NEW APPROACH TO THE USE OF 2-BROMOETHYL 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 January 18th, 1988
Accepted April 10th, 1988

Dedicated to the memory of Dr Karel Bláha.

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

The application of the 2-bromoethyl esters in peptide synthesis has been already described in connection: i) with a few model experiments to study its cleavage by using the supernucleophile cobalt(I) phthalocyanine anion[2,3]; ii) with its conversion into the corresponding 2-iodoethyl or choline esters[4].

In this paper we describe the synthesis of six new N-protected dipeptides 2-bromoethyl esters ($XHNCH_2CO—HNCHRCO_2CH_2CH_2Br$; R = H, CH_2Ph; X = Z, Boc, Trt) in order to study a new method for the ester selective cleavage. This was achieved by the action of the sulphide anion in aqueous acetonitrile, at room temperature for 1·5 to 3 h, similarly to the conditions used with the dipeptides 2-chloroethyl esters[5]. The N-protected dipeptides were isolated in 50 to 68% yields.

EXPERIMENTAL

The purity of all compounds was confirmed by TLC on kieselgel 60 F_{254}, usually in the three systems benzene–methanol (5 : 1), chloroform–ethyl acetate–methanol (95 : 3 : 5) and chloroform–methanol (9 : 1). The compounds were revealed by the $(NH_4)_2SO_4$–H_2SO_4 method[6]. 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. NMR spectra were recorded with a Bruker AC 200 MHz spectrometer. The microanalyses were carried out by Dr Ilse Beetz (Kronach, Germany). According to the procedures described in the literature N-benzyloxycarbonylglycine[7], N-t-butyloxycarbonylglycine[8], and N-tritylglycine[9] were prepared.

* A communication on this work was presented at the 19th European Peptide Symposium, Porto Carras, Chalkidiki, Greece, 1986 (ref.[1]).

Esterification of Aminoacids

The aminoacids esters were prepared as hydrochlorides by direct acid catalysed esterification based on the procedure referred to for glycine[10].

L-*Phenylalanine 2-bromoethyl ester hydrochloride.* A suspension of L-phenylalanine (3·30 g; 20 mmol) in 2-bromoethanol (25 ml; 340 mmol) was treated with dry HCl for 21 h at 40°C. The solution obtained was kept at 0°C overnight, precipitating a semi-solid, which after trituration with ethyl ether was filtered off, and well washed with ethyl ether. Yield 5·4 g (87%), m.p. 179 to 181°C, ^{1}H NMR ($(CD_3)_2SO$): 8·70 s, 3 H (NH_3), 7·32 complex, 5 H (Ph), 4·44—4·32 complex, 3 H (CH and CO_2CH_2), 3·59 t, 2 H (CH_2Br), 3·19 d, 2 H ($PhCH_2$). The product was slightly contaminated with L-phenylalanine hydrochloride and was used without further purification.

Glycine 2-bromoethyl ester hydrochloride, 89% yield, m.p. 139—142°C (ref.[4] 128—134°C impure and obtained by other method).

Synthesis of N-Protected Dipeptides 2-bromoethyl Esters

General Procedure: To a solution of N-protected aminoacid (5 mmol) in dry dichloromethane (10 ml), cooled to −10°C and stirred, N,N′-dicyclohexylcarbodiimide (5 mmol) was added followed by the aminoacid bromoethyl ester hydrochloride (5 mmol) and triethylamine (5 mmol). The mixture was kept at −10°C for 3 h and at room temperature for 3 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.

N-Benzyloxycarbonylglycylglycine 2-bromoethyl ester, 65% yield, m.p. 107—108°C (from ethanol), ^{1}H NMR ($CDCl_3$): 7·35 s, 5 H (Ph), 6·60 br; 1 H (CONH), 5·47 br, 1 H (OCONH), 5·14 s, 2 H ($PhCH_2$), 4·45 t, 2 H (CO_2CH_2), 4·09 d, 2 H (CH_2CO_2), 3·93 d, 2 H (CH_2CO), 3·51 t, 2 H (CH_2Br). For $C_{14}H_{17}BrN_2O_5$ (373·2) calculated: 45·06% C, 4·59% H, 21·41% Br, 7·51% N; found: 45·1% C, 4·6% H, 21·5% Br, 7·6% N.

