# 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 $CH_2Cl_2$ in the presence of a catalyic 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 derivertive **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-

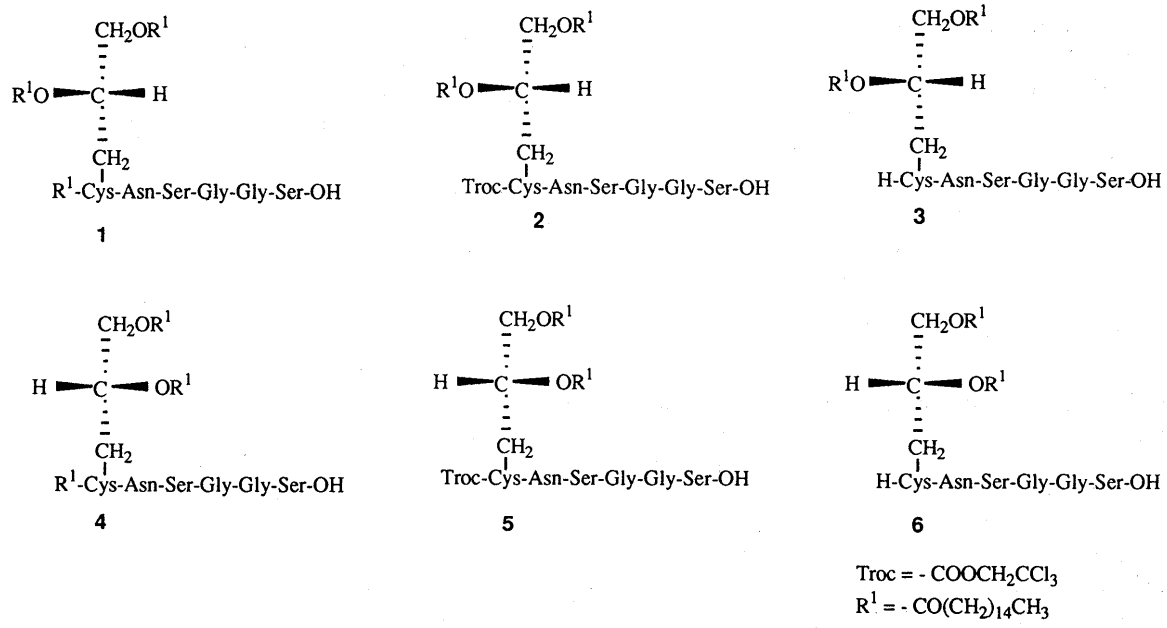



Fig. 1. Structures of Lipopeptide WS1279 and Its Derivatives

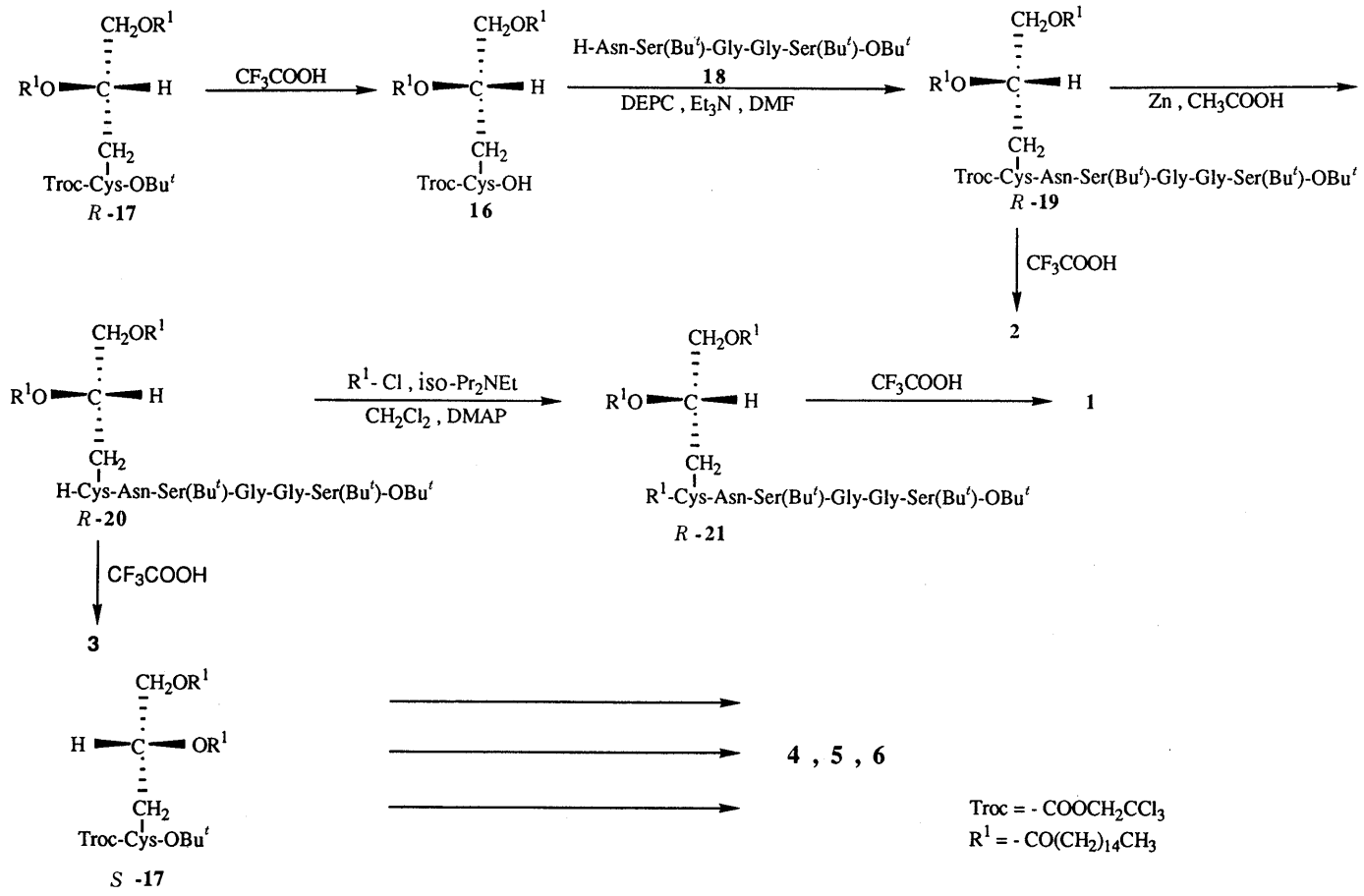
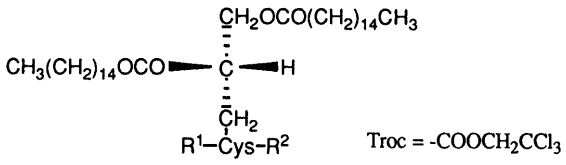



Chart 1. Synthesis of Chiral Lipopeptide WS1279 and Its Derivatives




**7**: $R^1$ = H , $R^2$ = - Asn-Ser-Gly-Gly-OH
**8**: $R^1$ = H, $R^2$ = -Asn-Ser-Gly-OH
**9**: $R^1$ = H , $R^2$ = -Asn-Ser-OH
**10**: $R^1$ = H , $R^2$= -Asn-OH
**11**: $R^1$ = H , $R^2$= - OH
**12**: $R^1$ =Troc, $R^2$ = - Asn-Ser-Gly-Gly-OH
**13**: $R^1$ =Troc, $R^2$ = -Asn-Ser-Gly-OH
**14**: $R^1$ =Troc, $R^2$ = -Asn-Ser-OH
**15**: $R^1$ =Troc , $R^2$= -Asn-OH
**16**: $R^1$ =Troc , $R^2$= - OH

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(But)–Gly–Gly–OBut **25**, which was prepared by stepwise chain elongation by the DEPC–TEA method as shown in Chart 3. H–Gly–OBut·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(But)–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–OBut·HCl and H–Ser(But)–OBut·HCl. Compounds **32**, **34**, **36** and **38** were obtained by the coupling of **16** with **25**, **28**, **30** and H–Asn–OBut·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

