

USE OF 4-CHLOROBUTYL ESTERS IN PEPTIDE SYNTHESIS

M. Joaquina S. A. AMARAL TRIGO and M. Isabel A. OLIVEIRA SANTOS

Centro de investigação em Química da Universidade do Porto,

Faculdade de Ciências do Porto, 4000 Porto, Portugal

Received December 12, 1989

Accepted March 17, 1990

Ho and Wong¹ synthesised 4-chlorobutyl esters of simple carboxylic acids and removed the ester group by the action of sodium sulfide under reflux conditions. We describe here the synthesis of the 4-chlorobutyl esters of glycine and L-phenylalanine and its use in the synthesis of six new N-protected dipeptides 4-chlorobutyl esters (XHNCH₂CO—HNCHRCO₂(CH₂)₄Cl; R = H, CH₂Ph; X = Z, Boc, Trt). The selective removal of the 4-chlorobutyl group can be achieved by the action of the sulfide anion in aqueous acetonitrile (room temperature, 1.5 to 5 h). The conditions are milder than those described¹, but similar to the conditions used with the dipeptides 2-bromoethyl esters². The N-protected dipeptides were isolated in 50 to 80% yield.

EXPERIMENTAL

The purity of all compounds was confirmed by TLC on Kieselgel 60 F₂₅₄, in the systems benzene-methanol (5 : 1) and chloroform-methanol (9 : 1). Spots were visualized by UV light or revealed by the (NH₄)₂SO₄-H₂SO₄ method³. 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. ¹H NMR spectra were recorded with a Bruker AC 200 MHz spectrometer. The microanalyses were carried out by Dr Ilse Beetz (Kronach, Germany).

L-Phenylalanine 4-chlorobutyl ester hydrochloride. A suspension of L-phenylalanine (3.30 g; 20 mmol) in 4-chlorobutanol (38.0 g; 350 mmol) was treated with dry HCl for 19 h at 40°C. After addition of the ethyl ether and cooling to 0°C overnight, a crystalline solid was obtained. Yield 8.2 g (95%) m.p. 139–141°C (from ethanol), [α]_D²⁵ +36.1° (c 1.0, CHCl₃), ¹H NMR ((CD₃)₂SO): 8.83 br, 3 H (NH₃); 7.33 complex, 5 H (Ph); 4.24–4.17 complex, 1 H (CH); 4.03 t, 2 H (CO₂CH₂); 3.59–3.53 t, 2 H, (CH₂Cl); 3.42–3.22 dd, 2 H (PhCH₂); 1.67 t, 4 H (CH₂.CH₂). For C₁₃H₁₉Cl₂NO₂ (292.2) calculated: 53.44% C, 6.55% H, 24.26% Cl, 4.79% N; found: 53.49% C, 6.54% H, 24.17% Cl, 4.76% N.

Glycine 4-chlorobutyl ester hydrochloride. The same method was used. Yield 66%, m.p. 90 to 92°C (from ethanol). ¹H NMR ((CD₃)₂SO): 8.52 br, 3 H (NH₃); 4.21–4.15 t, 2 H (CO₂CH₂); 3.78 s, 2 H (CH₂CO₂); 3.71–3.65 t, 2 H (CH₂Cl); 1.77 t, 4 H (CH₂CH₂). For C₆H₁₃Cl₂NO₂ (202.1) calculated: 35.66% C, 6.48% H, 35.08% Cl, 6.93% N; found: 34.95% C, 6.44% H, 34.84% Cl, 7.08% N.

Synthesis of N-Protected Dipeptides 4-Chlorobutyl Esters

To a stirred solution of N-protected aminoacid (5 mmol) in dry dichloromethane (10 ml), cooled

to -10°C , N,N' -dicyclohexylcarbodiimide (5 mmol) was added followed by the amino acid 4-chlorobutyl ester hydrochloride (5 mmol) and triethylamine (5 mmol). The mixture was kept at -10°C for 3 h and at 0°C for 4 days. The precipitated N,N' -dicyclohexylurea was filtered off and the filtrate was washed (water, aqueous 5% citric acid, aqueous 1M sodium hydrogen carbonate and water), dried (MgSO_4) and evaporated. The residue was dissolved in acetone and kept at 0°C for 24 h. After filtration, the solvent was removed and the fully protected peptide was crystallised or purified by column chromatography on silica gel.

