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NEW APPROACH TO THE USE OF 2-CHLOROETHYL GROUP AS CARBOXYL PROTECTION IN PEPTIDE SYNTHESIS (*)

The synthesis of six new fully protected dipeptides 2-chloroethyl esters and a new method for the removal of the C-protection by the action of the sulphide anion are described.

(*) A communication on this work was presented at the 17th European Peptide Symposium, Prague, Czechoslovakia, 1982, reference [1].

A number of compounds of biological and synthetic interest containing the carboxyl group often have to be suitably C-protected during synthesis procedures. The most representative example of this is verified in the field of peptide synthesis.

The carboxyl protection has been achieved mainly through esterification and there is a wide range of ester groups which can be cleaved under very diversified hydrolytic conditions [2, 3].

2-Haloethyl ester groups, mono or polysubstituted, have been used as they allow both solvolysis removal and reductive elimination. In principle, the most attractive of these groups would be the 2-chloroethyl ester, but being rather stable its removal has been described, lately, only for the corresponding benzoate, although achieved by a variety of reagents:

a) The binucleophiles trithiocarbonate ion [4] and ethanedithiolate ion [5].
b) The supernucleophile cobalt(I) phthalocyanine ion [6, 7].
c) The hydrogen selenide ion [8] and the hydrogen telluride ion [9].
d) The vitamin B_{12} derivatives as catalysts for the reductive elimination [10].

The 2-chloroethyl group has had little application in peptide synthesis as it is inert towards the deblocking reagents commonly used in this field.

However, 2-chloroethyl esters of some aminoacids and peptides were used as intermediates in the synthesis of other C-protected derivatives such as 2-(4-methylphenylthio)ethyl esters [11] and 2-iodoethyl or choline esters [12].

HO and WONG [13] described an interesting procedure for the deprotection of 4-chlorobutyl and 5-chloropentyl esters of simple carboxyl acids by the action of the bidentate nucleophile sulphide anion. The reaction proceeds by nucleophilic substitution of the chloride ion, followed by intramolecular substitution. This prompted us to investigate similar removing conditions with the 2-chloroethyl

ester group used as carboxyl protection in peptide synthesis. For this purpose the 2-chloroethyl esters of glycine and L-leucine (as hydrochlorides) were coupled with N-benzyloxycarbonyl, N-t-butyloxycarbonyl and N-trityl glycines, by the N,N'-dicyclohexylcarbodi-imide procedure, giving the six new fully protected dipeptides $XHNCH_2CO-HNCHRCOOCH_2CH_2Cl$; (R = H, $CH_2CH(CH_3)_2$; X = Z, Boc, Trt). The 2-chloroethyl ester group could be selectively removed from all these peptides by the action of sodium sulphide in aqueous acetonitrile with reflux for one hour. The N-protected dipeptides were isolated in 42 to 75% yields. The N-t-butyloxycarbonyl and the N-trityl groups were also selectively removed from the fully protected dipeptides under acidic conditions, by the action of hydrogen chloride in acetic acid and in ethanol, respectively, and 72 to 85% yields were obtained.

EXPERIMENTAL

The purity of all compounds was confirmed by t.l.c. on kieselgel 60 F_{254}, usually in the four systems n-butanol-acetic acid-water (4 : 1 : 5), benzene-methanol (5 : 1), chloroform-ethylacetate-methanol (95:3:5) and chloroform-methanol (9 : 1). The compounds were revealed by the $(NH_4)_2SO_4 - H_2SO_4$ method [14]. Evaporations and concentrations were all carried out under reduced pressure with a rotary evaporator. Optical rotations were measured with a Bellingham and Stanley Pepol 66 polarimeter. N.m.r. spectrà 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).

Glycine 2-chloroethyl ester hydrochloride and L-*leucine 2-chloroethyl ester hydrochoride* were both prepared by direct esterification of the aminoacids [11].

According to the procedures described in the literature N-*benzyloxycarbonylglycine* [15], N-*t-butyloxycarbonylglycine* [16] and *N-tritylglycine* [17 a] were prepared.

General Procedure for the Synthesis of 2-Chloroethyl Esters of N-Protected Dipeptides:

To a solution of N-protected aminoacid (0,025 mol) in dry dichloromethane (55 ml), cooled to -10°C and stirred, was added *N,N'*-dicyclohexylcarbodi-imide (0.025 mol). The aminoacid chloroethyl ester hydrochloride (0.025 mol) and triethylamine (0.025 mol) were added. The mixture was kept at 0°C for 4 hours and at room temperature for 3 to 4 days. The precipitated *N,N'*-dicyclohexylurea was filtered off and the filtrate was washed [saturated aqueous sodium chloride, aqueous 5% citric acid (with the exception of the Boc-derivatives), aqueous 1M-sodium hydrogen carbonate and saturated aqueous sodium chloride], dried ($MgSO_4$) and evaporated. The residue was dissolved in acetone and kept at 0°C for 24 hours. After filtration the solvent was removed and, when possible, the fully protected peptide was crystallised. The following dipeptides were prepared in this way:

N-*Benzyloxycarbonylglycylglycine 2-chloroethyl ester,* 74% yield, m. p. 110°C (from ethanol), δ(DMSO-d_6), 3.60-3.83 (4 H, complex, CH_2Cl and NCH_2CO), 4.23-4.45 (2 H, t, $COOCH_2$), 5.03 (2 H, s, $PhCH_2$), 7.20-7.50 (6H, complex, Ph and NH), 8.05-8.30 (1 H, t, NH) (Found: C, 51.4; H, 5.3; Cl, 10.6; N, 8.5. $C_{14}H_{17}ClN_2O_5$ requires C, 51.2; H, 5.2; Cl, 10.8; N, 8.5%).

