

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-(R)-cysteine tert-Butyl Ester (40) Compound 40 was obtained from R-17 by a procedure similar to that described for R-20, in 80% yield. 40: mp 37–38°C, $[\alpha]_D^{22} +4.7^\circ$ ($c=0.35$, CHCl₃). FAB-MS m/z : 729 (M+H)⁺.

S-[2,3-Bis(palmitoyloxy)-(2R)-propyl]-(R)-cysteine (11) Compound 11 was obtained from 40 by a procedure similar to that described for 1, in 51% yield. 11: mp 100–102°C, $[\alpha]_D^{22} -130.4^\circ$ ($c=0.22$, CHCl₃). FAB-MS m/z : 673 (M+H)⁺. IR (KBr): 3410 (OH, NH), 1736 (ester), 1630, 1536 (amide).

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