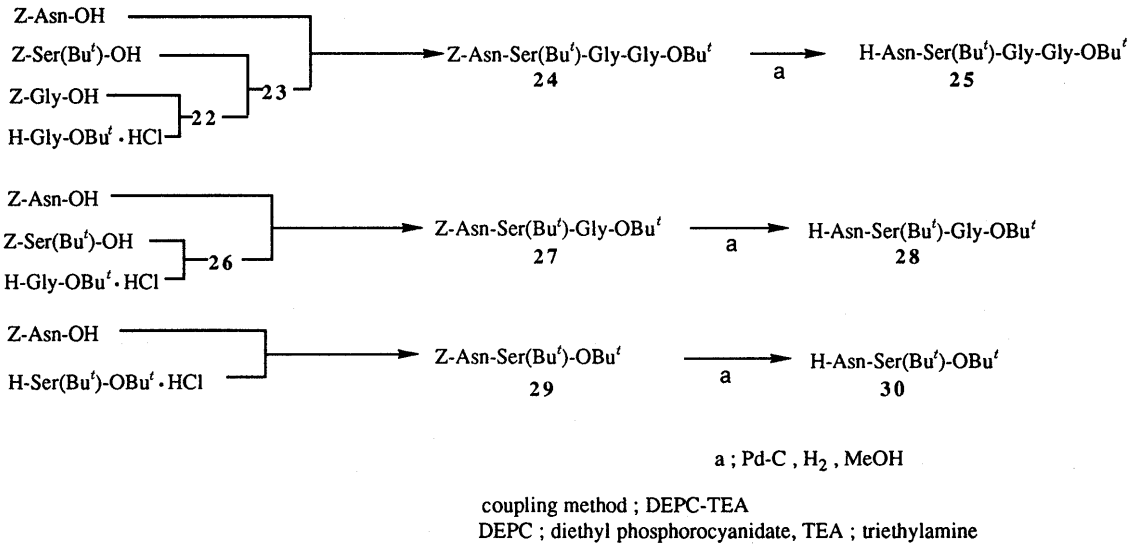



Chart 2. Synthesis of Peptides

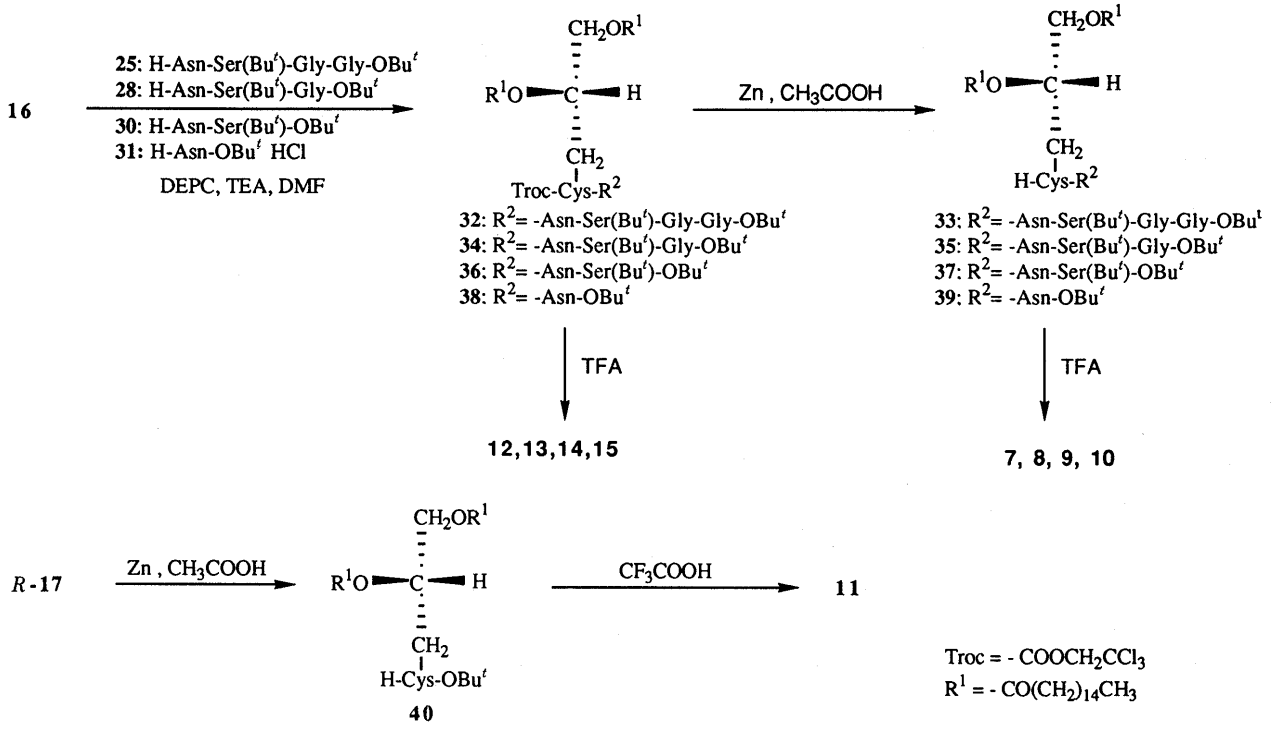



Chart 3. Synthesis of Lipopeptide WS1279 Derivatives

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 $60F_{254}$ 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 $60F_{254}$ 2 and 0.5 mm, Merck). Column chromatography was performed on silica gel (Kieselgel 60, 70—230 mesh, Merck).

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-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, $2.0 \times 10^{-4}$ mol) was added to a stirred solution of **16** (0.17 g, $2.0 \times 10^{-4}$ mol) and the protected pentapeptide **18**[1] (0.12 g, $2.0 \times 10^{-4}$ mol) in DMF (2 ml) at 0 °C, followed by the addition of TEA (20 mg, $2.0 \times 10^{-4}$ 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 $CH_2Cl_2$ (20 ml), the mixture was successively washed with 5% aqueous citric acid (×2), water (×2), 5% aqueous $NaHCO_3$ (×2), and saturated aqueous NaCl (×1), then dried over $MgSO_4$. Removal of the solvent by concentration *in vacuo* gave a white powder, which was subjected to column chromatography on silica gel with $CHCl_3$–MeOH (20:1) as an eluent to give *R*-**19** (0.22 g, 78%). *R*-**19**: mp 158—160 °C, $[\alpha]_D^{22}$ +3.3° (*c*=1.0, $CHCl_3$). FAB-MS *m/z*: 1417 $(M+H)^+$. IR (KBr): 3310 (NH), 1740 (ester), 1663, 1538 (amide). *Anal.