# 606. *Polypeptides. Part II.* The Preparation of Some Protected Peptides of Cysteine and Glycine.*

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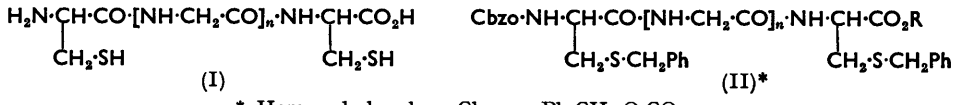
Syntheses of L-cysteinyl-L-cysteine, the *N*-benzyloxycarbonyl benzyl esters of L-cysteinyl-D-cysteine, of L-cysteinyl-glycyl-, -diglycyl-, -triglycyl-, and -tetraglycyl-L-cysteine, and of some related compounds, are described.

The five peptides of cysteine and glycine represented by the general formula (I; $n$ = 0—4) were required for a study of their oxidation;[1] the present paper is concerned with the synthesis of the fully protected compounds (II; $n$ = 0—4, R = $CH_2Ph$), this being the form in which the peptides were stored before use; in one case only, *viz.*, L-cysteinyl-L-cysteine (I; $n$ = 0), was the unstable free peptide prepared as such.



(I) (II)*

* Here and elsewhere Cbzo = $Ph{\cdot}CH_2{\cdot}O{\cdot}CO$.

When our work began the only known member of either series was L-cysteinyl-L-cysteine (I; $n$ = 0), which was prepared, as its hydrochloride, by Greenstein[2] in 1937; since the completion of our work, however, Izumiya and Greenstein[3] have synthesised derivatives (II; $n$ = 0, R = Et and H) of three of the four possible diastereoisomerides of cysteinylcysteine (I; $n$ = 0).

Before embarking on a detailed description of our synthetic work it seems desirable to make some general comments. *S*-Benzylcysteine[4] has been found preferable to cystine as a starting material, giving better yields of more easily crystallised products. We also chose to protect our final products as benzyl rather than ethyl esters, since the benzyl group can eventually be removed readily by treatment with sodium in liquid ammonia,[5] or with hydrogen bromide in acetic acid[6] or nitromethane,[7] whereas removal of an ethyl group requires alkaline hydrolysis, always a dubious process with cysteine derivatives; the necessary *S*-benzylcysteine benzyl ester was at first prepared from the *N*-carboxy-anhydride and benzyl alcohol,[8] but in later work the simple and elegant azeotropic procedure of Cipera and Nicholls[9] was used.

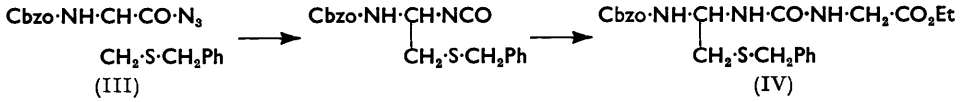
In the present work the most successful coupling procedures have been those of Curtius[10,11] and Boissonnas,[12] each of which has its own advantages and disadvantages. The great advantage of the Curtius azide procedure is its avoidance of the dangerous alkaline hydrolysis of intermediate ethyl esters, often inescapable in the Boissonnas procedure, since these are converted directly into the hydrazides and thence into the azides; fission of the *S*-benzyl linkage during hydrazide formation was negligible. The main disadvantage of the Curtius method is the tendency of certain azides to rearrange to the *iso*cyanates, with resultant formation of substituted ureas instead of peptides; thus, coupling *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine azide (III) with glycine ester led to the unwanted urea (IV).

We experienced considerable difficulty in applying the Boissonnas procedure in certain cases until the great instability of some of the intermediate mixed anhydrides, which has

* Part I, Rydon and Smith, *J.*, 1955, 2542.

