On Cysteine and Cystine Peptides. Part VI.[1] *S*-Acylcysteines in Peptide Synthesis [2]

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S-*p*-Methoxybenzyloxycarbonyl-L-cysteine (pMZ-Cys) (I), as its *N*-*o*-nitrophenylsulphenyl derivative (II), can be used as an intermediate for the incorporation of cysteine residues in peptide synthesis. The pMZ group can be removed either by methanolysis or by treatment with hydrogen halides in acetic acid.

S-Acylcysteines are useful intermediates in peptide synthesis.[3] The *S*-acyl groups can be easily and selectively removed by methanolysis in the presence of sodium methoxide without affecting *S*-aralkyl or S–S groups already existing in the molecule. However, although several peptides,[3b,c] including oxytocin,[4] have been prepared by use of *S*-acyl protecting groups, the method does have limitations. In particular, where a carboxy-group of the polypeptide is esterified with an alcohol other than methanol, methanolysis can give rise

[1] Part V, preceding paper.

[2] Presented in part at the Ninth European Peptide Symposium: I. Photaki, I. Phocas, J. Taylor-Papadimitriou, and L. Zervas, 'Peptides: Proceedings of the Ninth European Symposium,' Orsay, France, 1968, ed. E. Bricas, North-Holland Publishing Co., Amsterdam, 1968, p. 201.

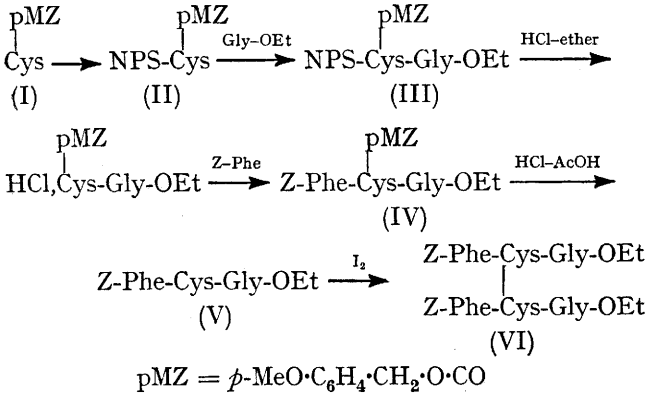
[3] (*a*) L. Zervas and I. Photaki, *J. Amer. Chem. Soc.*, 1965, **87**, 4922; (*b*) L. Zervas, I. Photaki, and N. Ghelis, 1963, **85**, 1337; (*c*) L. Zervas, I. Photaki, A. Cosmatos, and D. Borovas, 1965, **87**, 4922.

[4] I. Photaki, *J. Amer. Chem. Soc.*, 1966, **88**, 2292.

to transesterification.[3b] This is a serious disadvantage in cases where selectively removable ester groups such as diphenylmethyl, t-butyl, *etc.* are used. It can be avoided if, instead of the usual acyl groups such as benzoyl,[3] the *p*-methoxybenzyloxycarbonyl (pMZ) group is used for *S*-protection. In contrast to benzoyl, this group can be removed not only by methanolysis but also with 1·5N-hydrogen chloride in acetic acid or 0·2N-hydrogen bromide in acetic acid.

Because of the lability of the *S*-*p*-methoxybenzyloxycarbonyl group in acid, chain lengthening of a peptide containing this group is only feasible when an *N*-protecting group such as *o*-nitrophenylsulphenyl [5] is used.

The following example of synthesis of a tripeptide illustrates the use of the *p*-methoxybenzyloxycarbonyl group.



pMZ = *p*-MeO·C_6H_4·CH_2·O·CO

p-Methoxybenzyloxycarbonyl-L-cysteine (I) prepared by interaction of *p*-methoxybenzyl chloroformate [6] and cysteine at pH 7·5 was transformed into its *o*-nitrophenylsulphenyl derivative (II) according to a reported general procedure.[5a] Coupling of this *NS*-protected cysteine with glycine ethyl ester affords a protected dipeptide (III) from which the nitrophenylsulphenyl group is removed selectively by the action of 2 equiv. of hydrogen chloride in ether. The hydrochloride of the dipeptide ester thus formed is coupled with *N*-benzyloxycarbonyl-L-phenylalanine to give a protected tripeptide (IV). Removal of the *S*-*p*-methoxybenzyloxycarbonyl group, by methanolysis, by 1·5N-hydrogen chloride in acetic acid, or by 0·2N-hydrogen bromide in acetic acid, gives *N*-benzyloxycarbonyl-L-phenylalanyl-L-cysteinylglycine ethyl ester (V).[3a] Oxidation of this compound yields the corresponding cystine derivative (VI).[3a] If a peptide chain of greater length is to be made, the *o*-nitrophenylsulphenyl [5] (or trityl [7]) method must be used subsequently instead of the benzyloxycarbonyl method.[8]