N-*t-Butyloxycarbonylglycylglycine 2-chloroethyl ester*, 50% yield, m. p. 70-72°C (from ethyl ether), δ ($CDCl_3$) 1.45 (9 H, s, Bu^t), 3.58-3.77 (2 H, t, CH_2Cl), 3.77-3.95 (2 H, d, NCH_2CO), 4.03-4.18 (2 H, d, NCH_2COO), 4.32-4.50 (2H, t, $COOCH_2$), 5.28-5.53 (1H, t, NH), 6.80-7.05 (1 H, t, NH). (Found: C, 44.7; H, 6.3; Cl, 11.9; N, 8.9. $C_{11}H_{19}Cl\,N_2O_5$ requires C, 4.48; H, 6.5; Cl, 12.0; N, 9.5%).

N-*Tritylglycylglycine 2-chloroethyl ester*, 70% yield, m. p. 144-145°C (from ethanol), δ (DMSO-d_6) 2.65-2.90 (2 H, d , NCH_2CO), 3.70-3.90 (2 H, t, CH_2Cl), 3.9-4.05 (2 H, d , NCH_2COO), 4.25-4.45 (2 H, t, $COOCH_2$),

6.90-7.60 (16 H, complex, Ph and NH), 8.30-8.60 (1 H, t, NH). (Found: C, 69.2; H, 5.8; Cl, 7.4; N, 6.4. $C_{25}H_{25}ClN_2O_3$ requires C, 68.7; H, 5.8; Cl, 8.1; N, 6.4%).

N-*Benzyloxycarbonylglycyl-L-leucine 2-chloroethyl ester*, 58% yield, an oil, δ($CDCl_3$) 0.80-1.10 (6 H, d , CH_3), 1.15-1.90 (3 H, complex, CH_2CH), 3.50-3.75 (2 H, t, CH_2Cl), 3.75-4.00 (2 H, d, NCH_2CO), 4.20-4.45 (2 H, t , $COOCH_2$), 4.50-4.80 (2 H, complex, NCH), 5.10 (2 H, s, CH_2Ph), 5.55-5.80 (1H, t , NH), 6.60-6.80 (1 H, d, NH), 7.30 (5 H, s, Ph).

N-*t-Butyloxycarbonylglycyl-L-leucine 2-chloroethyl ester*, 72% yield, an oil, δ ($CDCl_3$) 0.97-1.10 (6 H, d, CH_3), 1.40-1.90 (12H, complex, Bu^t and CH_2CH), 3.58-3.75 (2H, t, CH_2Cl), 3.75-3.90 (2 H, d, NCH_2CO), 4.28-4.50 (2H, t, $COOCH_2$), 5.35-5.55 (1 H, t , NH), 6.70-6.95 (1 H, d, NH).

N-*Tritylglycyl-L-leucine 2-chloroethyl ester*, 75% yield, an oil δ ($CDCl_3$) 0.90-1.10 (6 H, d, CH_3), 1.50-1.85 (3 H, complex, CH_2CH), 2.95 (2 H, s, NCH_2CO), 3.50-3.85 (2H, t, CH_2Cl), 4.20-4.45 (2 H, t, $COOCH_2$), 4.50-6.90 (1H, complex, NCH), 6.60-7.00 (1 H, d, NH), 7.00-7.50 (15, complex, Ph), 7.50-7.90 (1 H, t, NH).

Removal of N-Protecting Groups from the Dipeptides Esters.

a) N-*t-Butyloxycarbonyl group*

N-t-Butyloxycarbonylglycylglycine 2-chloroethyl ester (0.45g;0.0015mol) was dissolved in a solution of 1M-HC1 in acetic acid (8,5ml) The reaction mixture was stirred for 20 minutes at room temperature and by addition of ethyl ether a white solid precipitated. After cooling, the product was filtered off and recrystallised from ethanol, giving the pure *glycylglycine 2-chloroethyl ester hydrochloride*, (0.30g;85%), m.p. 183-185°C, δ(DMSO-d_6) 3.10-3.50 (2H, broad, NCH_2CO), 3.75-3.90 (2H, t, CH_2Cl), 3.90-4.10 (2H, d, NCH_2CO), 4.25-4.50 (2H, t, $COOCH_2$), 8.10-8.65 (3H, broad, $\overset{+}{N}H_3$), 8.90-9.20(1H, t, NH) (Found: C,31.3; H,5.2; Cl,30.8; N,12.2. $C_6H_{12}Cl_2N_2O_3$ requires C,31.2; H,5.2; Cl,30.7; N, 12.1%).