[1] Heaton, Rydon, and Schofield, following paper.
[2] Greenstein, *J. Biol. Chem.*, 1937, **118**, 321.
[3] Izumiya and Greenstein, *Arch. Biochem. Biophys.*, 1954, **52**, 203.
[4] Wood and Du Vigneaud, *J. Biol. Chem.*, 1939, **130**, 109.
[5] Sifferd and Du Vigneaud, *ibid.*, 1935, **108**, 753.
[6] Ben-Ishai and Berger, *J. Org. Chem.*, 1952, **17**, 1564; Ben-Ishai, *ibid.*, 1954, **19**, 62.
[7] Albertson and McKay, *J. Amer. Chem. Soc.*, 1953, **75**, 5323.
[8] Bergmann, Zervas and Ross, *J. Biol. Chem.*, 1935, **111**, 245.
[9] Cipera and Nicholls, *Chem. and Ind.*, 1955, 16.
[10] Curtius, *Ber.*, 1902, **35**, 3226.
[11] Harington and Pitt-Rivers, *Biochem. J.*, 1944, **38**, 417.
[12] Boissonnas, *Helv. Chim. Acta*, 1951, **34**, 874.

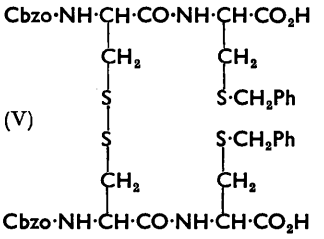
not been sufficiently emphasised in the literature, was realised; thereafter, coupling was always put in hand immediately the mixed anhydride had been formed and no further difficulty was experienced. In certain cases the method of Wieland, Schäfer, and Bokelmann,[13] in which the intermediate anhydride is converted into the phenyl thiolester,




was used; this method, excellent though it is in some cases, is not generally very suitable for the preparation of peptides of *S*-benzylcysteine owing to their tendency to decompose under the influence of the alkali present in the reaction mixture.

In a few instances Goldschmidt and Lautenschlager's phosphorazo-method [14] was used; the method gave easily crystallised products, but the yields were only moderate. In the early stages of our work the classical acid chloride procedure,[15, 16] used in Greenstein's early work,[2] was found to be of little value. Attempts to use Cook and Levy's thiothiazolidone method [17] to add glycine residues to *S*-benzylcysteine were completely unsuccessful, while the polymerisation [18] of *S*-benzylcysteine *N*-carboxy-anhydride proved insufficiently controllable to be of value for the synthesis of *S*-benzylcysteinyl-*S*-benzylcysteine.

*S*-Benzyl-*N*-benzyloxycarbonyl-L-cysteinyl-*S*-benzyl-L-cysteine benzyl ester (II; $n = 0$, R = $CH_2Ph$) was prepared both by coupling *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine azide (III) with *S*-benzyl-L-cysteine benzyl ester, and by the Boissonnas procedure from *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine (VI; R = OH) and *S*-benzyl-L-cysteine benzyl ester; the former procedure gave the better yield and was also employed for the preparation of the diastereoisomeride, *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteinyl-*S*-benzyl-D-cysteine benzyl ester. Treatment of the LL-benzyl ester (II; $n = 0$, R = $CH_2Ph$) with hydrogen bromide in warm acetic acid [6] afforded *S*-benzyl-L-cysteinyl-*S*-benzyl-L-cysteine, while fission with sodium in liquid ammonia gave the unstable L-cysteinyl-L-cysteine (I; $n = 0$). The protected LL-ethyl ester (II; $n = 0$, R = Et) was likewise prepared by both the azide and the Boissonnas procedure, the former again proving the more satisfactory in our hands; the unesterified LL-peptide (II; $n = 0$, R = H) was also prepared, both by the original Boissonnas procedure [12] and by its phenyl thiolester modification.[13] Finally in this series we prepared *NN'*-dibenzyloxycarbonyl-L-cystinylbis-(*S*-benzyl-L-cysteine) (V) by coupling *NN'*-dibenzyloxycarbonyl-L-cystine with *S*-benzyl-L-cysteine by the original procedure of Wieland, Kern, and Sehring,[19] which employs the mixed anhydride of the first-named component with benzoic acid.

