42. *Elimination–Addition. Part III.*[1] *New Procedures for the Protection of Amino-groups.**

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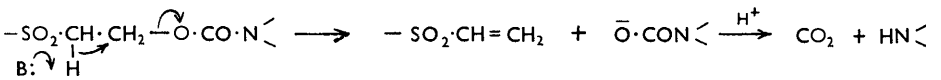
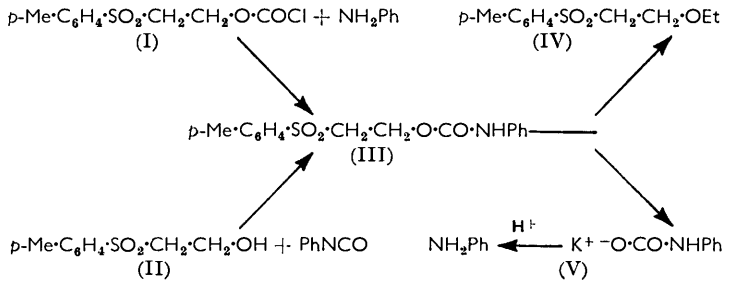
Use of the 2-*p*-tolylsulphonylethoxycarbonyl and 2-*p*-tolylthioethoxycarbonyl groups for protection of amino-groups, in particular during peptide synthesis, is reported. When attached to nitrogen, the former group is stable to acids, to catalytic hydrogenation, and during azide and *p*-nitrophenyl ester coupling procedures, but is removed in mildly basic conditions. The latter group is stable to bases but oxidation to the alkali-labile sulphonyl derivatives is readily carried out at an appropriate stage.

ELIMINATION of certain groups, unfamiliar as leaving groups, occurs readily when they are situated β to a sulphonyl group. Alkoxy-,[2] phenoxy-,[2] arylsulphonyl,[3] and *N*-alkylsulphonamido-groups [4] fall into this category and elimination of benzoate ion from 2-benzoyloxyethyl phenyl sulphone has been reported by Mamalis and Rydon.[5] The last-mentioned observation suggested a study of the reactions of β-carbamoyloxy-sulphones with bases, as elimination would constitute a method for the protection of amino-groups:



In preliminary experiments, the β-carbamoyloxy-sulphone (III) was obtained from the chloroformate (I) and aniline, or from 2-hydroxyethyl *p*-tolyl sulphone (II) and phenyl



isocyanate. Treatment of the sulphone (III) with a slight excess of cold N-ethanolic potassium hydroxide gave 2-ethoxyethyl *p*-tolyl sulphone [3] (IV) and the salt (V), which with acid gave aniline in 88% yield.

This successful outcome led us to apply the reaction to amino-group protection during

* A preliminary account of part of this work was given in *Proc. Chem. Soc.*, 1962, 363.

[1] Part II, Kader and Stirling, *J.*, 1962, 3425.
[2] Stirling, *Chem. and Ind.*, 1960, 933.
[3] Kader and Stirling, *J.*, 1962, 3686.
[4] Stirling, *J.*, 1962, 3676.
[5] Mamalis and Rydon, *J.*, 1955, 1049.

peptide synthesis. For a protecting group to be of use for this purpose, protected amino-acids and their esters must be easily prepared, and the protecting group must be stable under the coupling conditions but selectively removable from the coupled product. Selective removal is essential so that *N*-protected peptide acids or peptides with protected carboxyl groups and free amino-groups are available for further coupling. Further, all three stages should give good yields without racemisation. Synthesis of simple peptides has been investigated with reference to these requirements.

Amino-acid Derivatives Protected by the 2-p-*Tolylsulphonylethoxycarbonyl Group.*—Treatment of amino-acids with the chloroformate (I) in aqueous dioxan in the presence of magnesium oxide [6] gave the *N*-protected amino-acids (Table 1) in good yields. Esters (VII)

TABLE 1.

Protected amino-acids.

Amino-acid	M. p. of deriv.	Cryst. from	[α]	Yield (%)	Found (%) C	Found (%) H	Formula	Reqd. (%) C	Reqd. (%) H
			Z·NH·CHR·CO_2H						
DL-Alanine	133—134°	EtOAc–Pet †	—	89	49·8	5·4	$C_{13}H_{17}NO_6S$	49·5	5·4
L-Alanine	120—121	EtOAc–Pet	−16·2°	87	49·7	5·5	$C_{13}H_{17}NO_6S$	49·5	5·4
L-Proline	89—90	EtOAc–Pet	+65·6	92	52·5	5·5	$C_{15}H_{19}NO_6S$	52·8	5·6
L-Leucine	147—148 *	C_6H_6–Pet	−16·5	87	62·5	8·6	$C_{28}H_{46}N_2O_6S$	62·45	8·55
L-Leucine (ethyl ester)	55—56	C_6H_6–Pet	+23·2	91	55·9	7·3	$C_{18}H_{27}NO_6S$	56·1	7·0
			Z′·NH·CHR·CO_2H						
L-Alanine	92—93	Et_2O–Pet	−15·9	77	55·0	5·8	$C_{13}H_{17}NO_4S$	55·1	6·0

* Dicyclohexylamine salt. † Pet = light petroleum.