N-Benzoyloxycarbonyl-glycylglycine 4-chlorobutyl ester. Yield 65%, m.p. $67-68^{\circ}\text{C}$ (from ethanol). $^1\text{H NMR}$ (CDCl_3): 7.35 s, 5 H (Ph); 6.61 br, 1 H (CONH); 5.50 br, 1 H (OCONH); 5.13 s, 2 H (PhCH_2); 4.19 t, 2 H (CO_2CH_2); 4.04 d, 2 H (CH_2CO_2); 3.94 d, 2 H (CH_2CO); 3.57 t, 2 H (CH_2Cl); 1.83 t, 4 H (CH_2CH_2). For $\text{C}_{16}\text{H}_{21}\text{ClN}_2\text{O}_5$ (356.8) calculated: 53.85% C, 5.93% H, 9.93% Cl, 7.85% N; found: 53.94% C, 5.90% H, 9.93% Cl, 7.77% N.

N-tert-Butyloxycarbonyl-glycylglycine 4-chlorobutyl ester. Yield 60%, an oil homogeneous by TLC. The analytical specimen was readily obtained by chromatography on silica with CHCl_3 as eluant. $^1\text{H NMR}$ (CDCl_3): 7.05 t, 1 H (CONH); 5.55 br, 1 H (OCONH); 4.20, 2 H (CO_2CH_2); 4.04 d, 2 H (CH_2CO_2); 3.85 d, 2 H (CH_2CO); 3.58 t, 2 H (CH_2Cl); 1.84 t, 4 H (CH_2CH_2); 1.44 s, 9 H (Bu^t). For $\text{C}_{13}\text{H}_{23}\text{ClN}_2\text{O}_5$ (322.8) calculated: 48.37% C, 7.18% H, 10.98% Cl, 8.68% N; found: 48.56% C, 7.20% H, 10.74% Cl, 8.75% N.

N-Tritylglycylglycine 4-chlorobutyl ester. Yield 50%, m.p. $143-144^{\circ}\text{C}$ (from ethanol). $^1\text{H NMR}$ (CDCl_3): 7.76 t, 1 H (CONH); 7.44–7.21 complex, 16 H (Ph and CNH); 4.24 t, 2 H (CO_2CH_2); 4.11 d, 2 H (CH_2CO_2); 3.59 t, 2 H (CH_2Cl); 3.01 d, 2 H (CH_2CO); 1.88 t, 4 H (CH_2CH_2). -or $\text{C}_{27}\text{H}_{29}\text{ClN}_2\text{O}_3$ (465.0) calculated: 69.74% C, 6.28% H, 7.62% Cl, 6.02% N; found: 69.79% C, 6.11% H, 7.53% Cl, 6.05% N.

N-Benzoyloxycarbonyl-glycyl-L-phenylalanine 4-chlorobutyl ester. Yield 83%, an oil homogeneous by TLC. The analytical sample was obtained by column chromatography (silica; CHCl_3). $^1\text{H NMR}$ (CDCl_3): 7.34–7.24 complex, 10 H (Ph); 6.51 d, 1 H (CONH); 5.42 t, 1 H (OCONH); 5.12 s, 2 H (CH_2OCO); 4.84 q, 1 H (CH); 4.10 t, 2 H (CO_2CH_2); 3.83 d, 2 H (CH_2CO); 3.50 t, 2 H (CH_2Cl); 3.09 d, 2 H (PhCH_2); 1.73 t, 4 H (CH_2CH_2). For $\text{C}_{23}\text{H}_{27}\text{ClN}_2\text{O}_5$ (446.9) calculated: 61.81% C, 6.09% H, 7.93% Cl, 6.27% N; found: 61.89% C, 6.22% H, 7.97% Cl, 6.38% N.

