The 2-(*p*-Nitrophenylthio)ethyl Group for Carboxy-group Protection in Peptide Synthesis

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The 2-(*p*-nitrophenylthio)ethyl group may be used, as an alternative to the 2-methylthioethyl group, for carboxy-group protection in peptide synthesis and has certain advantages. The group is selectively removed after conversion into the corresponding sulphone by treatment with alkali (pH 10—10·5 at room temperature).

THE 2-methylthioethyl group is very useful for carboxy-group protection in stepwise peptide synthesis.[1,2] It is (*a*) stable under the conditions of the usual coupling procedures; (*b*) readily removed after conversion into the corresponding methiodide or sulphone under very mild alkaline conditions (pH 10—10·5 at room temperature for a short time) without the side reactions that may occur during the removal of other ester groupings; (*c*) removed selectively in the presence of acid-labile protecting groups, or groups removed under strong alkaline conditions; and (*d*) removed independently of steric factors.[3]

The cleavage of 2-*p*-tolylsulphonylethyl esters of amino-acids,[4] also used in peptide synthesis, is based on the same principle.

This paper describes a preliminary study of the use of the 2-(*p*-nitrophenylthio)ethyl group for carboxy-group protection. This group, which has chemical properties similar to those of the 2-methylthioethyl group, can be used as an alternative and seems to have some advantages. The corresponding derivatives show higher crystallinity and the 2-(*p*-nitrophenylthio)ethyl ester of glycine can be obtained by direct esterification in good yield; this is not so in the case of the 2-methylthioethyl and 2-*p*-tolylsulphonylethyl groups.

The 2-(*p*-nitrophenylthio)ethyl esters of glycine and L-leucine were prepared as toluene-*p*-sulphonates in good yields by direct azeotropic esterification with 2-(*p*-nitrophenylthio)ethanol, by means of the procedure of Cipera

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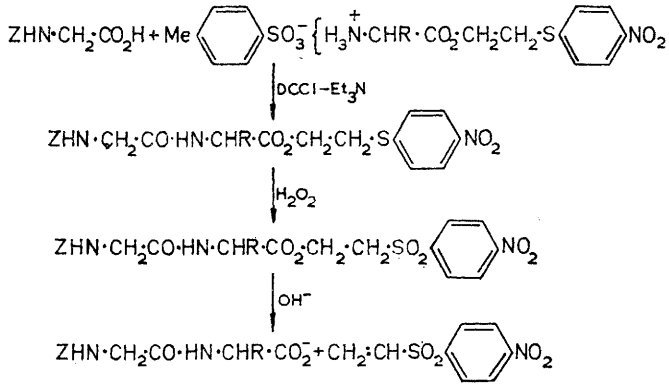
and Nicholls.[5] The yield of the ester of DL-phenylalanine under these conditions was, however, low.

The group was used in peptide synthesis in conjunction with benzyloxycarbonyl and triphenylmethyl *N*-protecting groups, by use of the *NN'*-dicyclohexylcarbodiimide coupling procedure.[6] The 2-(*p*-nitrophenylthio)ethyl ester group was successfully removed from the fully protected dipeptides, *via* the corresponding sulphone (method described by Rydon and his co-workers [2]).



R = H or $PhCH_2$, Z = $PhCH_2$·O·CO

DCCI = Dicyclohexylcarbodi-imide

In the case of *N*-triphenylmethylglycylglycine 2-(*p*-nitrophenylthio)ethyl ester, the *N*-protecting group and the carboxy-protecting group could both be removed selectively.

All the dipeptide 2-(*p*-nitrophenylthio)ethyl esters and the corresponding sulphones described were obtained crystalline and in good yield.

EXPERIMENTAL

Evaporations and concentrations were all effected under reduced pressure. Organic extracts were dried over magnesium sulphate. The purity of all products was confirmed by t.l.c. in acetic acid–n-butanol–water (2 : 6 : 2) and chloroform–methanol (95 : 5). M.p.s were taken by the capillary method. Microanalyses were carried out by Dr. I. Beetz, Kronach, Germany.

2-(*p*-Nitrophenylthio)ethanol was prepared by a modification of the procedure of Ishida.[7] To a solution of *p*-nitrobenzenethiol [8] (8·7 g., 0·056 mole) in hot ethanol (50 ml.), 2-chloroethanol (5·4 g., 0·067 mole) was added. Aqueous 10M-sodium hydroxide (5·6 ml.) was added dropwise with stirring and cooling and then the mixture was refluxed for 40 min. After cooling and addition of water the derivative was isolated quantitatively as a yellow solid. Recrystallisation from benzene yielded chromatographically pure material (solvent benzene) (9·6 g., 86%), m.p. 59—60·5° (lit.,[7] 60—62°).