Z = *p*-Me·C_6H_4·SO_2·CH_2·CH_2·O·CO; Z′ = *p*-Me·C_6H_4·S·CH_2·CH_2·O·CO.

of *N*-protected amino-acids were obtained from the chloroformate and amino-acid ester salts in triethylamine. Hydrolysis of these esters with hydrochloric acid in acetic acid [7] afforded the protected acids.

Coupling. Two methods for formation of the peptide link have been used. Conversion of the protected acids into their *p*-nitrophenyl esters (VIII) was accomplished with *p*-nitrophenol and dicyclohexylcarbodi-imide.[8] Subsequent treatment of the esters with the ester of a second amino-acid yielded the protected peptide esters (X) (Table 2). Alternatively, treatment of the protected amino-acid ester (VII) with hydrazine gave the hydrazide (IX); hydrazine, fortunately, is insufficiently basic to remove the protecting group. Treatment of the hydrazide with nitrous acid and then the ester of the second amino-acid gave the protected peptide ester.[9] In preliminary work with glycine, the protected acid (VI; R = H) was converted into the acid chloride and thence into *N*-protected glycylaniline and *N*-protected glycylglycine ethyl ester (X; R = R′ = H). This coupling method was not, however, further investigated as it is less convenient than other methods and is unsuitable for optically active peptides.[9] Coupling of the protected amino-acid and free amino-ester directly [9] with dicyclohexylcarbodi-imide was also tried but gave unsatisfactory yields.

Removal of the protecting groups. Appropriate treatment of protected peptide esters produced by the coupling procedures yielded free peptides (XII), protected peptide acids (XIII), or unblocked peptide esters (XIV). Free peptides were obtained from the protected peptide esters with an excess (3 mol.) of aqueous-ethanolic N-sodium hydroxide: elimination of the carbamoyloxy-group occurred rapidly and the ester group was slowly (1 hr.) saponified to give the double salt (XI). Removal of the ethoxy-sulphone (IV) and

[6] Sheehan and Frank, *J. Amer. Chem. Soc.*, 1949, **71**, 1856.
[7] Cf. Ehrensvärd, *Nature*, 1947, **159**, 500.
[8] Bodanszky and du Vigneaud, *J. Amer. Chem. Soc.*, 1959, **81**, 5688.
[9] Albertson, *Org. Reactions*, 1962, **12**, 157.

TABLE 2.

Protected peptide esters.

Amino-acids	M. p. of deriv.	Cryst. from	[α]	Yield (%)	Found (%) C	Found (%) H	Formula	Reqd. (%) C	Reqd. (%) H
			Z·NHCHR·CO·NH·CHR′·CO_2R″						
R″ = Et									
Glycyl-DL-alanine	93—94°	EtOAc–Pet	—	90 *	51·2	6·2	$C_{17}H_{24}N_2O_7S$	51·0	6·0
Glycyl-L-alanine	91—92	EtOAc–Pet	−23·6° *	80 *	51·4	6·3	$C_{17}H_{24}N_2O_7S$	51·0	6·0
DL-Alanylglycine	79—80	EtOAc–Pet	—	85 *	51·3	5·8	$C_{17}H_{24}N_2O_7S$	51·0	6·0
L-Alanylglycine	112—113	EtOAc	−21·6	93 *	51·2	5·95	$C_{17}H_{24}N_2O_7S$	51·0	6·0
L-Alanyl-L-alanine	126—128	EtOAc–Pet	−39·3 * −39·0 †	83 * 91 †	51·7	6·5	$C_{18}H_{26}N_2O_7S$	52·1	6·3
R″ = CH_2Ph									
L-Alanylglycine	115—116	EtOAc–Pet	−19·1	76 *	57·4	5·7	$C_{22}H_{26}N_2O_7S$	57·1	5·6
			Z′·NH·CHR·CO·NH·CHR′·CO_2Et						
Glycyl-L-alanine	110—111	EtOAc–Pet	−30·7	87 *	55·2	6·5	$C_{17}H_{24}N_2O_5S$	55·4	6·8
L-Alanyl-L-alanine	103—104	EtOAc–Pet	−41·6	90 *	56·7	6·6	$C_{18}H_{26}N_2O_5S$	56·5	6·8

* *p*-Nitrophenyl ester coupling. † Oxidation of Z′-derivative.

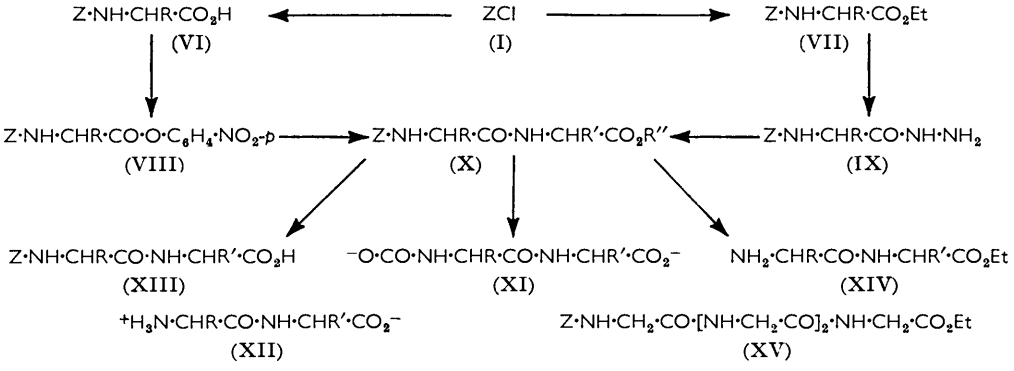
TABLE 3.