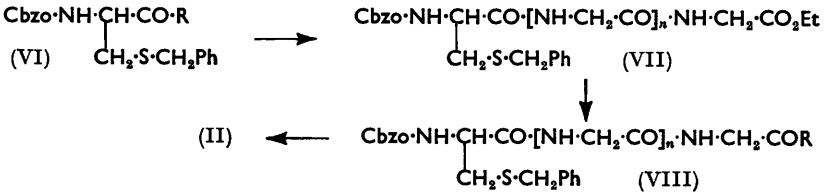


(V)

The route employed for the synthesis of the protected tri-, tetra-, and penta-peptides (II; $n = 1$—3) is outlined in the annexed scheme. In the tripeptide series, Boissonnas condensation of the mixed anhydride (VI; R = $O{\cdot}CO_2Et$), from *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine (VI; R = OH) and ethyl chloroformate, with glycine ethyl ester yielded the ester (VII; $n = 0$); as already mentioned, an attempt to prepare this ester by the appropriate azide coupling failed owing to urea formation. The ester (VII; $n = 0$) was converted, through the hydrazide (VIII; $n = 0$, R = $NH{\cdot}NH_2$), into the azide (VIII; $n = 0$, R = $N_3$) which was then coupled with *S*-benzyl-L-cysteine benzyl ester to give the required product (II; $n = 1$, R = $CH_2Ph$) in almost theoretical yield; the corresponding ethyl ester (II; $n = 1$, R = Et) was prepared similarly, using *S*-benzyl-L-cysteine ethyl

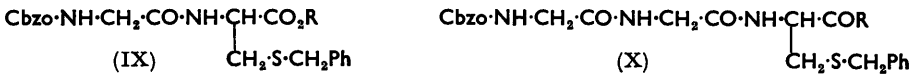
[13] Wieland, Schäfer, and Bokelmann, *Annalen*, 1951, **573**, 99.
[14] Goldschmidt and Lautenschlager, *ibid.*, 1953, **580**, 68.
[15] Fischer, *Ber.*, 1903, **36**, 2094.
[16] Bergmann and Zervas, *ibid.*, 1932, **65**, 1192.
[17] Cook and Levy, *J.*, 1950, 646; cf. Rydon and Smith, *J.*, 1955, 2542.
[18] Bailey, *J.*, 1950, 3461.
[19] Wieland, Kern, and Sehring, *Annalen*, 1950, **569**, 117.

ester. The unesterified tripeptide (II; $n = 1$, R = H) was prepared by a different procedure: condensation of *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine phenyl thiolester (VI; R = SPh) with glycine afforded the peptide (VIII; $n = 0$, R = OH); this, in its turn, was converted into its phenyl thiolester (VIII; $n = 0$, R = SPh) which was coupled




with *S*-benzyl-L-cysteine to give the desired product, but only in poor yield. An alternative route to the acid (II; $n = 1$, R = H) was also explored; condensation of *N*-benzyloxycarbonylglycine phenyl thiolester with *S*-benzyl-L-cysteine yielded the peptide (IX; R = H); the benzyloxycarbonyl group was removed by the method of Albertson and McKay,[7] but condensation of the product with *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine phenyl thiolester (VI; R = SPh) gave only a small amount of an uncrystallisable oil; in view of this disappointing result, the condensations of the last-mentioned compound with the ethyl and benzyl esters (IX; R = Et and $CH_2Ph$), which had been prepared for this purpose, were not investigated.

In the tetrapeptide series, the intermediate ester (VII; $n = 1$) was prepared in three ways from glycylglycine ethyl ester and a suitable derivative of *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine; the best yield was obtained by using the Boissonnas procedure with the mixed anhydride (VI; R = O·$CO_2$Et), but this was only a little better than that obtained by means of the azide (VI; R = $N_3$); the phosphorazo-method, applied to the free acid (VI; R = OH), was rather less successful. The carboxylic acid corresponding to (VII; $n = 1$) was also prepared, by condensation of the phenyl thiolester (VI; R = SPh) with glycylglycine, but was not used further in the present investigation. The ester (VII; $n = 1$) was finally converted into the azide (VIII; $n = 1$, R = $N_3$) which was then coupled, in very satisfactory yield, with *S*-benzyl-L-cysteine benzyl ester to give the required ester (II; $n = 2$, R = $CH_2Ph$).