EXPERIMENTAL

Anhydrous solvents were used for the coupling reactions and for removal of the *N*- or *S*-protecting groups. Evaporations were carried out *in vacuo* at 35—40°. M.p.s were taken for samples in capillary tubes. Before analysis compounds were dried (P_2O_5) at room temperature under high vacuum. Microanalyses were performed by Dr. H. Mantzos, Analytical Laboratory, Royal Hellenic Research Foundation. T.l.c. was performed in the solvent systems (*a*) n-butanol–acetic acid–water (100 : 10 : 30), (*b*) toluene–acetic acid–pyridine (80 : 1 : 10), (*c*) chloroform–carbon tetrachloride–methanol (6 : 3 : 1); substances with a free amino-group were detected with ninhydrin and *N*-protected derivatives with iodine. Free SH groups were determined by titration with 0·1N-iodine.[3b]

S-p-*Methoxybenzyloxycarbonyl*-L-*cysteine* (I).—To a solution of anhydrous L-cysteine hydrochloride (15·7 g., 0·1 mole) in ice-cold aqueous M-sodium hydrogen carbonate (180 ml.), an ethereal solution (80 ml.) of *p*-methoxybenzyl chloroformate [6] (18 g., 0·09 mole) was added. The mixture was vigorously shaken for 1 hr. at 0° and then for 1 hr. at 10—15°. The precipitated S-p-*methoxybenzyloxycarbonyl*-L-*cysteine* was filtered off and washed with acetone and then with ether; yield 7·8 g. (30%), m.p. 180—182° (decomp., sinters at 165—166°), R_F 0·45 in system (*a*). A sample was purified by dissolution in dimethylformamide–2N-hydrochloric acid (1 : 1), filtration, and reprecipitation with sodium acetate; m.p. 180—182°, $[\alpha]_D^{22}$ −46·5° (*c* 1 in AcOH) (Found: C, 50·8; H, 5·4; N, 5·1. $C_{12}H_{15}NO_5S$ requires C, 50·5; H, 5·3; N, 4·9%).

N-o-*Nitrophenylsulphenyl*-S-p-*methoxybenzyloxycarbonyl*-L-*cysteine Dicyclohexylammonium Salt* (II).—This was prepared from *o*-nitrophenylsulphenyl chloride (1·9 g., 0·01 mole) and compound (I) (2·85 g., 0·01 mole) by the procedure (C) described by Zervas *et al.*[5a] The crude product (4·8 g.), m.p. 152—155°, was triturated with boiling methanol (20 ml.); after cooling, filtration gave *dicyclohexylammonium salt* (3·9 g., 63%), m.p. 154—155° (from ethanol), $[\alpha]_D^{19}$ −30·6° (*c* 1 in Me_2N·CHO), R_F 0·1 in system (*b*) (Found: C, 58·5; H, 6·6; N, 7·1; S, 10·6. $C_{30}H_{41}N_3O_7S_2$ requires C, 58·1; H, 6·7; N, 6·8; S, 10·3%).

N-o-*Nitrophenylsulphenyl*-S-p-*methoxybenzyloxycarbonyl*-L-*cysteinylglycine Ethyl Ester* (III).—To a precooled (0°) solution of compound (II) (6·2 g., 0·01 mole) and glycine ethyl ester hydrochloride (1·39 g., 0·01 mole) in chloroform (50 ml.), *NN'*-dicyclohexylcarbodi-imide [9] (2·2 g., 0·011 mole) was added, and the mixture was kept at room temperature overnight. The precipitate (of *NN'*-dicyclohexylurea and dicyclohexylamine hydrochloride) was filtered off and the filtrate was washed successively with water, cold dilute sulphuric acid, water, aqueous potassium hydrogen carbonate, and water again, dried, and evaporated to dryness. The residue gave the N-*protected dipeptide ester* (4·2 g., 80%), m.p. 135—137° (from methanol), unchanged after further recrystallisation from ethanol, $[\alpha]_D^{22}$ −18·3° (*c* 2 in tetrahydrofuran) (Found: C, 50·7; H, 4·8; N, 8·0; S, 12·4. $C_{22}H_{25}N_3O_8S_2$ requires C, 50·5; H, 4·8; N, 8·0; S, 12·2%).

N-*Benzyloxycarbonyl*-L-*phenylalanyl*-S-p-*methoxybenzyloxycarbonyl*-L-*cysteinylglycine Ethyl Ester* (IV).—To a solution of the dipeptide derivative (III) (2·35 g., 0·0045

[5] (*a*) L. Zervas, D. Borovas, and E. Gazis, *J. Amer. Chem. Soc.*, 1963, **85**, 3660; (*b*) L. Zervas and Ch. Hamalidis, 1965, **87**, 99.