Similarly, the *glycyl-L-leucine 2-chloroethyl ester hydrochoride* was prepared, 72% yield, m.p. 167-168°C, $[\alpha]_D^{20}$ – 37.4° (c 0.5 in MeOH), δ(DMSO-d_6) 0.75-1.10 (6H, complex, CH_3), 1.40-1.85 (3H, complex, CH_2CH), 3.10-3.60 (2H, complex, NCH_2CO), 3.70-4.00 (2H, t, CH_2Cl), 4.15-4.60 (3H, complex, NCH and $COOCH_2$), 8.00-8.60 (3H, broad, $\overset{+}{N}H_3$), 8.90-9.10 (1H, d, NH). (Found: C,42.0; H,7.0; Cl,24.3; N,9.7. $C_{10}H_{20}Cl_2N_2O_3$ requires C,41.8; H,7.0; Cl,24.7; N,9.8%).

b) N-*Trityl group.*

N-Tritylglycylglycine 2-chloroethyl ester (0.44g; 0.001 mol) in ethanol (5ml) and 1.5M-ethanolic HCl (2.8ml) were heated at 60°C for 3 minutes. Concentration to half volume and precipitation with ether gave a solid. This, after recrystallisation from ethanol, gave glycylglycine 2-chloroethyl ester hydrochloride (0.18g; 78%), m.p. and mixed m.p. 183-185°C.

Similarly, the glycyl-L-leucine 2-chloroethyl ester hydrochloride was obtained, 79% yield, m.p. and mixed m. p. 167-168°C.

Removal of the 2-Chloroethyl Ester Group.

General Procedure: A mixture of the fully protected dipeptide (0.005 mol) and sodium sulphide nonahydrate (0.006mol) in 10 ml of aqueous acetonitrile (2:3) was refluxed for 1 hour with stirring. The reaction mixture was cooled and, after addition of water (30ml), extracted with diethyl ether. The aqueous layer was mixed with ethyl acetate, titrated with aqueous 2M-HCl at pH3 with stirring, separated from the organic solvent and washed several times with ethyl acetate. The combined organic extracts were dried ($MgSO_4$) and evaporated do dryness to yield the N-protected dipeptide which was further recrystallised from a suitable solvent.

From the corresponding fully protected dipeptides 2-chloroethyl esters, using the described method for the ester removal, the following N-protected dipeptides were prepared:

N-Benzyloxycarbonylglycylglycine, 75% yield, m.p. 175-178°C (from ethanol) (lit.[18], m.p. 177-178°C).

N-t-Butyloxycarbonylglycylglycine, 50% yield, m.p. 135-136°C (from ethyl acetate), δ(DMSO-d_6) 1.20-1.50 (9H, s, Bu^t), 3.45-3.68 (2H, d, NCH_2CO), 3.68-3.88 (2H, d, CH_2COO), 6.70-7.00 (1H, tripleto, NH), 7.85-8.10 (1H, t, NH). (Found: C,46.0; H,6.8; N,12.0. $C_9H_{16}N_2O_5$ requires C,46.5; H,7.0; N,12.1%).

N-Tritylglycylglycine, 70% yield, m.p. 178-180°C (from ethanol) (lit. [17b], 174-177°C).

N-Benzyloxycarbonylglycyl-L-leucine, 55% yield, m.p. 100-101°C, $[\alpha]_D^{23}$ – 9.7°(c 2 in EtOH) (lit.[19], m.p. 100-101°C; $[\alpha]_D^{25}$ – 9.5°(c 5 in EtOH). N-t-Butyloxycarbonylglycyl-L-leucine, 68% yield, m.p. 135-137°C (from ethyl acetate), $[\alpha]_D^{22}$ – 15.6° (c 0.5 in MeOH), δ(DMSO-d_6) 0.70-1.05 (6H, d, CH_3), 1.35-1.80 (3H, complex, CH_2CH), 3.55-3.80 (2H, d, NCH_2CO), 4.05-4.50 (1H, complex NCH), 4.90-5.10 (2H, s, $PhCH_2$), 7.15-7.50 (6H, complex, Ph and NH), 7.80-8.05 (1H, d, NH) (Found: C,54.2; H,8.4; N,9.8. $C_{13}H_{24}N_2O_5$ requires C,54.2; H,8.4; N,9.7%).

N-Tritylglycyl-L-leucine, 42% yield, m.p.73-83°C (from ethyl acetate/light petroleum ether), $[\alpha]_D^{22}$ – 4.0° (c 0.2 in MeOH) (lit.[20], m.p. 75-85°C).

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RESUMO

Novo estudo para o uso do grupo 2-cloroetilo como protecção em síntese peptídica

Neste trabalho descreve-se a síntese de seis novos ésteres 2-cloroetílicos de dipéptidos N-protegidos e um novo método para remover a C-protecção, utilizando o anião sulfureto.