



The cyclic protected peptides (II), *S,S'*,*S'*-bis (*N*-*t*-butyloxycarbonyl-L-hemicystinyl-L-hemicystine *t*-butyl ester), *S,S'*,*S'*-bis (*N*-*t*-butyloxycarbonyl-L-hemicystinylglycyl-L-hemicystine-*t*-butyl ester) and *S,S'*,*S'*-bis (*N*-*t*-butyloxycarbonyl-L-hemicystinylglycylglycyl-L-hemicystine *t*-butyl ester), were obtained by direct oxidative removal of the *S*-trityl group from the corresponding peptides (I). The reactions proceeded in good yields to give crystalline products. The peptides with *n* = 0 and 2 were purified by column chromatography (silica gel) and that with *n* = 1 by crystallization from methanol. The molecular masses of the pure cyclic peptides were determined by mass spectrometry.

## EXPERIMENTAL

The purity of all compounds was confirmed by t.l.c. on Kieselgel 60 F<sub>254</sub>, usually in the four systems chloroform-methanol (9:1), benzene-chloroform-ethanol (12:12:1), acetic acid-chloroform (1:9), and ethyl acetate-methanol (40:1). The compounds were revealed by the (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>-H<sub>2</sub>SO<sub>4</sub> method [5]. Evaporations and concentrations were all carried out under reduced pressure with a rotary evaporator. Extracts were dried over magnesium sulphate. Light petroleum was the fraction b.p. 40-60°C. When purification was achieved by column chromatography, silica gel (<0.08 mm) from Merck was used. Optical rotations were measured with a Bellingham and Stanley Pepol 66 polarimeter. N.m.r. spectra were recorded by Dr. J.A.B. Baptista at 33°C with a Perkin-Elmer R32 90 MHz spectrometer. The microanalyses were carried out by Dr. Ilse Beetz (Kronach, Germany).

### *N*-*t*-Butyloxycarbonyl-*S*-trityl-L-cysteinylglycylglycine.

*N*-*t*-Butyloxycarbonyl-*S*-trityl-L-cysteine *N*-hidroxysuccinimidyl ester [6] was coupled with glycylglycine hydrochloride [7], yielding the peptide (62%), m.p. 104°C (softening from 82°C),  $[\alpha]_D^{20} + 19.1^\circ$  (c 1.00 in MeOH) (Found: C, 64.8; H, 6.3; N, 7.2; S, 5.1. C<sub>31</sub>H<sub>35</sub>N<sub>3</sub>O<sub>16</sub>S requires C, 64.6; H, 6.1; N, 7.3; S, 5.5%).

### *N,N'*-Bis (*N*-*t*-butyloxycarbonyl-*S*-trityl-L-cysteinyl)-L-cystine bis-*t*-butyl ester.

To a solution of *N*-*t*-butyloxycarbonyl-*S*-trityl-L-cysteine [8,9] (1.42 g, 0.0031 mol) in dichloromethane (4 ml), cooled to -10°C and stirred, was added *N,N'*-dicyclohexylcarbodi-imide (0.63 g, 0.0031 mol). A solution of L-cystine bis-*t*-butyl ester [10] (0.53 g, 0.0015 mol), recently prepared, in dichloromethane (3 ml) was added. The mixture was kept at -10°C for 2 h and at room temperature for 4 days. The precipitated *N,N'*-dicyclohexylurea was filtered off and the filtrate was washed (saturated aqueous sodium chloride, aqueous 5% citric acid, aqueous *M*-sodium hydrogen carbonate, and saturated aqueous sodium chloride), dried and evaporated. The residue was dissolved in acetone and kept at 0°C for 24 h. The solution was filtered and evaporated and the residue triturated with light petroleum, giving a solid. Two recrystallisations from diethyl ether gave the pure peptide (0.84 g, 50%), m.p. 114°C (softening from 99°C),  $[\alpha]_D^{20} - 3.4^\circ$  (c 0.5 in MeOH),  $\tau$ (CDCl<sub>3</sub>), 2.40-3.00 (30 H, complex, Ph), 3.00-3.18 (2 H, d, NH), 4.70-5.00 (2 H, d, NH), 5.27-5.60 (2 H, complex, CH), 5.94-6.28 (2 H, complex, CH), 6.80-7.10 (4 H, d, CH<sub>2</sub>), 7.22-7.50 (4 H, d, CH<sub>2</sub>), 8.40-8.73 (36 H, s, Bu<sup>t</sup>) (Found: C, 66.0; H, 6.5; N, 4.5; S, 11.2. C<sub>68</sub>H<sub>82</sub>N<sub>4</sub>O<sub>10</sub>S<sub>4</sub> requires C, 65.7; H, 6.6; N, 4.5; S, 10.8%).