Peptides.

Peptide	M. p. Found	M. p. Lit.	Cryst. from	[α] Found	[α] Lit.	Yield (%)	Found (%) C	Found (%) H	Formula	Calc. (%) C	Calc. (%) H
Glycyl-L-alanine ...	235—237°	235° *	H_2O–EtOH	−49·9°	−50·4°	91	40·9	6·9	$C_5H_{10}N_2O_3$	41·1	6·85
L-Alanylglycine ...	255—256	256 †	H_2O–EtOH	+49·6	+50·6	93	41·0	7·15	$C_5H_{10}N_2O_3$	41·1	6·85
L-Alanyl-L-alanine	295—297	298 ‡	H_2O–EtOH	−21·2	−21·2	88	44·9	7·8	$C_6H_{12}N_2O_3$	45·0	7·5

* Fischer and Schulze, *Ber.*, 1907, **40**, 943. † Fischer, *Ber.*, 1908, **41**, 850. ‡ Fischer and Raske, *Ber.*, 1906, **39**, 3981.

treatment of the aqueous phase with a weak acid ion-exchange resin neutralised the excess of base and decarboxylated the carbamic acid giving the free peptides in good yield (Table 3). Dipeptides containing L-alanine residues had rotations within 1° of the recorded values,[10] showing that racemisation at any stage is insignificant. In an alternative procedure,



acid hydrolysis of the protected peptide ester gave the acid (XIII) and subsequent brief (5 min.) treatment with base and then acid gave the peptide. Treatment of the protected peptide acid with a strongly basic ion-exchange resin also removed the protecting group, the peptide being recovered from the resin by elution with dilute acid.

[10] Erlanger and Brand, *J. Amer. Chem. Soc.*, 1951, **73**, 3508.

Protected peptide acids (XIII) (Table 4) were obtained either by acid hydrolysis of the esters (X) as above, or preferably, by catalytic hydrogenation of benzyl esters (X; R″ = CH_2Ph) over palladium–charcoal in t-butyl alcohol [11] (to prevent transesterification).

TABLE 4.

Protected peptides.

Peptide	M. p. of deriv.	Cryst. from	[α]	Yield (%)	Found (%) C	Found (%) H	Formula	Reqd. (%) C	Reqd. (%) H
Z·NH·CHR·CO·NH·CHR′·CO_2H									
Glycyl-DL-alanine	165—168°	MeOH	—	85 *	48·1	5·6	$C_{15}H_{20}N_2O_7S$	48·4	5·4
Glycyl-L-alanine	138—140	Dioxan–Et_2O	−8·9° *	87 *	48·2	5·5	$C_{15}H_{20}N_2O_7S$	48·4	5·4
			−8·7 †	85 †					
L-Alanylglycine	150—151	EtOAc–Pet	−22·1 *	86 *	48·1	5·1	$C_{15}H_{20}N_2O_7S$	48·4	5·4
			−22·2 ‡	84 ‡					
Z′·NH·CHR·CO·NH·CHR′·CO_2H									
Glycyl-L-alanine	97—98	EtOAc–Pet	−19·3	97 §	52·7	5·7	$C_{15}H_{20}N_2O_5S$	52·9	5·9

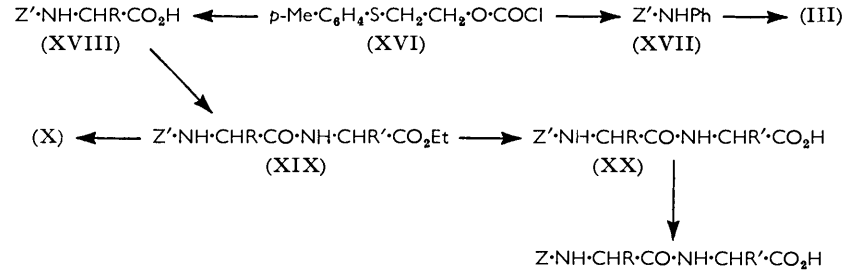
* From acid hydrolysis of ethyl ester. † Oxidation of Z′-derivative. ‡ Hydrogenation of benzyl ester. § Alkaline hydrolysis of protected ethyl ester.

The 2-*p*-tolylsulphonylethoxycarbonyl group is completely resistant to hydrogenation and the acids were obtained in good yield. Neither procedure caused racemisation with the L-alanyl derivative (X; R = Me, R′ = H, R″ = CH_2Ph) (cf. Table 4).

Removal of the protecting group without hydrolysis of the ester group was achieved by brief treatment of the protected peptide ester with one mol. of base. Glycylglycine ethyl ester was obtained in this way in 92% yield as the *N*-2,4-dinitrophenyl derivative,[12] and in a separate experiment the ester (X; R = R′ = H, R″ = Et) was converted by the azide procedure into the tetrapeptide ester (XV) which in turn gave an 83% yield of glycyldiglycylglycine ethyl ester.

The 2-p-*Tolylthioethoxycarbonyl Protecting Group.*—The use of this group allows variation in the above procedures. Elimination of groups situated β to an arylthio-group is facilitated [4] but to a much smaller extent than in sulphones. Consequently, this protecting group is resistant towards the basic conditions which cause removal of the 2-*p*-tolylsulphonylethoxycarbonyl group. At an appropriate stage of the synthesis, oxidation of the *N*-protected derivative yields the 2-*p*-tolylsulphonylethoxycarbonyl compound.