* Calcd for $C_{67}H_{120}Cl_3N_7O_{16}S \cdot H_2O$: C, 56.03; H, 8.56; N, 6.82. Found: C, 55.96; H, 8.40; H, 6.58.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-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, $6.8 \times 10^{-5}$ mol) in $CH_3COOH$ (2 ml). The mixture was stirred for 15 h at room temperature, then $CH_2Cl_2$ (50 ml) was added and zinc powder was filtered off. The filtrate was washed with saturated aqueous $NaHCO_3$ (50 ml × 3) and brine. The $CH_2Cl_2$ layer was dried over $MgSO_4$ 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, $[\alpha]_D^{22}$ −12.6° (*c*=1.16, $CHCl_3$). FAB-MS *m/z*: 1243 $(M+H)^+$. IR (KBr): 3290 ($NH_2$),

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

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-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, $5.1 \times 10^{-5}$ mol) in $CH_2Cl_2$ (2 ml) was added to a stirred solution of *R*-**20** (58 mg, $4.7 \times 10^{-5}$ mol), 4-dimethylaminopyridine (1.4 mg, $1.2 \times 10^{-5}$ mol) and *N*,*N*-diisopropylethylamine (24 mg, $1.9 \times 10^{-4}$ 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 (×2), 5% aqueous $NaHCO_3$ (×2) and saturated aqueous NaCl (×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, $[\alpha]_D^{22}$ +7.38° ($c$=2.68, $CHCl_3$). FAB-MS *m/z*: 1481 $(M+H)^+$. IR (KBr): 3296 (NH), 1730 (ester), 1628, 1540 (amide).