N-tert-Butyloxycarbonyl-glycyl-L-phenylalanine 4-chlorobutyl ester. Yield 84%, an oil homogeneous by TLC. The analytical sample was obtained by chromatography on silica with CHCl_3 as eluant. $^1\text{H NMR}$ (CDCl_3): 7.29–7.14 complex, 5 H (Ph); 6.69 d, 1 H (CONH); 5.21 br, 1 H (OCONH); 4.88 q, 1 H (CH); 4.14 t, 2 H (CO_2CH_2); 3.78 d, 2 H (CH_2CO); 3.51 t, 2 H (CH_2Cl); 3.11 d, 2 H (PhCH_2); 1.78 t, 4 H (CH_2CH_2); 1.50 s, 9 H (Bu^t). For $\text{C}_{20}\text{H}_{29}\text{ClN}_2\text{O}_5$ (412.9) calculated: 58.18% C, 7.08% H, 8.59% Cl, 6.78% N; found: 58.32% C, 7.15% H, 8.67% Cl, 6.80% N.

N-Tritylglycyl-L-phenylalanine 4-chlorobutyl ester. Yield 55% m.p. $99-101^{\circ}\text{C}$ (from ethanol), $[\alpha]_{\text{D}}^{25} + 22.6^{\circ}$ (c 1.0, CHCl_3). $^1\text{H NMR}$ (CDCl_3): 7.75 d, 1 H (CONH); 7.27 complex, 21 H (Ph and CNH); 4.89 q, 1 H (CH); 4.19 t, 2 H (CO_2CH_2); 3.49 t, 2 H (CH_2Cl); 3.20 d, 2 H (PhCH_2); 2.89 d, 2 H (CH_2CO); 1.78 t, 4 H (CH_2CH_2). For $\text{C}_{34}\text{H}_{35}\text{ClN}_2\text{O}_3$ (555.1) calculated: 73.56% C, 6.35% H, 6.38% Cl, 5.04% N; found: 73.40% C, 6.38% H, 6.29% Cl, 4.96% N.

Cleavage of the 4-Chlorobutyl Ester Group

To a solution of the fully protected dipeptide (3 mmol) in acetonitrile (9.0 ml), sodium sulfide nonahydrate (3.5 mmol) in water (6.0 ml) was added with stirring. The reaction mixture was

kept at room temperature for 1.5 h for the N-benzyloxycarbonyl and N-tert-butyloxycarbonyl dipeptides and for 5 h the N-trityl dipeptides. The solution was diluted with water (12 ml), extracted with diethyl ether, pH was adjusted to 3 with aqueous 2M-HCl and the dipeptide precipitated as a solid on cooling. In the case of the N-tert-butyloxycarbonyl dipeptides the aqueous layer was mixed with ethyl acetate, pH was adjusted to 3 with aqueous 2M-HCl with stirring. The aqueous layer was separated from the organic solvent and washed several times with ethyl acetate. Combined organic extracts were dried (MgSO_4) and evaporated to dryness to yield the N-protected dipeptide which was further recrystallised from a suitable solvent.

The following N-protected dipeptides were obtained (yields of the product identical to that described in literature are given in parentheses): Z-Gly-Gly-OH (60%), Boc-Gly-Gly-OH (50%), Trt-Gly-Gly-OH (65%), Z-Gly-Phe-OH (77%), Boc-Gly-Phe-OH (50%), Trt-Gly-Phe-OH (60%).

This research was supported by the Instituto Nacional de Investigação Científica, Lisboa, Portugal (Research Project L1 of CIQ, U.P.) to whom we express our thanks. We thank to Mrs. M. M. M. V. Braga and Mr. E. N. Domingues for collaboration.

REFERENCES

1. Ho T.-L., Wong C. M.: *Synth. Commun.* **4**, 307 (1974).
2. Amaral Trigo M. J. S. A., Oliveira Santos M. I. A.: *Collect. Czech. Chem. Commun.* **53**, 2787 (1988).
3. Zimiński T., Borowski E.: *J. Chromatogr.* **23**, 480 (1966).