### *N,N'*-Bis (*N*-*t*-butyloxycarbonyl-*S*-trityl-L-cysteinylglycyl)-L-cystine bis-*t*-butyl ester.

*N*-*t*-Butyloxycarbonyl-*S*-trityl-L-cysteinylglycine [6] was coupled with L-cystine bis-*t*-butyl ester [10] by the *N,N'*-dicyclohexylcarbodi-imide method, as described above. The crude compound after trituration with light petroleum and diethyl ether, was recrystallised from ethyl acetate, giving the peptide (40%), m.p. 155°C (softening from 112°C),  $[\alpha]_D^{25} + 25.7^\circ$  (c 1.00 in CHCl<sub>3</sub>),  $\tau$ (CDCl<sub>3</sub>) 2.43-3.00 (34 H, complex, Ph and NH), 4.72-5.02 (2 H, d, NH), 5.12-5.50 (2 H, complex, CH), 5.90-6.30 (6 H, complex, CH and CH<sub>2</sub>), 6.90-7.15 (4 H, d, CH<sub>2</sub>S), 7.20-7.50 (4 H, d, CH<sub>2</sub>S), 8.40-8.68 (36 H, 2 s, Bu<sup>t</sup>) (Found: C, 62.4; H, 6.5; N, 6.2; S, 9.8. C<sub>72</sub>H<sub>88</sub>N<sub>6</sub>O<sub>12</sub>S<sub>4</sub> requires C, 62.6; H, 6.5; N, 6.2; S, 9.4%).

### *N,N'*-Bis (*N*-*t*-butyloxycarbonyl-*S*-trityl-L-cysteinylglycylglycyl)-L-cystine bis-*t*-butyl ester

*N*-*t*-Butyloxycarbonyl-*S*-trityl-L-cysteinylglycylglycine was coupled with L-cystine bis-*t*-butyl ester [10] by the *N,N'*-dicyclohexyl-

carbodi-imide method, keeping the temperature at  $-15^{\circ}\text{C}$  for 10 days. The crude solid isolated from the reaction was applied to a column of silica gel. Gradient elution with chloroform to chloroform-ethanol (9:1), yielded a chromatographically homogeneous *peptide* (55%), which after crystallisation from acetone had m.p.  $122\text{--}124^{\circ}\text{C}$  (softening from  $104^{\circ}\text{C}$ ),  $[\alpha]_{\text{D}}^{25} + 18.0^{\circ}$  (c 0.90 in  $\text{CHCl}_3$ ),  $\tau(\text{CDCl}_3)$  2.20-3.20 (36 H, complex, Ph and NH), 4.68-4.96 (2 H, d, NH), 5.20-5.56 (2 H, complex, CH), 5.82-6.50 (10 H, complex, CH and  $\text{CH}_2$ ), 6.80-7.10 (4 H, d,  $\text{CH}_2\text{S}$ ), 7.23-7.53 (4 H, d,  $\text{CH}_2\text{S}$ ), 8.30-8.83 (36 H, 2 s,  $\text{Bu}^t$ ) (Found: C, 61.4; H, 6.3; N, 7.8; S, 8.4.  $\text{C}_{76}\text{H}_{94}\text{N}_8\text{O}_{14}\text{S}_4$  requires C, 61.0; H, 6.4; N, 7.6; S, 8.7%).

*S,S'-S',S'-Bis (N-t-butyloxycarbonyl-L-hemicystinyl-L-hemicystine t-butyl ester).*