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-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, $5.7 \times 10^{-3}$ 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 $(M+H)^+$. IR (KBr): 3286 (OH, NH), 1737 (ester), 1662, 1540 (amide). *Anal.* Calcd for $C_{68}H_{125}N_7O_{15} \cdot H_2O$: C, 61.37; H, 9.62; N, 7.06. Found: C, 60.83; H, 9.52; N, 7.06.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-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, $[\alpha]_D^{22}$ −14.8° ($c$=0.21, $CHCl_3$). FAB-MS *m/z*: 1249 $(M+H)^+$. IR (KBr): 3284 (OH, NH), 1737 (ester), 1662, 1538 (amide). *Anal.* Calcd for $C_{55}H_{96}Cl_3N_7O_{16}S \cdot H_2O$: C, 52.10; H, 7.79; N, 7.73. Found: C, 52.32; H, 7.76; N, 7.26.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-(*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 $(M+H)^+$. IR (KBr): 3296 (OH, NH), 1737 (ester), 1668, 1537 (amide).

***S*-[2,3-Bis(palmitoyloxy)-(2*S*)-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, $1.3 \times 10^{-4}$ mol) was added to a stirred solution of *S*-**16** (0.11 g, $1.3 \times 10^{-4}$ mol) and the protected pentapeptide **18** (75 mg, $1.3 \times 10^{-4}$ mol) in DMF (2 ml) at 0 °C, followed by the addition of TEA (13 mg, $2.0 \times 10^{-4}$ 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, $[\alpha]_D^{22}$ +4.9° ($c$=2.2, $CHCl_3$). FAB-MS *m/z*: 1417 $(M+H)^+$. IR (KBr): 3288 (NH), 1742 (ester), 1663, 1537 (amide). *Anal.* Calcd for $C_{67}H_{120}Cl_3N_7O_{16}S \cdot H_2O$: C, 56.03; H, 8.56; N, 6.82. Found: C, 55.96; H, 8.40; N, 6.58.

***S*-[2,3-Bis(palmitoyloxy)-(2*S*)-propyl]-(*R*)-cysteinyl-(*S*)-asparaginyl-*O*-*tert*-butyl-(*S*)-seryl-glycyl-glycyl-*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, $[\alpha]_D^{22}$ +1.3° ($c$=1.6, $CHCl_3$). FAB-MS *m/z*: 1243 $(M+H)^+$. IR (KBr): 3296 ($NH_2$), 1739 (ester), 1640, 1538 (amide).

***S*-[2,3-Bis(palmitoyloxy)-(2*S*)-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, $[\alpha]_D^{22}$ +2.2° ($c$=0.84, $CHCl_3$). FAB-MS *m/z*: 1481 $(M+H)^+$. IR (KBr): 3294 (NH), 1734 (ester), 1628, 1543 (amide).

***S*-[2,3-Bis(palmitoyloxy)-(2*S*)-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 $(M+H)^+$. IR (KBr): 3288 (OH, NH), 1737 (ester), 1661, 1536 (amide). *Anal.* Calcd for $C_{68}H_{125}N_7O_{15}S \cdot 3H_2O$: C, 59.75; H, 9.66; N, 7.17. Found: C, 59.78; H, 9.32; N, 7.15.

***S*-[2,3-Bis(palmitoyloxy)-(2*S*)-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, $[\alpha]_D^{22}$ −20.5° ($c$=0.21, $CHCl_3$). FAB-MS *m/z*: 1249 $(M+H)^+$. IR (KBr): 3282 (OH, NH), 1738 (ester), 1660, 1538 (amide). *Anal.* Calcd for $C_{55}H_{96}Cl_3N_7O_{16}S \cdot H_2O$: C, 52.10; H, 7.79; N, 7.73. Found: C, 52.23; H, 7.75; N, 7.41.

***S*-[2,3-Bis(palmitoyloxy)-(2*S*)-propyl]-(*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 $(M+H)^+$. IR (KBr): 3290 (OH, NH), 1738 (ester), 1661, 1537 (amide).

***N*-Carbobenzyloxy-glycyl-glycine *tert*-Butyl Ester (22)** DEPC (0.36 g, $2.2 \times 10^{-3}$ mol) was added to a stirred solution of Z–Gly–OH (0.38 g, $1.8 \times 10^{-3}$ mol) and H–Gly–OBu$^t$·HCl (0.31 g, $1.8 \times 10^{-3}$ mol) in DMF (2 ml) at 0 °C, followed by the addition of TEA (0.37 g, $3.6 \times 10^{-3}$ 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 $(M+H)^+$. IR (neat): 3328 (NH), 1731 (ester), 1533 (amide).

