Synthesis of L-Cystine Bis-t-butyl Ester and its Application to Peptide Synthesis

By **M. Joaquina S. A. Amaral,* M. Adelina Macedo,** and **M. Isabel A. Oliveira,** Centro de Investigação em Química da Universidade do Porto, Faculdade de Ciências, Porto, Portugal

The preparation of L-cystine bis-t-butyl ester and its use in the synthesis of *NN'*-bis(tritylglycyl)-L-cystine bis-t-butyl ester and *NN'*-bis(tritylglycylglycyl)-L-cystine bis-t-butyl ester are described.

L-CYSTINE BIS-T-BUTYL ESTER PERCHLORATE has been synthesized in good yield by a modification of the procedure of Chimiak *et al.*,[1] which involves treatment of the amino-acid with t-butyl acetate and aqueous perchloric acid. The ester perchlorate was converted into the free bis-t-butyl ester, usually isolated as an oil but occasionally as a low melting solid. Both forms were used in synthesis with identical results. The free ester does not have the exceptional stability characteristic of other free amino-acid t-butyl esters; it decomposes slowly in a desiccator under vacuum. Attempts to convert it into other salts such as the acetate, oxalate, and hydrochloride, in order to allow further purification and characterization, were unsatisfactory; the ester group appeared to be partially removed during crystallization.

In order to verify its applicability in peptide synthesis, L-cystine bis-t-butyl ester, chromatographically homogeneous and characterized by n.m.r. spectroscopy, was coupled with *N*-tritylglycine and *N*-tritylglycylglycine by the *NN'*-dicyclohexylcarbodi-imide procedure. The reactions proceeded in good yields to give crystalline products.

EXPERIMENTAL

The purity of all compounds was confirmed by t.l.c. on kieselgel 60 F_{254}, usually in the three systems pyridine–butan-1-ol–water (1 : 2 : 2), benzene–chloroform–ethanol (12 : 12 : 1), and chloroform–methanol (9 : 1). Compounds with free amino-groups were located by spraying with 0.3% ninhydrin in butan-1-ol and heating for 10 min at 100 °C. Other compounds were revealed by the $(NH_4)_2SO_4$–H_2SO_4 method,[2] which can be applied subsequently. Evaporations and concentrations were all carried out under reduced pressure with a rotary evaporator. Extracts were dried over magnesium sulphate. Optical rotations were measured with a Bellingham and Stanley Pepol 66 polarimeter. N.m.r. spectra were recorded at 33 °C with a Perkin-Elmer R32 90 MHz spectrometer. The microanalyses were carried out by Dr. Ilse Beetz (Kronach, Germany).

L-Cystine Bis-t-butyl ester.—L-Cystine (1.92 g, 0.008 mol; finely powdered) was dissolved in aqueous 60% perchloric acid (5.88 g, 0.035 2 mol) with stirring. t-Butyl acetate (50 ml) was added and stirring was continued until a homogeneous solution was obtained (2 h). The mixture was kept at room temperature for 2 days, during which a white solid crystallised out. After cooling to 0 °C for 24 h, the solid was filtered off, washed with diethyl ether, and dissolved in diethyl ether (50 ml) and aqueous M-sodium hydrogen carbonate (25 ml). The organic layer was washed in succession with aqueous M-sodium hydrogen carbonate (15 ml) and saturated aqueous sodium chloride until it was no longer alkaline, dried, and evaporated, yielding a chromatographically homogeneous *oil* (1.9 g, 67%) (occasionally the product was a low melting, white solid); $[\alpha]_D^{20}$ −8.2° (*c* 2.02 in MeOH), τ [$(CD_3)_2SO$] 8.50—8.62 (18 H, s, Bu^t), 6.33—6.55 (2 H, t, CH), and 6.7—7.2 (8 H, complex, NH_2 and CH_2). The compound should be used within a few days otherwise it decomposes, becoming yellowish.