The chloroformate (XVI) with aniline gave the carbamate (XVII), which, with peracetic acid, gave the sulphonyl derivative (III). Similarly, protected amino-acids (XVIII) were converted in good yield *via* their *p*-nitrophenyl esters into the protected peptide



esters (XIX) which, on *alkaline* hydrolysis gave the protected peptide acids (XX). Oxidation of the protected acids and their esters gave the sulphonyl derivatives (XII) and (X), respectively, in good yield and without racemisation.

[11] Crofts, Markes, and Rydon, *J.*, 1959, 3610.
[12] Rydon and Smith, *J.*, 1955, 2542.

Conclusions.—The most popular [13] groups for the protection of nitrogen in peptide syntheses are benzyloxycarbonyl, t-butoxycarbonyl, and trityl. Each is removed under acidic or neutral (catalytic hydrogenation) conditions. The 2-*p*-tolylsulphonylethoxycarbonyl group, because of its acid stability and resistance to catalytic hydrogenation provides a practical alternative to these groups, and in conjunction with them will allow selective unmasking of amino-groups. This new method has advantages in that the reagent is stable and crystalline and the derivatives generally crystallise well. The 2-*p*-tolylthioethoxycarbonyl group should provide a useful adjunct to the sulphonyl series, allowing selective unblocking particularly in diacidic amino-acids.

Elimination of carboxylate ion from 2-sulphonylethyl esters mentioned earlier constitutes a method for carboxyl protection which we shall report in a subsequent paper. Sulphonium salts derived from 2-methylthioethyl esters have recently been employed for this purpose.[14]

Experimental

The light petroleum used had b. p. 40—60°, and solvents were anhydrous. Extracts were dried over Na_2SO_4. Glycine ethyl ester was distilled just before use. Rotations were determined with sodium D light at 18—20°. " Amberlite " ion-exchange resins were used throughout. Examples of procedures for glycine derivatives are given in full; details of derivatives of other amino-acids are given in the Tables.

2-p-*Tolylsulphonylethyl Chloroformate.*—2-Hydroxyethyl *p*-tolyl sulphone (70% yield) was obtained by heating sodium toluene-*p*-sulphinate (1 mol.) and 2-chloroethanol (3 mol.) in dimethylformamide for 3 hr. The sulphone (60 g.) in dry benzene (250 ml.) was stirred with an excess of phosgene for 2 hr. at 0°. The mixture was warmed to 25° and then set aside for 12 hr. The excess of phosgene and solvent were removed under reduced pressure, and addition of ether to the residue gave the *chloroformate* (64·2 g., 82%), m. p. 48° (from ether) (Found: C, 46·0; H, 4·3. $C_{10}H_{11}ClO_4S$ requires C, 45·7; H, 4·2%).

Reaction of 2-p-*Tolylsulphonylethyl Benzoate with Sodium Ethoxide.*—The sulphone [15] (1·52 g.) was treated with sodium ethoxide (1·7 g.) in 1,2-dimethoxyethane (25 ml.) and water (3·5 ml.). The mixture was kept at 20° for 10 min., poured into saturated brine, acidified, and extracted with chloroform. The extracts were washed with saturated aqueous sodium hydrogen carbonate, and evaporation gave 2-ethoxyethyl *p*-tolyl sulphone (1·08 g., 95%), b. p. 126—128°/0·01 mm., n_D^{20} 1·5220 (lit.,[3] b. p. 135°/0·05 mm., n_D^{20} 1·5218).

The aqueous extract was saturated with sodium chloride, acidified with hydrochloric acid, and extracted with methylene chloride. Evaporation at 20° gave benzoic acid (0·52 g., 84%), m. p. and mixed m. p. 121—122°. Ethyl benzoate under the same conditions gave only 10% of benzoic acid.

N-2-p-*Tolylsulphonylethoxycarbonylaniline.*—(i) 2-Hydroxyethyl *p*-tolyl sulphone (10 g.), phenyl isocyanate (7·15 g.), and benzene (50 ml.) were refluxed together for 30 min. Water (3 ml.) was added and the mixture was kept at 80° for 5 min. The *carbamate* (13·95 g., 88%) separated from the cold mixture. It had m. p. 115° (from benzene) (Found: C, 60·4; H, 5·35. $C_{16}H_{17}NO_4S$ requires C, 60·2; H, 5·3%).

(ii) 2-*p*-Tolylsulphonylethyl chloroformate (1 g.) and aniline (0·74 g.) in dry ether (20 ml.) were refluxed for 5 min. The mixture was washed with hydrochloric acid, and evaporation of the ethereal solution gave the carbamate (1·08 g., 90%), m. p. 115°.

Reactions of N-2-p-*Tolylsulphonylethoxycarbonylaniline.*—(*a*) *With sodium ethoxide.* The carbamate (1·595 g., 0·005 mole) and sodium ethoxide (1·7 g., 0·025 mole) in ethanol (30 ml.) were kept at 80° for 30 min. The mixture was poured into acidified, saturated brine and extracted with benzene. Evaporation of the extract gave 2-ethoxyethyl *p*-tolyl sulphone (95%). The aqueous layer was basified, saturated with sodium chloride, and extracted with ether. Evaporation of the extract and treatment of the residue with benzoyl chloride (Schotten–Baumann conditions) gave benzanilide (73%), m. p. and mixed m. p. 164—165°.