N-t-Butyloxycarbonylglycylglycine 2-bromoethyl ester, 52% yield, m.p. 60—62°C (from ethyl acetate). ^{1}H NMR ($CDCl_3$): 6·84 br, 1 H (CONH), 5·32 br, 1 H (OCONH), 4·46 t, 2 H (CO_2CH_2), 4·09 d, 2 H (CH_2CO_2), 3·86 d, 2 H (CH_2CO), 3·50 t, 2 H (CH_2Br), 1·45 s, 9 H (Bu^t). For $C_{11}H_{19}BrN_2O_5$ (339·2) calculated: 38·95% C, 5·65% H, 23·56% Br, 8·26% N; found: 39·4% C, 5·6% H, 23·2% Br, 8·19% N.

N-Tritylglycylglycine 2-bromoethyl ester, 72% yield, m.p. 139—140°C (from ethanol), ^{1}H NMR ($CDCl_3$): 7·75 t, 1 H (CONH), 7·33—7·20 complex, 16 H (Ph and CNH), 4·50 t, 2 H (CO_2CH_2), 4·16 d, 2 H (CH_2CO_2), 3·55 t, 2 H (CH_2Br), 3·02 d, 2 H (CH_2CO). For $C_{25}H_{25}BrN_2O_3$ (481·4) calculated: 62·38% C, 5·23% H, 16·60% Br, 5·82% N; found: 62·9% C, 5·2% H, 16·2% Br, 5·7% N.

N-Benzyloxycarbonylglycyl-L-phenylalanine 2-bromoethyl ester, 70% yield, m.p. 88—91°C (from ethanol), $[\alpha]_D^{22}$ +12·5° (*c* 1·0, $CHCl_3$), ^{1}H NMR ($CDCl_3$): 7·35—7·23 complex, 10 H (Ph), 6·4 d, 1 H (CONH), 5·30 t, 1 H (OCONH), 5·12 s, 2 H (CH_2OCO), 4·90 q, 1 H (CH), 4·40 t, 2 H (CO_2CH_2), 3·86 d, 2 H (CH_2CO), 3·45 t, 2 H (CH_2Br), 3·14 d, 2 H ($PhCH_2$). For $C_{21}H_{23}BrN_2O_5$ (463·3) calculated: 54·44% C, 5·00% H, 17·25% Br, 6·05% N; found: 55·0% C, 5·1% H, 17·1% Br, 6·1% N.

N-t-Butyloxycarbonylglycyl-L-phenylalanine 2-bromoethyl ester, 76% yield, an oil chromatographically homogeneous (from trituration with petroleum ether). ^{1}H NMR ($CDCl_3$): 7·27

complex, 5 H (Ph), 6·68 d, 1 H (CONH), 5·17 br, 1 H (OCONH), 4·90 q, 1 H (CH), 4·40 t, 2 H (CO_2CH_2), 3·80 d, 2 H (CH_2CO), 3·48 t, 2 H (CH_2Br), 3·14 d, 2 H ($PhCH_2$), 1·44 s, 9 H (Bu^t).

*N-Tritylglycyl-*L*-phenylalanine 2-bromoethyl ester*, 80% yield, m.p. 73–74°C (from ethanol), $[\alpha]_D^{22}$ +12·7° (*c* 1·0, $CHCl_3$). ^{1}H NMR ($CDCl_3$): 7·78 d, 1 H (CONH), 7·31 complex, 20 H (Ph), 5·07 q, 1 H (CH), 4·51 t, 2 H (CO_2CH_2), 3·54 t, 2 H (CH_2Br), 3·30 d, 2 H ($PhCH_2$), 2·95 d, 2 H (CH_2CO). For $C_{32}H_{31}BrN_2O_3$ (571·5) calculated: 67·25% C, 5·47% H, 13·98% Br, 4·90% N; found: 67·7% C, 5·6% H, 13·8% Br, 5·0% N.