***N*-Carbobenzyloxy-*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, $1.4 \times 10^{-3}$ mol) was added to a stirred solution of the resulting free base (0.22 g, $1.2 \times 10^{-3}$ mol) and Z–Ser(Bu$^t$)–OH (0.35 g, $1.2 \times 10^{-3}$ mol) in DMF (2 ml) at 0 °C, followed by the addition of TEA (0.14 g, $1.4 \times 10^{-3}$ 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**: $[\alpha]_D^{22}$ +14.1° ($c$=0.61, $CHCl_3$). FAB-MS *m/z*: 466 $(M+H)^+$.

***N*-Carbobenzyloxy-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, $8.9 \times 10^{-4}$ mol) was added to a stirred solution of the resulting free base (0.24 g, $7.4 \times 10^{-4}$ mol) and Z–Asn–OH (0.20 g, $7.4 \times 10^{-4}$ mol) in DMF (2 ml) at 0 °C, followed by the addition of TEA (89 mg, $8.9 \times 10^{-4}$ 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, $[\alpha]_D^{22}$ −9.51° ($c$=1.0, $CHCl_3$). FAB-MS *m/z*: 580 $(M+H)^+$. 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, $2.6 \times 10^{-4}$ 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*-Carbobenzyloxy-*O*-*tert*-butyl-L-seryl-glycine *tert*-Butyl Ester (26)** DEPC (0.23 g, $1.4 \times 10^{-3}$ mol) was added to a stirred solution of Z–Ser(Bu$^t$)–OH (0.35 g, $1.2 \times 10^{-3}$ mol) and HCl·H–Gly–OBu$^t$ (0.20 g, $1.2 \times 10^{-3}$ mol) in DMF (2 ml) at 0 °C, followed by the addition of TEA (0.24 g, $2.4 \times 10^{-3}$ 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**: $[\alpha]_D^{22}$ +21.6° ($c$=0.64, $CHCl_3$). FAB-MS *m/z*: 409 $(M+H)^+$.

***N*-Carbobenzyloxy-L-asparaginyl-*O*-*tert*-butyl-L-seryl-glycine *tert*-Butyl Ester (27)** Compound **26** was hydrogenated with Pd–C in MeOH, and DEPC (53 mg, $3.2 \times 10^{-4}$ mol) was added to a stirred solution of the resulting free base (0.11 g, $2.7 \times 10^{-4}$ mol) and Z–Asn–OH (72 mg, $2.7 \times 10^{-4}$ mol) in DMF (2 ml) at 0 °C, followed by the addition of TEA (32 mg, $3.2 \times 10^{-4}$ 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, $[\alpha]_D^{22}$ +22.6° ($c$=1.0, $CHCl_3$). FAB-MS *m/z*: 523 $(M+H)^+$. IR (KBr): 3292 (NH), 1742 (ester), 1642, 1540 (amide). *Anal.* Calcd for $C_{25}H_{38}N_4O_8 \cdot 1/2H_2O$: 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-glycine *tert*-Butyl Ester (28)** Compound **27** (0.11 g, $2.1 \times 10^{-4}$ 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*-Carbobenzyloxy-L-asparaginyl-*O*-*tert*-butyl-L-serine *tert*-Butyl Ester (29)** DEPC (0.21 g, $1.3 \times 10^{-3}$ mol) was added to a stirred solution of HCl·H–Ser(Bu$^t$)–OBu$^t$ (0.30 g, $1.2 \times 10^{-3}$ mol) and Z–Asn–OH (0.32 g, $1.2 \times 10^{-3}$ mol) in DMF (2 ml) at 0 °C, followed by the addition of TEA (0.26 g, $2.6 \times 10^{-3}$ 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, $[\alpha]_D^{22}$ +22.6° ($c$=1.0, $CHCl_3$). FAB-MS *m/z*: 466 $(M+H)^+$. IR (KBr): 3312 (NH), 1741 (ester), 1675, 1538 (amide). *Anal.* Calcd for $C_{23}H_{35}N_3O_7 \cdot 1/2H_2O$: 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, $3.6 \times 10^{-4}$ 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)-(2*R*)-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, $3.2 \times 10^{-4}$ mol) was added to a stirred solution of **16** (0.23 g, $2.7 \times 10^{-4}$ mol) and **25** (0.12 g, $2.7 \times 10^{-4}$ mol) in DMF (2 ml) at 0 °C, followed by the addition of TEA (33 mg, $3.2 \times 10^{-4}$ mol) in DMF (1 ml) at 0 °C. After the usual work-up, the product was precipitated from MeOH–$CHCl_3$ (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° ($c$=1.0, $CHCl_3$). FAB-MS *m/z*: 1274 $(M+H)^+$. IR (KBr): 3296 (NH), 1743 (ester), 1664, 1532 (amide). *Anal.* Calcd for $C_{60}H_{107}Cl_3N_6O_{14}S \cdot H_2O$: C, 56.53; H, 8.46; N, 6.59. Found: C, 56.36; H, 8.39; N, 6.33.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-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° ($c$=1.34, $CHCl_3$). FAB-MS *m/z*: 1200 $(M+H)^+$.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-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° ($c$=0.20, $CHCl_3$). FAB-MS *m/z*: 988 $(M+H)^+$. IR (KBr): 3308 (OH, NH), 1737 (ester), 1669, 1557 (amide).