The most satisfactory method for the preparation of the intermediate (VII; $n = 2$) required for the synthesis of the protected pentapeptide (II; $n = 2$, R = $CH_2Ph$) was by the Boissonnas procedure from *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine (VI; R = OH) and diglycylglycine ethyl ester, the azide route in this case giving a considerably poorer yield; as before, the ester (VII; $n = 2$) was converted into the azide (VIII; $n = 2$, R = $N_3$) which was then condensed, although in only moderate yield, with *S*-benzyl-L-cysteine benzyl ester to give the fully protected pentapeptide (II; $n = 3$, R = $CH_2Ph$).

A different approach was used in the synthesis of the protected hexapeptide (II; $n = 4$, R = $CH_2Ph$). *N*-Benzyloxycarbonyldiglycyl-*S*-benzyl-L-cysteine benzyl ester (X; R = O·$CH_2Ph$) was prepared, in moderate yield, by the phosphorazo-method [14] from *S*-benzyl-L-cysteine benzyl ester and *N*-benzyloxycarbonylglycyl-glycine * and, in poor yield, from the first-named intermediate and the azide of the second. The *N*-benzyloxycarbonyl group was then removed by treatment with hydrogen bromide in acetic acid,[6] and the product coupled with the azide (VIII; $n = 1$, R = $N_3$) to give the required product. The free acid (X; R = OH) was prepared, in good yield, by condensing *N*-benzyloxycarbonylglycylglycine phenyl thiolester with *S*-benzyl-L-cysteine for an alternative projected synthesis of the benzyl ester (II; $n = 4$, R = $CH_2Ph$) which was, however, not pursued owing to the achievement of our objective by the method already described.

* Here and elsewhere hyphens are inserted into names where it is believed that it will help to elucidate the positions of substituents.

We record also the preparation of the ethyl and benzyl esters of *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteinyl-tetraglycylglycine.

## Experimental

Solutions were dried over sodium sulphate.

### *Derivatives of* S-*Benzylcysteine.*

*S*-Benzyl-L-cysteine, m. p. 214°, was prepared in an average yield of 92% by the method of Wood and Du Vigneaud [4] and converted into the ethyl ester hydrochloride, m. p. 155°, $[\alpha]_D$ −25·4° (*c* 2·27 in $H_2O$) (84% yield), by the method of Harington and Pitt-Rivers [11] and into the *N*-benzyloxycarbonyl derivative, m. p. 98° (60—85% yield), by the method of Harington and Mead.[20]

Racemisation of *S*-benzyl-L-cysteine (50 g.) by the procedure of Wood and Du Vigneaud [4] afforded, not *S*-benzyl-DL-cysteine, but *acetyl*-S-*benzyl*-DL-*cysteine* [22] (35 g., 60%), m. p. 155° (from acetone) (Found: N, 5·8. $C_{12}H_{15}O_3NS$ requires N, 5·5%); Stekol [21] obtained this compound but, despite the discrepant m. p., regarded it as *S*-benzyl-DL-cysteine and used it as such in his biological experiments. Hydrolysis of the compound (30 g.) by 3 hours' refluxing with 20% hydrochloric acid (300 ml.) afforded *S*-benzyl-DL-cysteine (27·2 g., 92%), m. p. 213° (Wood and Du Vigneaud [4] give m. p. 213—215°). Resolution, by Wood and Du Vigneaud's method,[4] afforded *S*-benzyl-D-cysteine, m. p. 216°, $[\alpha]_D^{20}$ +21·0° (*c* 1·00 in N-NaOH).