Cleavage of the 2-Bromoethyl Ester Group

General procedure: To a solution of the fully protected dipeptide (1·77 mmol) in acetonitrile (5·3 ml), sodium sulfide nonahydrate (2 mmol) in water (5·3 ml) was added with stirring. The reaction mixture was kept at room temperature for 1·5 h for the dipeptides containing only glycine and 3 h for the other dipeptides. The solution was diluted with water (9 ml),extracted with diethyl ether, titrated with 2M-HCl at pH 3 and, on cooling, the dipeptide precipitated as a solid. In the case of the N-t-butyloxycarbonyl dipeptides the aqueous layer was mixed with ethyl acetate, titrated with aqueous 2M-HCl at pH 3 with stirring, separated from the organic solvent and washed several times with ethyl acetate. These 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.

N-Benzyloxycarbonylglycylglycine, 65% yield, m.p. 178–179°C (from ethanol) (ref.[11] m.p. 186°C).

N-t-Butyloxycarbonylglycylglycine, 55% yield, m.p. 135–136°C (from ethyl acetate) (ref.[5] m.p. 135–136°C).

N-Tritylglycylglycine, 60% yield, m.p. 180–181°C (from ethanol) (ref.[9] m.p. 180°C).

*N-Benzyloxycarbonylglycyl-*L*-phenylalanine*, 68% yield, m.p. 129°C (from $CHCl_3$), $[\alpha]_D^{22}$ +40·2° (*c* 2·0, EtOH) (ref.[12] m.p. 129–130°C, $[\alpha]_D^{22}$ +38·9° (*c* 2·0, EtOH)).

*N-t-Butyloxycarbonylglycyl-*L*-phenylalanine*, 50% yield, m.p. 140–142°C (ethyl acetate), $[\alpha]_D^{22}$ +29·0° (*c* 0·4, $CHCl_3$), ^{1}H NMR ($CDCl_3$) 7·38–7·08 complex, 5 H (Ph), 6·75 br, 1 H (CONH), 5·32 br, 1 H (OCONH), 4·85 br, 1 H (CH), 3·70 t, 2 H (CH_2CO), 3·17 t, 2 H ($PhCH_2$), 1·43 s, 9 H (Bu^t). For $C_{16}H_{22}N_2O_5$ (322·4) calculated: 59·62% C, 6·88% H, 8·69% N; found: 59·5% C, 6·7% H, 8·5% N.

*N-Tritylglycyl-*L*-phenylalanine*, 60% yield, m.p. 211–215°C dec., $[\alpha]_D^{22}$ +50·8° (*c* 1·0, $CHCl_3$) (ref.[9] m.p. 210–215°C dec.).

This research was supported by the Instituto Nacional de Investigação Cientifica, Lisboa, Portugal (Research Project L1 of CIQ, U.P.) and, in part, by the Universidade do Porto (C.l.n° 2/85), to whom we express our thanks.

REFERENCES

1. Amaral Trigo M. J. S. A., Oliveira Santos M. I. A. in: *Peptides 1986* (D. Theodoropoulos, Ed.), p. 61. de Gruyter, Berlin 1987.
2. Eckert H., Ugi I.: Angew. Chem. Int. Ed. *15*, 681 (1976).
3. Eckert H., Lagerlund I., Ugi I.: Tetrahedron *33*, 2243 (1977).
4. Kunz H., Buchholz M.: Chem. Ber. *112*, 2145 (1979).

5. Amaral Trigo M. J. S. A., Barbedo M. I. M. R. E. in: *Peptides 1982* (K. Bláha and P. Maloň Eds), p. 133. de Gruyter, Berlin 1983.
6. Zimiński T., Borowski E.: J. Chromatogr. *23*, 480, (1966).
7. Bergmann M., Zervas L.: Chem. Ber. *65*, 1192 (1932).
8. Schnabel E.: Ann. Chim. *702*, 188 (1967).
9. Zervas L., Theodoropoulos D. M.: J. Am. Chem. Soc. *78*, 1359 (1956).
10. Blinova G. G., Erkina G. V., Sochilin E. G.: Chem. Abst. *82*, 43704t (1975).
11. Maclaren J. A.: Aust. J. Chem. *11*, 360 (1958).
12. Kemp D. S., Wrobel S. J. jr, Wang S.-W., Bernstein Z., Rebek J. jr: Tetrahedron *30*, 3969 (1974).