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-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° ($c$=0.21, $CHCl_3$). FAB-MS *m/z*: 1161 $(M+H)^+$. IR (KBr): 3304 (OH, NH), 1736 (ester), 1639, 1541 (amide). *Anal.* Calcd for $C_{52}H_{91}Cl_3N_6O_{14}S \cdot 2H_2O$: C, 52.10; H, 7.99; N, 7.01. Found: C, 52.27; H, 8.07; N, 6.75.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-*N*-2,2,2-trichloro-ethoxycarbonyl-(*R*)-cysteinyl-(*S*)-asparaginyl-*O*-*tert*-butyl-(*S*)-seryl-glycine *tert*-Butyl Ester (34)** DEPC (50 mg, $3.1 \times 10^{-4}$ mol) was added to a stirred solution of **16** (0.22 g, $2.6 \times 10^{-4}$ mol) and **28** (0.10 g, $2.6 \times 10^{-4}$ mol) in DMF (2 ml) at 0 °C, followed by the addition of TEA (31 mg, $3.1 \times 10^{-4}$ mol) in DMF (1 ml) at 0 °C. After the usual work-up, the product was precipitated as a solid from MeOH–$CHCl_3$ (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° ($c$=1.2, $CHCl_3$). FAB-MS *m/z*: 1217 $(M+H)^+$. IR (KBr): 3292 (OH, NH), 1742 (ester), 1639, 1540 (amide). *Anal.* Calcd for $C_{58}H_{104}Cl_3N_5O_{13}S$: C, 57.20; H, 8.61; N, 5.75. Found: C, 57.49; H, 8.66; N, 5.80.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-(*R*)-cysteinyl-(*S*)-asparaginyl-*O*-*tert*-butyl-(*S*)-seryl-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° ($c$=0.6, $CHCl_3$). FAB-MS *m/z*: 1043 $(M+H)^+$.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-(*R*)-cysteinyl-(*S*)-asparaginyl-(*S*)-seryl-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° ($c$=0.24, $CHCl_3$). FAB-MS *m/z*: 930 $(M+H)^+$. IR (KBr): 3308 (OH, NH), 1741 (ester), 1639, 1539 (amide).