*S*-Benzyl-*N*-benzyloxycarbonyl-L-cysteine ethyl ester, m. p. 50°, was prepared in 73% yield by the method of Harington and Pitt-Rivers [11] and also by azeotropic distillation of *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine (10·4 g.) in benzene (60 ml.) and ethanol (10 ml.) in the presence of a few drops of sulphuric acid; evaporation and trituration of the residue with light petroleum (b. p. 40—60°) afforded the ester (9·9 g., 89%), m. p. 50°. The hydrazide, m. p. 135°, was prepared from this ester in 91% yield by the method of Harington and Pitt-Rivers [11] and also, more conveniently, in 79% yield directly from *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine by the procedure of Hegedüs.[23]

S-*Benzyl*-L-*cysteine* N-*Carboxy-anhydride*.—Carbonyl chloride was passed into a stirred suspension, at 70°, of *S*-benzyl-L-cysteine (1·5 g.) in dry dioxan (50 ml.). Fifteen minutes after dissolution was complete the passage of carbonyl chloride was discontinued and the excess removed by means of dry nitrogen. Evaporation at <40° in nitrogen, followed by recrystallisation from ether, afforded the *anhydride* (1·6 g., 95%), m. p. 104° (Found: C, 55·3; H, 4·9; S, 13·9. $C_{11}H_{11}O_3NS$ requires C, 55·8; H, 4·65; S, 13·5%).

S-*Benzyl*-L-*cysteine Benzyl Ester* (with G. S. Heaton).—(*a*) The crude *N*-carboxy-anhydride from *S*-benzyl-L-cysteine (24 g.) in benzyl alcohol (120 ml.) was treated with a solution of hydrogen chloride (5 g.) in benzyl alcohol (50 ml.). After being warmed to 50° until gas evolution ceased, the mixture was kept overnight, concentrated under reduced pressure, and treated with ether. Recrystallisation from ethanol–ether afforded the *ester hydrochloride* (33 g., 86%), m. p. 134° (Found: C, 60·2; H, 6·2. $C_{17}H_{20}O_2NSCl$ requires C, 60·4; H, 5·9%).

(*b*) *S*-Benzyl-L-cysteine (10 g.) was refluxed overnight with benzene (200 ml.), benzyl alcohol (30 ml.), and toluene-*p*-sulphonic acid monohydrate (9·75 g.) in a Dean and Stark apparatus. Addition of light petroleum (b. p. 40—60°; 200 ml.) and recrystallisation of the precipitate from ethanol gave the *ester toluene*-p-*sulphonate* (20·8 g., 93%), m. p. 159° (Found: C, 61·0; H, 5·8; N, 3·0. $C_{24}H_{27}O_5NS_2$ requires C, 60·9; H, 5·7; N, 3·0%).

S-*Benzyl*-D-*cysteine Benzyl Ester Hydrochloride*.—This *salt*, m. p. 126°, $[\alpha]_D^{22}$ +29·0° (*c* 1·00 in $H_2O$) (Found: C, 60·6; H, 6·3. $C_{17}H_{20}O_2NSCl$ requires C, 60·4; H, 5·9%), was prepared, in 92% overall yield, from *S*-benzyl-D-cysteine through the *N*-carboxy-anhydride, m. p. 104°, as described for the L-compound.

### *Synthesis of Cysteinylcysteine and its Derivatives.*

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl*-S-*benzyl*-L-*cysteine Benzyl Ester* (II; $n = 0$, R = $CH_2Ph$) (with G. S. Heaton).—(*a*) *S*-Benzyl-*N*-benzyloxycarbonyl-L-cysteine hydrazide (1·8 g.), in 50% acetic acid (50 ml.) containing 2N-hydrochloric acid (5 ml.), was cooled and

[20] Harington and Mead, *Biochem. J.*, 1936, **30**, 1598.
[21] Stekol, *J. Biol. Chem.*, 1946, **164**, 651.
[22] Du Vigneaud, Wood, and Irish, *ibid.*, 1949, **179**, 529.
[23] Hegedüs, *Helv. Chim. Acta*, 1948, **31**, 737.

treated dropwise, with stirring, with a solution of sodium nitrite (0·35 g.) in water (5 ml.). The insoluble azide was extracted with ether and dried for a short time.