[13] Rydon, *Roy. Inst. Chem., Lectures*, 1962, No. 5.

[14] Rydon and Willett, Proc. Fifth European Peptide Symposium, Oxford, 1962, Pergamon Press, Oxford. We thank Professor Rydon for providing a copy of the M.S. of this paper before its publication.

[15] Otto, *J. prakt. Chem.*, 1884, **30**, 321.

(*b*) *With potassium hydroxide.* The carbamate (3·19 g., 0·01 mole) was kept with potassium hydroxide (0·616 g., 0·011 mole) in ethanol (30 ml.) at 20° for 15 min. Ether (100 ml.) was added and the potassium salt of the carbamic acid was filtered off. The salt dissolved in dilute hydrochloric acid (evolution of carbon dioxide), and treatment of the solution as in (*a*) gave benzanilide (1·7 g., 88%).

N-2-p-*Tolylsulphonylethoxycarbonylglycine Ethyl Ester.*—Glycine ethyl ester hydrochloride (1·59 g., 0·0114 mole), triethylamine (2·32 g., 0·0228 mole), and 2-*p*-tolylsulphonylethyl chloroformate (3 g., 0·0114 mole) in chloroform (25 ml.) were kept at 20° for 1 hr. Ether (100 ml.) was added and triethylamine hydrochloride was filtered off. The filtrate was evaporated, and the residue, in chloroform, was washed with dilute hydrochloric acid and water. Evaporation of the extract gave the *ester* (3·2 g., 89%), m. p. 54—55° (from ether) (Found: C, 51·0; H, 5·6. $C_{14}H_{19}NO_6S$ requires C, 51·1; H, 5·8%).

Reaction of N-2-p-*Tolylsulphonylethoxycarbonylglycine Ethyl Ester with Sodium Ethoxide.*—The ester (1·645 g., 0·005 mole) was kept with sodium ethoxide (1·02 g., 0·015 mole) in ethanol (30 ml.) for 15 min. at 20°. Ether (70 ml.) was added and the precipitate was separated by centrifugation. Dissolution of the precipitate in 3% hydrochloric acid (2 moles) and subsequent evaporation gave a residue which was extracted with ethanol. Evaporation of the extract gave glycine ethyl ester hydrochloride (0·42 g., 60%), m. p. and mixed m. p. 133—135°. 2-Ethoxyethyl *p*-tolyl sulphone (1·01 g., 90%) was obtained from the original ethereal solution.

N-2-p-*Tolylsulphonylethoxycarbonylglycine.*—(i) 2-*p*-Tolylsulphonylethyl chloroformate (3 g.) in dioxan (20 ml.) was added dropwise during 30 min. to a stirred and cooled (5°) suspension of glycine (0·86 g., 1 mol.) and magnesium oxide (0·7 g., 1·5 mol.) in water (50 ml.). The mixture was stirred for a further 10 min. at 20°, acidified, and extracted with chloroform. Evaporation of the extract gave the *acid* (3·1 g., 90%) m. p. 156—157° (from ethyl acetate–light petroleum) (Found: C, 48·2; H, 5·2. $C_{12}H_{15}NO_6S$ requires C, 47·8; H, 5·0%).

(ii) *N*-2-p-Tolylsulphonylethoxycarbonylglycine ethyl ester (21·3 g.) in acetic acid (75 ml.) and concentrated hydrochloric acid (11 ml.) was refluxed for 10 min. The mixture was poured into water and extraction with ethyl acetate gave the acid (18·5 g., 92%), m. p. and mixed m. p. 156—157°.

Reaction of N-2-p-*Tolylsulphonylethoxycarbonylglycine with Sodium Ethoxide.*—The acid (1·505 g.) and sodium ethoxide (0·37 g.) in ethanol (20 ml.) were kept at 20° for 5 min. Water (20 ml.) and ether (100 ml.) were added, and the layers were separated. Passage of the aqueous layer through a column of *IRC*-50(H^+) resin caused evolution of carbon dioxide, and evaporation of the aqueous eluate gave glycine (0·31 g., 83%), m. p. and mixed m. p. 225—232°.

N-2-p-*Tolylsulphonylethoxycarbonylglycylaniline.*—Phosphorus pentachloride (6·93 g.) was added in portions during 1 hr. to a suspension of the preceding acid (10 g.) in cold ether (300 ml.). The mixture was stirred for 4 hr. and subsequently filtered and evaporated. Extraction of the residue with light petroleum gave the crude acid chloride (9·15 g.), m. p. 86—88°. This (5 g.) was refluxed with aniline (5·82 g.) in ether (100 ml.) for 5 min., and kept at 20° for a further 10 min. Addition of dilute hydrochloric acid and filtration of the mixture gave the *anilide* (5·05 g., 86%), m. p. 163° (from ethanol) (Found: C, 57·4; H, 5·5. $C_{18}H_{20}N_2O_5S$ requires C, 57·45; H, 5·3%).