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-*N*-2,2,2-trichloro-ethoxycarbonyl-(*R*)-cysteinyl-(*S*)-asparaginyl-(*S*)-seryl-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° ($c$=0.20, $CHCl_3$ : MeOH = 1 : 1). FAB-MS *m/z*: 1105 $(M+H)^+$. IR (KBr): 3296 (ON, NH), 1734 (ester), 1655, 1540 (amide). *Anal.* Calcd for $C_{50}H_{88}Cl_3N_5O_{13}S \cdot H_2O$: C, 53.44; H, 8.07; N, 6.23. Found: C, 53.50; H, 8.03; N, 6.30.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-*N*-2,2,2-trichloro-ethoxycarbonyl-(*R*)-cysteinyl-(*S*)-asparaginyl-*O*-*tert*-butyl-(*S*)-serine *tert*-Butyl Ester (36)** DEPC (65 mg, $4.0 \times 10^{-4}$ mol) was added to a stirred solution of **16** (0.28 g, $3.3 \times 10^{-4}$ mol) and **29** (0.11 g, $3.3 \times 10^{-4}$ mol) in DMF (2 ml) at 0 °C, followed by the addition of TEA (40 mg, $4.0 \times 10^{-4}$ 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 (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° ($c$=1.1, $CHCl_3$). FAB-MS *m/z*: 1160 $(M+H)^+$. IR (KBr): 3314 (OH, NH), 1741 (ester), 1641, 1538 (amide). *Anal.* Calcd for $C_{56}H_{101}Cl_3N_4O_{12}S$: C, 57.94; H, 8.77; N, 4.83. Found: C, 57.92; H, 8.74; N, 4.61.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-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° ($c$=0.54, $CHCl_3$). FAB-MS *m/z*: 986 $(M+H)^+$.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-(*R*)-cysteinyl-(*S*)-asparaginyl-(*S*)-serine (9)** $CF_3COOH$ (2 ml) was added to **37** (27 mg, $2.7 \times 10^{-6}$ 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° ($c$=0.22, $CHCl_3$). FAB-MS *m/z*: 874 $(M+H)^+$. IR (KBr): 3404 (OH, NH), 1742 (ester), 1664, 1538 (amide).

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-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° ($c$=0.28, $CHCl_3$). FAB-MS *m/z*: 1047 $(M+H)^+$. IR (KBr): 3302 (OH, NH), 1739 (ester), 1659, 1537 (amide). *Anal.* Calcd for $C_{48}H_{85}Cl_3N_4O_{12} \cdot S \cdot H_2O$: C, 54.98; H, 8.17; N, 5.34. Found: C, 55.02; H, 8.30; N, 5.08.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-propyl]-*N*-2,2,2-trichloro-ethoxycarbonyl-(*R*)-cysteinyl-(*S*)-asparagine *tert*-Butyl Ester (38)** DEPC (33 mg, $2.0 \times 10^{-4}$ mol) was added to a stirred solution of **16** (0.17 g, $2.0 \times 10^{-4}$ mol) and H–Asn–OBu$^t$·HCl (42 mg, $2.0 \times 10^{-4}$ mol) in DMF (2 ml) at 0 °C, followed by the addition of TEA (40 g, $4.0 \times 10^{-4}$ 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 (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° ($c$=0.81, $CHCl_3$). FAB-MS *m/z*: 1017 $(M+H)^+$. IR (KBr): 3376 (OH, NH), 1727 (ester), 1680, 1665 (amide); *Anal.* Calcd for $C_{49}H_{88}Cl_3N_3O_{10}S \cdot 1/2H_2O$: C, 57.32; H, 8.74; N, 4.09. Found: C, 57.36; H, 8.62; N, 3.88.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-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° ($c$=0.88, $CHCl_3$). FAB-MS *m/z*: 843 $(M+H)^+$.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-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° ($c$=0.20, $CHCl_3$). FAB-MS *m/z*: 787 $(M+H)^+$. IR (KBr): 3404 (OH, NH), 1741 (ester), 1630, 1536 (amide).

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-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° ($c$=0.20, $CHCl_3$). FAB-MS *m/z*: 960 $(M+H)^+$. IR (KBr): 3348 (OH, NH), 1740 (ester), 1666 (amide). *Anal.* Calcd for $C_{45}H_{80}Cl_3N_3O_{10}S$: C, 56.21; H, 8.39; N, 4.37. Found: C, 56.04; H, 8.63; N, 4.22.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-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° ($c$=0.35, $CHCl_3$). FAB-MS *m/z*: 729 $(M+H)^+$.

***S*-[2,3-Bis(palmitoyloxy)-(2*R*)-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° ($c$=0.22, $CHCl_3$). FAB-MS *m/z*: 673 $(M+H)^+$. IR (KBr): 3410 (OH, NH), 1736 (ester), 1630, 1536 (amide).

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