To a solution of *N,N'*-bis-(*N*-t-butyloxycarbonyl-S-trityl-L-cysteinyl)-L-cystine bis-t-butyl ester (0.747 g, 0.0006 mol) in methanol (300 ml), was added a solution of iodine (0.735 g, 0.003 mol) in methanol (75 ml), dropwise and with stirring. The reaction mixture was, then, kept with stirring for a further 30 min. After cooling to  $0^{\circ}\text{C}$ , aqueous M-sodium thiosulphate was added until a colourless solution was obtained. Evaporation of the solvent to about a volume of 30 ml, followed by the addition of water (200 ml) yielded a white solid. This was filtered off, washed thoroughly with water, and dried. The purification of the compound was achieved by column chromatography (silica gel), using chloroform as eluent, followed by two recrystallisations from chloroform-ethyl acetate. The pure *peptide* was obtained in 56% yield, m.p.  $198\text{--}199^{\circ}\text{C}$ ,  $[\alpha]_{\text{D}}^{25} + 102.4^{\circ}$  (c 0.50 in  $\text{CHCl}_3$ ),  $\tau(\text{CDCl}_3)$  2.42-2.67 (2 H, d, NH), 3.72-4.00 (2 H, d, NH), 5.07-5.47 (4 H, complex, CH), 6.00-6.45 (4 H, 2 d,  $\text{CH}_2\text{S}$ ), 6.76-7.02 (4 H, complex,  $\text{CH}_2\text{S}$ ), 8.30-8.62 (36 H, s,  $\text{Bu}^t$ ) (Found: C, 47.4; H, 6.9; N, 7.6; S, 16.4;  $\text{C}_{30}\text{H}_{52}\text{N}_4\text{O}_{10}\text{S}_4$  requires C, 47.6; H, 6.9; N, 7.4; S, 16.9%), molecular-mass (EI-MS):  $m/e = 757$  (calc. 757).

*S,S'-S',S'-Bis (N-t-butyloxycarbonyl-L-hemicystinylglycyl-L-hemicystine t-butyl ester).*

The same procedure was applied to *N,N'*-bis-(*N*-t-butyloxycarbonyl-S-trityl-L-cysteinylglycyl)-L-cystine bis-t-butyl ester. The isolated crude

material on crystallisation from methanol and trituration with ethanol and diethyl ether gave the *cyclic peptide*, chromatographically homogeneous, in 53% yield, m.p.  $200\text{--}202^{\circ}\text{C}$  (decomp.),  $\tau[(\text{CD}_3)_2\text{SO}]$  1.60-2.00 (4 H, d, NH), 2.80-3.20 (2 H, d, NH), 5.30-5.83 (4 H, complex, CH), (6.10-6.35 (4 H, d,  $\text{CH}_2$ ), 6.85-7.15 (8 H, d,  $\text{CH}_2\text{S}$ ), 8.30-8.90 (36 H, s,  $\text{Bu}^t$ ) (Found: C, 46.9; H, 6.7; N, 9.6; S, 14.3.  $\text{C}_{34}\text{H}_{58}\text{N}_6\text{O}_{12}\text{S}_4$  requires C, 46.9; H, 6.7; N, 9.6; S, 14.7%), molecular-mass (FD-MS):  $m/e = 871$  (calc. 871).

*S,S'-S',S'-Bis (N-t-butyloxycarbonyl-L-hemicystinylglycylglycyl-L-hemicystine t-butyl ester).*

Identical procedure was applied to *N,N'*-bis(*N*-t-butyloxycarbonyl-S-trityl-L-cysteinylglycylglycyl)-L-cystine bis-t-butyl ester. The compound was purified by column chromatography (silica gel), using chloroform-ethanol (19:1) as eluent and chrysallisation from methanol. The *cyclic peptide*, chromatographically homogeneous was obtained in 73% yield, m.p.  $164^{\circ}\text{C}$  (softening from  $154^{\circ}\text{C}$ ),  $\tau(\text{CD}_3\text{OD})$  5.30-5.60 (8 H, complex, NH), 5.86-6.26 (12 H, complex, CH and  $\text{CH}_2$ ), 6.88-7.16 (8 H, complex,  $\text{CH}_2\text{S}$ ), 8.40-8.76 (36 H, s,  $\text{Bu}^t$ ) (Found: C, 44.8; H, 6.6; N, 11.4; S, 12.0.  $\text{C}_{38}\text{H}_{64}\text{N}_8\text{O}_{14}\text{S}_4 \cdot 2\text{H}_2\text{O}$  requires C, 44.6; H, 6.7; N, 11.0; S, 12.5%), molecular-mass (FD-MS):  $m/e = 985$  (calc. 985).

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## RESUMO

### Novo método de síntese de peptídeos cíclicos simétricos de L-cistina

Neste trabalho descreve-se um novo método de síntese de péptidos cíclicos simétricos, protegidos, de L-cistina, contendo duas ligações dissulfureto, usando derivados de L-cistina e L-cisteína, simultaneamente.