Reaction of N-2-p-*Tolylsulphonylethoxycarbonylglycylaniline with Sodium Ethoxide.*—The anilide (1·87 g.) was kept with sodium ethoxide (1·7 g.) in ethanol (50 ml.) for 15 min. at 40°. The mixture was cooled to 0° and decanted; the residue was washed with ice-cold ethanol and then treated with an excess of ethanolic hydrogen chloride. Evaporation gave a residue which was extracted with hot ethanol. Evaporation of the extract and treatment of the residue with ether left *N*-glycylaniline hydrochloride (0·81 g., 87%), m. p. 192—196° (from ethanol) (lit.,[6] m. p. 192—195°). Evaporation of the ethereal solution gave 2-ethoxyethyl *p*-tolyl-sulphone (1·02 g., 90%).

N-2-p-*Tolylsulphonylethoxycarbonylglycylglycine.*—(i) The acid chloride (above) (2·7 g.) in 1,2-dimethoxyethane (25 ml.) was added dropwise with stirring during 30 min. to a suspension of glycine (1 g.) and magnesium oxide (0·92 g.) in water (10 ml.) at 0°. The mixture was stirred for a further 10 min. at 20° acidified with hydrochloric acid, and evaporated to dryness. Addition of water to the residue gave the protected peptide acid (1·4 g.), m. p. 166—167° (from dioxan–light petroleum) (Found: C, 46·6; H, 5·2. $C_{14}H_{18}N_2O_7S$ requires C, 46·9; H, 5·0%). Extraction of the aqueous mother-liquors with ethyl acetate, and evaporation of the extract gave *N*-2-*p*-tolylsulphonylethoxycarbonylglycine (1·16 g.), m. p. 155—157°.

(ii) Reaction of 2-*p*-tolylsulphonylethyl chloroformate (8 g.) in dioxan (40 ml.) with glycylglycine (4 g.), and magnesium oxide (1·84 g.) in water (35 ml.), as for the experiment with glycine, gave the acid (9·3 g., 86%), m. p. 166—168°.

Reactions of N-2-p-*Tolylsulphonylethoxycarbonylglycylglycine.*—(*a*) The acid (1·79 g.) and sodium ethoxide (1·02 g.) in ethanol (30 ml.) were kept for 10 min. at 20°. Treatment of the mixture as for the glycine derivative gave glycylglycine (0·63 g., 96%), m. p. and mixed m. p. 225—230° (infrared spectrum identical with that of an authentic specimen).

(*b*) The acid (0·9 g.) in methanol (80 ml.) and water (20 ml.) was poured on to a column of strongly basic ion-exchange resin (*IRA*-400). The column was washed with aqueous methanol (100 ml.) and then eluted successively with N-hydrochloric acid (evolution of carbon dioxide), and water. The aqueous eluates, on evaporation, gave glycylglycine hydrochloride monohydrate (0·366 g., 80%) m. p. and mixed m. p. 110°. Glycylglycine (0·23 g., 96%) was obtained from the hydrochloride (0·34 g.) by treatment of an aqueous solution with the weakly basic resin *IR*-4*B*.

N-2-p-*Tolylsulphonylethoxycarbonylglycylglycine Ethyl Ester.*—(i) The protected ester (VII; R = H) (3 g.) in ethanol (40 ml.) was treated with hydrazine hydrate (1 g.) and the mixture was kept at 20° overnight. Filtration gave the *hydrazide* (2·5 g., 88%), m. p. 136—137° (from dioxan) (Found: C, 45·8; H, 5·2. $C_{12}H_{17}N_3O_5S$ requires C, 45·7; H, 5·4%). Sodium nitrite (0·55 g.) in water (3 ml.) was added to the hydrazide (2·25 g.) in a mixture of acetic acid (9 ml.), 5N-hydrochloric acid (4 ml.), and water (36 ml.) at −5°. The mixture was extracted with cold ethyl acetate (200 ml.) and the extract was washed successively with water, 3% aqueous sodium hydrogen carbonate, and water. Glycine ethyl ester (2·5 g.) in ethyl acetate (20 ml.) was added and the mixture was kept for 2 hr. at 20°, then it was washed with 0·5N-hydrochloric acid and with 3% aqueous sodium hydrogen carbonate. Evaporation gave the protected *ester* (2·18 g., 81%), m. p. 93—94° (from ethyl acetate–light petroleum) (Found: C, 49·3; H, 5·75. $C_{16}H_{22}N_2O_7S$ requires C, 49·7, H, 5·7%).

For other *hydrazides* see Table 5.

TABLE 5.

Protected amino-acid hydrazides and *p*-nitrophenyl esters.

Amino-acids	M. p. of deriv.	Cryst. from	[α]	Yield (%)	Found (%) C	Found (%) H	Formula	Reqd. (%) C	Reqd. (%) H
			Z·NH·CHR·CO·NH·NH$_2$						
L-Leucine	160—162°	EtOH *	+66·3°	60	51·7	6·8	$C_{16}H_{25}N_3O_5S$	51·75	6·7
Glycylglycine ...	149—150	MeOH	—	57	45·35	5·3	$C_{14}H_{20}N_4O_6S$	45·4	5·4
			Z·NH·CHR·CO·O·C$_6$H$_4$–NO$_2$-*p*						
DL-Alanine	121—122	EtOH	—	80	52·55	4·8	$C_{19}H_{20}N_2O_8S$	52·3	4·6
L-Alanine	138—139	MeOH	−41·0	82	52·2	4·5	$C_{19}H_{20}N_2O_8S$	52·3	4·6
			Z′·NH·CHR·CO·O·C$_6$H$_4$–NO$_2$-*p*						
L-Alanine	100—101	C$_6$H$_6$–Pet	+67·7	52	56·7	5·0	$C_{19}H_{20}N_2O_6S$	56·45	4·95

* From crude ester.