[6] J. H. Jones and G. T. Young, *Chem. and Ind.*, 1966, 1722.

[7] L. Zervas and D. M. Theodoropoulos, *J. Amer. Chem. Soc.*, 1956, **78**, 1359; G. C. Stelakatos, D. M. Theodoropoulos, and L. Zervas, *ibid.*, 1959, **81**, 2884; L. Velluz, G. Amiard, and R. Heymès, *Bull. Soc. chim. France*, 1955, 1283.

[8] M. Bergmann and L. Zervas, *Ber.*, 1932, **65**, 1192; Ger.P. 556,798/1932.

[9] J. C. Sheehan and G. P. Hess, *J. Amer. Chem. Soc.*, 1955, **77**, 1067.

mole) in tetrahydrofuran (14 ml.), ether was added (70 ml.) followed by an ethereal solution of hydrogen chloride (0·01 mole). Light petroleum (240 ml.) was added after 30 sec. to precipitate the *hydrochloride*. The supernatant liquid was decanted and the hydrochloride was triturated once with light petroleum, then several times with ether, and was dried *in vacuo* (KOH and P_2O_5). To a solution of this peptide ester hydrochloride in chloroform (10 ml.) and triethylamine (0·15 ml.) the mixed anhydride, prepared at 0°, from benzyloxycarbonyl-L-phenylalanine (1·35 g., 0·0045 mole) in chloroform (5 ml.) containing triethylamine (0·63 ml., 0·0045 mole) and isobutyl chloroformate (0·59 ml., 0·0045 mole) was added, followed by triethylamine (0·7 ml., 0·005 mole; added dropwise). The mixture was set aside at room temperature overnight and worked up as described for compound (III). The residue was crystallised from ethanol and recrystallised from ethyl acetate giving the N-*protected tripeptide ester* (1·8 g., 61%), m.p. 164—165°, $[\alpha]_D^{20}$ −46·6° (*c* 1 in $Me_2N{\cdot}CHO$) (Found: C, 60·7; H, 5·55; N, 6·6. $C_{33}H_{37}N_3O_9S$ requires C, 60·8; H, 5·7; N, 6·4%).

Removal of the S-p-*Methoxybenzyloxycarbonyl Group*.—(*a*) To a mixture of compound (IV) (0·65 g., 0·001 mole) and anisole (0·45 ml.), trifluoroacetic acid (2·2 ml.) was added at −10°. The solution was set aside for 30 min. at room temperature then concentrated *in vacuo* at 35°. After addition of acetic acid (5 ml.) and water (2 ml.), titration revealed 50—55% cleavage. The crystalline oxidation product, isolated by filtration, was washed with dilute sodium thiosulphate solution. Recrystallisation from ethanol yielded pure *NN'*-bisbenzyloxycarbonyl-L-phenylalanyl-L-cystinyldiglycine diethyl ester (VI) (0·3 g., 30%), m.p. 212—214° (lit.,[3a] 214—215°), R_F 0·55 in system (*b*), 0·98 in system (*c*) (the same R_F values were obtained with an authentic sample).

(*b*) Compound (IV) (0·001 mole) was dissolved in 0·2N-hydrogen bromide in acetic acid (20 ml.). After 5 min. at room temperature, water (5 ml.) was added and the mixture was titrated with iodine. 85% Cleavage had occurred. The mixture was extracted with chloroform and the extract was washed with water, dilute sodium sulphate solution, water, potassium hydrogen carbonate, and water again. After evaporation to dryness, light petroleum was added to the residue. The crude product was recrystallised from ethanol yielding the pentapeptide (VI) (0·29 g., 60%), m.p. 215—216°, $[\alpha]_D^{20}$ −81·3° (*c* 1 in $Me_2N{\cdot}CHO$) {lit.,[3a] m.p. 214—215°, $[\alpha]_D^{23}$ −82·8° (*c* 3 in $Me_2N{\cdot}CHO$)}.

(*c*) Compound (IV) (0·001 mole) was dissolved in 1·5N-hydrogen chloride in acetic acid (15 ml.) at room temperature. After 15 min., water (5 ml.) was added and the mixture was titrated to reveal 75% cleavage.

(*d*) The same percentage of cleavage as in (*c*) was obtained by treatment with hydrogen chloride in nitromethane.

(*e*) To a solution of compound (IV) (0·001 mole) in dimethylformamide (4 ml.), methanolic 0·5N-sodium methoxide (10 ml.) was added with stirring, under hydrogen, at room temperature. The mixture was acidified after 15 min. with acetic acid (5 ml.); titration then revealed 92—95% cleavage.

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