Esterification of Amino-acids.—L-*Leucine* 2-(p-*nitrophenylthio*)*ethyl ester toluene*-p-*sulphonate*. To L-leucine (1·3 g., 0·01 mole) and toluene-*p*-sulphonic acid (2·1 g., 0·011 mole) in hot benzene (60 ml.), 2-(*p*-nitrophenylthio)ethanol (4·5 g., 0·023 mole) was added and the solution was refluxed for 9 hr. with use of a Dean-Stark apparatus. A solid crystallised from the cooled product, which was kept at 0° overnight then filtered off, yielding material which gave the *ester* (3·9 g., 81%), m.p. 169—169·5° (from ethanol). A further recrystallised sample had m.p. 169·5—170° $[\alpha]_D^{22}$ −1·2° (*c* 3·40 in MeOH) (Found: C, 52·1; H, 5·8; N, 5·8; S, 13·2. $C_{21}H_{28}N_2O_7S_2$ requires C, 52·1; H, 5·8; N, 5·8; S, 13·2).

Glycine 2-(p-*nitrophenylthio*)*ethyl ester toluene*-p-*sulphonate* was prepared in a similar way (66%); m.p. 167—168° (from acetone) (Found: C, 47·2; H, 4·6; N, 6·6; S, 15·1. $C_{17}H_{20}N_2O_7S_2$ requires C, 47·6; H, 4·7; N, 6·5; S, 15·0).

DL-*Phenylalanine* 2-(p-*nitrophenylthio*)*ethyl ester toluene*-p-*sulphonate*. The same procedure was applied to DL-phenylalanine but after the mixture had been refluxed the unchanged DL-phenylalanine toluene-*p*-sulphonate in suspension was filtered off. The filtrate was concentrated, extracted successively with saturated aqueous sodium carbonate and water, dried, and treated with ethereal toluene-*p*-sulphonic acid. Crystallisation occurred after addition of light petroleum (b.p. 60—80°). The *ester* obtained (19%) had m.p. 139·5—140·5° (from benzene) (Found: C, 55·7; H, 5·1; N, 5·3; S, 12·0. $C_{24}H_{26}N_2O_7S_2$ requires C, 55·6; H, 5·1; N, 5·4; S, 12·4).

Coupling Reactions with 2-(p-*Nitrophenylthio*)*ethyl Esters.*—N-*Triphenylmethylglycylglycine* 2-(p-*nitrophenylthio*)*ethyl ester*. *N*-Triphenylmethylglycine [9a] (1·59 g., 0·005 mole) was dissolved in dry dichloromethane (30 ml.) containing triethylamine (0·51 g., 0·005 mole). Glycine 2-(*p*-nitrophenylthio)ethyl ester toluene-*p*-sulphonate (2·14 g., 0·005 mole) was added and the mixture was cooled to 0°. *NN'*-Dicyclohexylcarbodi-imide (1·14 g., 0·0055 mole) was added and the mixture was left at room temperature for 24 hr. The precipitated urea was filtered off and the filtrate was washed successively with water, aqueous sodium hydrogen carbonate, and water. The dried organic layer was evaporated to dryness and the solid obtained was triturated with ethanol (10 ml.) to yield the *dipeptide ester* (2·5 g., 90%), recryst. m.p. 160—161° (softening from 155°) (from ethyl acetate) (Found: C, 67·0; H, 5·3; N, 7·7; S, 5·8. $C_{31}H_{29}N_3O_5S$ requires C, 67·0; H, 5·3; N, 7·6; S, 5·8).

The following derivatives were obtained by a similar procedure: N-*benzyloxycarbonylglycylglycine* 2-(p-*nitrophenylthio*)*ethyl ester* (72%), m.p. 109·5—110° (from acetone) (Found: C, 53·8; H, 4·8; N, 9·5; S, 7·3; $C_{20}H_{21}N_3O_7S$ requires C, 53·7; H, 4·7; N, 9·4; S, 7·2); N-*benzyloxycarbonylglycyl*-DL-*phenylalanine* 2-(p-*nitrophenylthio*)*ethyl ester* (80%), m.p. 80—81° (from ethanol) (Found: C, 60·7; H, 5·1; N, 7·7; S, 5·8. $C_{27}H_{27}N_3O_7S$ requires C, 60·3; H, 5·1; N, 7·8; S, 6·0).