(ii) The protected acid (VI; R = H) (5 g.), *p*-nitrophenol (2·8 g., 1·2 mol.), and dicyclohexylcarbodi-imide (3·42 g.) in tetrahydrofuran (56 ml.) were kept at 0° for 30 min. and then at 20° overnight. *NN′*-Dicyclohexylurea was filtered off and evaporation of the filtrate gave a residue, which on treatment with ethanol, gave the p-*nitrophenyl ester* (6·15 g., 88%), m. p. 112—113° (from ethanol) (Found: C, 51·4; H, 4·55. $C_{18}H_{18}N_2O_8S$ requires C, 51·2; H, 4·3%). The *p*-nitrophenyl ester (2 g.) in chloroform (20 ml.) was added to glycine ethyl ester (0·67 g.) in chloroform (10 ml.), and the mixture was kept for 16 hr. at 20°. Solvent was removed under reduced pressure and ethyl acetate (100 ml.) and water (50 ml.) were added to the residue. The organic layer was washed with N-aqueous ammonia, water, and hydrochloric acid; evaporation and addition of light petroleum to the residue gave the protected ester (1·32 g., 77%), m. p. 93—94°.

For analogous *esters* see Table 5.

(iii) 2-*p*-Tolylsulphonylethyl chloroformate (3 g.) in chloroform (20 ml.) was added to glycylglycine ethyl ester hydrochloride (2 g.) and triethylamine (1·58 g., 1 mol.) in chloroform (40 ml.). After the addition of a further mol. of triethylamine the mixture was set aside for

1 hr. at 20°, and then was washed with water and dilute hydrochloric acid. Evaporation gave the protected ester (3·12 g., 71%), m. p. 92—94°.

(iv) Hydrogen chloride was passed through a solution of the protected peptide acid (XII; R = R′ = H) (3 g.) in ethanol (50 ml.) for 5 hr. at 0°. After 24 hr. at 20° the solvent was removed under reduced pressure and the residue was dissolved in methylene chloride. The solution was washed with aqueous sodium hydrogen carbonate and evaporation gave the ester (2·9 g., 89%), m. p. 93—94°.

Reactions of N-2-p-*Tolylsulphonylethoxycarbonylglycylglycine Ethyl Ester.*—(*a*) *Conversion into glycylglycine ethyl ester.* The ester (3 g.) in ethanol (10 ml.) was treated with sodium ethoxide (0·523 g., 1 mol.) in ethanol (6 ml.). The mixture was kept for 5 min. at 20°, then hydrogen chloride was passed into the solution until the latter was acidic. Solvent was removed under reduced pressure and the residue was extracted with hot ethanol. Evaporation of the extracts gave glycylglycine ethyl ester hydrochloride (1·49 g.), m. p. 181—182° (lit.,[16] m. p. 182°).

In a separate experiment, the reaction mixture was treated with N-hydrochloric acid (2 mol.) and then with aqueous sodium hydrogen carbonate (4 mol.) and 1-fluoro-2,4-dinitrobenzene (1·4 mol.). The mixture was shaken for 15 min. and set aside for 3 hr. at 20°. Filtration gave *N*-2,4-dinitrophenylglycylglycine ethyl ester (92%), m. p. and mixed m. p. 210—212° (from methanol) (Found: C, 44·1; H, 4·1. Calc. for $C_{11}H_{14}N_4O_7$: C, 44·2; H, 4·3%).

(*b*) *Conversion into glycylglycine.* Sodium hydroxide (0·6 g., 3 mol.) in water (5 ml.) was added to the ester (1·93 g.) in ethanol (10 ml.), and the mixture was kept for 1 hr. at 20°. Extraction was with ether and the extract was washed with water (2 × 25 ml.). The aqueous layer and washings were combined and passed through a column of IRC-50(H^+) resin; evaporation of the eluates, and addition of ethanol to the residue, gave glycylglycine (0·6 g., 91%), m. p. and mixed m. p. 233—234°.

N-2-p-*Tolylsulphonylethoxycarbonylglycylglycine Benzyl Ester.*—This protected peptide *ester* was obtained in 80% yield from glycylglycine benzyl ester toluene-*p*-sulphonate,[11] the chloroformate, and triethylamine as above. It had m. p. 116—117° (from ethyl acetate) (Found: C, 56·6; H, 5·4. $C_{21}H_{24}N_2O_7S$ requires C, 56·25; H, 5·4%). Hydrogenation of the ester (2 g.) in t-butyl alcohol (120 ml.) over 5% palladium–charcoal gave the acid (1·42 g., 89%), m. p. and mixed m. p. 166—167°.