NN'-Bis(tritylglycyl)-L-cystine Bis-t-butyl Ester.—*NN'*-Dicyclohexylcarbodi-imide (4.5 g, 0.021 8 mol) was added to a stirred suspension of *N*-tritylglycine [3] (6.9 g, 0.021 7 mol) in dry dichloromethane (50 ml) at −10 °C. To the mixture was added a solution of L-cystine bis-t-butyl ester (3.8 g, 0.010 7 mol) in dichloromethane (30 ml). After 48 h at 0 °C and 6 days at room temperature, *NN'*-dicyclohexylurea was filtered off and the filtrate was extracted successively with aqueous 0.024M-citric acid, M-sodium hydrogen carbonate, and saturated aqueous sodium chloride, dried, and evaporated to dryness. The residue solidified under light petroleum (b.p. 40—60 °C). After decantation of the solvent the solid was recrystallised from ethyl acetate–light petroleum (b.p. 40—60 °C), giving the *peptide* (8.0 g, 79%), m.p. 126—129°. Further recrystallisation gave the pure compound, m.p. 129—132° (6.7 g, 66%), $[\alpha]_D^{25}$ −43.9° (*c* 1.00 in MeOH), τ [$(CD_3)_2SO$] 1.85—2.05 (2 H, d, NH), 2.50—3.00 (32 H, complex, Ph and NH), 5.10—5.35 (2 H, q, CH), 6.68—6.80 (4 H, d, CH_2S), 6.98—7.20 (4 H, d, CH_2), and 8.45—8.60 (18 H, s, Bu^t) (Found: C, 70.4; H, 6.9; N, 5.7; S, 6.6. $C_{56}H_{62}N_4O_6S_2$ requires C, 70.7; H, 6.7; N, 5.9; S, 6.7%).

N-Tritylglycylglycine.—*N*-Tritylglycine [3] was coupled with glycine ethyl ester hydrochloride by the *NN'*-dicyclohexylcarbodi-imide method, yielding *N*-tritylglycylglycine ethyl ester (80%), m.p. 160—162° (lit.,[4] 159°; lit.,[3] yield 83%, m.p. 163—164°). The peptide was hydrolysed [3] giving *N*-tritylglycylglycine (93%), m.p. 179° (lit.,[4] 168°).

NN'-Bis(tritylglycylglycyl)-L-cystine Bis-t-butyl Ester.—To a suspension of *N*-tritylglycylglycine (3.37 g, 0.009 mol) in dichloromethane (20 ml), cooled to −10 °C and stirred, was added *NN'*-dicyclohexylcarbodi-imide (1.85 g, 0.009 mol). A solution of L-cystine bis-t-butyl ester (1.59 g, 0.004 5 mol) in dichloromethane (10 ml) was added. The mixture was kept at −10 °C for 2 h and at room temperature for 6 days. The precipitated *NN'*-dicyclohexylurea was filtered off and the filtrate was washed (saturated aqueous sodium chloride, 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 was triturated with light petroleum (b.p. 40—60 °C), giving a solid. Four recrystallisations from ethyl acetate gave the pure *peptide* (2.04 g, 43%), m.p. 161—162°, $[\alpha]_D^{25}$ −24.75° (*c* 1.00 in MeOH), τ ($CDCl_3$) 1.70—2.20 (4 H, t, NH), 2.50—3.00 (32 H, complex, Ph and NH), 5.10—5.40 (2 H, q, CH),

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5.80—6.10 (4 H, t, CH_2), 6.80—6.98 (4 H, d, CH_2S), 7.00—7.20 (4 H, d, CH_2), and 8.30—8.70 (18 H, s, Bu^t) (Found: C, 67.5; H, 6.5; N, 7.9; S, 6.0. $C_{60}H_{68}N_6O_8S_2$ requires C, 67.6; H, 6.4; N, 7.9; S, 6.0%).

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