Removal of the C-*Protecting Groups.*—(a) *Preparation of sulphone derivatives*. N-*Triphenylmethylglycylglycine* 2-(p-*nitrophenylsulphonyl*)*ethyl ester*. The corresponding sulphide (0·56 g., 0·001 mole) was dissolved in 4% aqueous acetone (26 ml.). Aqueous 30% hydrogen peroxide (2·3 ml.) containing aqueous 0·3M-ammonium molybdate (0·3 ml.) was added and the solution was kept for 2 hr. at room temperature. The acetone was evaporated off and the

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[8] C. C. Price and G. W. Stacy, *J. Amer. Chem. Soc.*, 1946, **68**, 498.

[9] J. P. Greenstein and M. Winitz, ' Chemistry of the Amino-acids,' Wiley, New York, 1961, vol. II, (*a*) p. 909; (*b*) p. 1170; (*c*) p. 1184; (*d*) p. 1172.

precipitate obtained was redissolved by addition of acetone to the hot mixture. Cooling yielded material which gave the *sulphone* (0·52 g., 89%), m.p. 176—178° (from ethanol) (Found: C, 63·6; H, 4·9; N, 7·0; S, 5·7. $C_{31}H_{29}N_3O_7S$ requires C, 63·4; H, 5·0; N, 7·1; S, 5·5).

The following derivatives were prepared similarly: *N-benzyloxycarbonylglycylglycine* 2-(p-*nitrophenylsulphonyl*)-*ethyl ester* (81%), m.p. 104—105° (from ethanol) (Found: C, 49·8; H, 4·5; N, 8·9; S, 6·9. $C_{20}H_{21}N_3O_9S$ requires C, 50·1; H, 4·4; N, 8·8; S, 6·7); *N-benzyloxycarbonylglycyl-*DL*-phenylalanine* 2-(p-*nitrophenylsulphonyl*)*ethyl ester* (87%), m.p. 118—119° (from ethanol) (Found: C, 57·1; H, 4·9; N, 7·3; S, 5·5. $C_{27}H_{27}N_3O_9S$ requires C, 57·0; H, 4·8; N, 7·4; S, 5·6).

(b) *Alkaline hydrolysis*. *N*-Benzyloxycarbonylglycylglycine 2-(*p*-nitrophenylsulphonyl)ethyl ester (0·48 g., 0·001 mole) dissolved in 80% aqueous acetone (25 ml.) was automatically titrated with aqueous 0·1M-potassium hydroxide at pH 10—10·5. The organic solvent was evaporated off and the aqueous solution after extraction with ether was acidified with 0·2M-hydrochloric acid to yield *N*-benzyloxycarbonylglycylglycine (0·23 g., 85%), m.p. 176·5—177°, identical with an authentic sample [9b] (mixed m.p., chromatography, i.r. spectroscopy). From the ethereal extract *p*-nitrophenyl vinyl sulphone was obtained by evaporation followed by recrystallisation from ethanol; m.p. 111—112° (lit.,[10] 100°) (Found: C, 45·2; H, 3·5; N, 6·4; S, 15·1. Calc. for $C_8H_7NO_4S$: C, 45·1; H, 3·3; N, 6·6; S, 15·0).

In a similar way *N*-triphenylmethylglycylglycine (67%), m.p. 179—180° (lit.,[9c] 180°) and *N*-benzyloxycarbonylglycyl-DL-phenylalanine (80%), m.p. 157·5° (lit.,[9d] 161°) were obtained from the corresponding fully protected dipeptides.

Detritylation of N-*Triphenylmethylglycylglycine* 2-(p-*Nitrophenylthio*)*ethyl Ester*.—The fully protected peptide (0·56 g., 0·001 mole) was dissolved by warming in a mixture of ethanol (30 ml.) and acetone (15 ml.). After addition of toluene-*p*-sulphonic acid (0·19 g., 0·001 mole) the solution was refluxed for 4 min. Addition of ether and cooling yielded material which gave the crystalline *toluene*-p-*sulphonate of glycylglycine* 2-(p-*nitrophenylthio*)*ethyl ester* (0·42 g., 86%), m.p. 191—192° (from ethanol) (Found: C, 46·9; H, 4·8; N, 8·7; S, 13·2. $C_{19}H_{23}N_3O_8S_2$ requires C, 47·0; H, 4·8; N, 8·7; S, 13·2).

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