N-2-p-*Tolylsulphonylethoxycarbonylglycyldiglycylglycine Ethyl Ester.*—The ester (X; R = R′ = H, R″ = Et) (3 g.) and hydrazine hydrate (1 g.) in ethanol (40 ml.) were refluxed for 1 hr. and kept for 20 hr. at 20°. Filtration and concentration of the filtrate gave the *hydrazide* (1·6 g.), m. p. 149—150° (from methanol) (Found: C, 45·35; H, 5·3. $C_{14}H_{20}N_4O_6S$ requires C, 45·4; H, 5·4%). The hydrazide (1·22 g.) was coupled with glycylglycine ethyl ester as above, to give the protected tetrapeptide *ester* (1·23 g., 77%), m. p. 201—203° (from *NN*-dimethylformamide) (Found: C, 48·4; H, 5·4. $C_{20}H_{28}N_4O_9S$ requires C, 48·0; H, 5·6%).

Reaction of N-2-p-*Tolylsulphonylethoxycarbonylglycyldiglycylglycine Ethyl Ester with Sodium Ethoxide.*—The ester (800 mg.) in ethanol (20 ml.) and *NN*-dimethylformamide (20 ml.), was treated with sodium ethoxide (109 mg., 1 mol.) in ethanol (5 ml.). The mixture was kept for 15 min. at 20° and worked up as for the glycylglycine derivative; *N*-2,4-dinitrophenylglycyldiglycylglycine ethyl ester (588 mg., 83%) was obtained, having m. p. 217—218° (from methanol) (Found: C, 43·9; H, 4·4. Calc. for $C_{16}H_{20}N_6O_9$: C, 43·6; H, 4·5%) (lit.,[12] m. p. 218°).

N-2-p-*Tolylthioethoxycarbonyl Derivatives.*—Crude 2-*p*-tolylthioethyl chloroformate was prepared as for the sulphonyl compound. It decomposed on attempted distillation at 0·1 mm.

N-2-p-*Tolylthioethoxycarbonylaniline.* (i) Treatment of the chloroformate with aniline as for the sulphonyl derivative gave the *carbamate* (88%), m. p. 85—86° (from ether) (Found: C, 67·0; H, 5·7. $C_{16}H_{17}NO_2S$ requires C, 66·9; H, 5·9%).

(ii) Phenyl isocyanate and 2-hydroxyethyl *p*-tolyl sulphide in benzene gave the carbamate (80%), m. p. and mixed m. p. 85—86°.

Oxidation of the carbamate in acetic acid with aqueous potassium permanganate gave *N*-2-*p*-tolylsulphonylethoxycarbonylaniline (81%), m. p. and mixed m. p. 115°. Oxidation with hydrogen peroxide in acetic acid gave 96% of the sulphone.

N-2-p-*Tolylthioethoxycarbonylglycine.*—The chloroformate (10 g.) in dry dioxan (50 ml.) was

[16] Fischer and Fourneau, *Ber.*, 1901, **34**, 2868.

added dropwise to a stirred and cooled suspension of glycine (3·26 g.) and magnesium oxide (2·64 g.) in water (50 ml.). The usual working up gave the *acid* (10·18 g.), m. p. 104—105° (from ethyl acetate) (Found: C, 53·5; H, 5·3. $C_{12}H_{15}NO_4S$ requires C, 53·5; H, 5·6%).

Oxidation of the product in acetic acid with aqueous potassium permanganate gave 80% of the sulphonyl derivative (VI; R = H), m. p. and mixed m. p. 155—156°.

N-2-p-*Tolylthioethoxycarbonylglycylglycine Ethyl Ester.*—(i) The chloroformate (6 g.) in chloroform (20 ml.) was added to glycylglycine ethyl ester hydrochloride (5·13 g.) and triethylamine (5·27 g.) in chloroform (60 ml.), the temperature of the mixture rising to 50°. After 30 min. dry ether (100 ml.) was added, and the mixture was filtered. The filtrate was washed with dilute hydrochloric acid, and on evaporation gave the *ester* (6·42 g.), m. p. 92—93° (from ethyl acetate) (Found: C, 54·3; H, 6·1. $C_{16}H_{22}N_2O_5S$ requires C, 54·2; H, 5·8%).

(ii) The glycine derivative (XVIII; R = H) was treated with *p*-nitrophenol and dicyclohexylcarbodi-imide in tetrahydrofuran as above, giving the p-*nitrophenyl ester* (76%) m. p. 94—95° (from ethanol) (Found: C, 55·2; H, 4·4. $C_{18}H_{18}N_2O_6S$ requires C, 55·4; H, 4·6%). Reaction of the *p*-nitrophenyl ester with glycine ethyl ester as above gave the peptide ester (93%), m. p. and mixed m. p. 92—93°.

Oxidation of the peptide ester with hydrogen peroxide in acetic acid gave the sulphonyl derivative (95%), m. p. and mixed m. p. 93—94°.

Alkaline Hydrolysis of N-2-p-*Tolylthioethoxycarbonylglycylglycine Ethyl Ester.*—Sodium hydroxide (0·272 g.) in water (4 ml.) was added to the ester (2 g.) in ethanol (10 ml.). The mixture was kept at 20° for 1 hr., poured into saturated brine, and acidified. Filtration gave the *acid* (1·66 g.), m. p. 156—160° (from aqueous ethanol) (Found: C, 51·5; H, 5·1. $C_{14}H_{18}N_2O_5S$ requires C, 51·5; H, 5·5%).

Oxidation of the acid with hydrogen peroxide in acetic acid gave *N*-2-*p*-tolylsulphonylethoxycarbonylglycylglycine (93%), m. p. and mixed m. p. 167—168°.

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