A suspension of *S*-benzyl-L-cysteine benzyl ester hydrochloride (1·7 g.), or toluene-*p*-sulphonate (2·3 g.), in ether (25 ml.) was shaken with triethylamine (0·7 ml.) and water (10 ml.) until the free ester had passed into the ether layer, which was then separated and dried.

The filtered ethereal solutions were mixed and kept at 0° overnight. Recrystallisation of the precipitate from benzene–light petroleum (b. p. 60—80°) yielded the *ester* (2·5—2·8 g., 80—90%), m. p. 129—130°, $[\alpha]_D^{21}$ −45·0° (*c* 1·00 in acetone) (Found : C, 66·7; H, 6·0. $C_{35}H_{36}O_5N_2S_2$ requires C, 66·8; H, 5·75%).

(*b*) *S*-Benzyl-*N*-benzyloxycarbonyl-L-cysteine (10·35 g.) was dissolved in chloroform (40 ml.) containing triethylamine (3·26 g.) and cooled to 0°; ethyl chloroformate (3·0 ml.) was added and the mixture kept at 0° for 10 min. An ice-cold solution of *S*-benzyl-L-cysteine benzyl ester hydrochloride (10·13 g.) in chloroform (40 ml.) containing triethylamine (3·03 g.) was then added and the mixture kept at 0° for 10 min. and then at 60° for 45 min. The mixture was evaporated under reduced pressure and the residue taken up in ether and washed successively with water, potassium hydrogen carbonate solution, 2N-hydrochloric acid, and water. Evaporation of the dried extract and recrystallisation from ether gave the *ester* (10·4 g., 57%), m. p. 127° (Found : N, 4·4. $C_{35}H_{36}O_5N_2S_2$ requires N, 4·5%).

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl*-S-*benzyl*-D-*cysteine benzyl ester*, m. p. 128—129°, $[\alpha]_D^{24}$ +2·5° (*c* 1·00 in acetone) (Found : C, 66·7; H, 6·0%), was prepared similarly (procedure *a*), in 81% yield, from *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine hydrazide and *S*-benzyl-D-cysteine benzyl ester hydrochloride and recrystallised from acetone–ether.

S-*Benzyl*-L-*cysteinyl*-S-*benzyl*-L-*cysteine* (with G. S. Heaton).—*S*-Benzyl-*N*-benzyloxycarbonyl-L-cysteinyl-*S*-benzyl-L-cysteine benzyl ester (1·0 g.), in acetic acid (10 ml.) saturated with hydrogen bromide, was kept at 60° for 1 hr. The product was poured into anhydrous ether (50 ml.); an equal volume of light petroleum (b. p. 40—60°) was added and the precipitated hydrobromide filtered off and washed with 1 : 1 ether–light petroleum. Addition of pyridine to a warm solution in aqueous dioxan liberated the peptide, which crystallised (0·4 g., 62%) on cooling and had m. p. 170—171°, $[\alpha]_D^{20}$ −18·0° (*c* 0·81 in acetic acid) (Found : C, 59·5; H, 5·8; N, 7·0. Calc. for $C_{20}H_{24}O_3N_2S_2$ : C, 59·4; H, 5·9; N, 6·9%) (Izumiya and Greenstein [3] give m. p. 170—172°).

In another experiment the crude hydrobromide was dissolved in ethanol (20 ml.), and water added to the boiling solution to turbidity. Addition of excess of pyridine precipitated a solid which was collected after cooling and recrystallised from aqueous ethanol and proved to be 3 : 6-*di*(*benzylthiomethyl*)-2 : 5-*dioxopiperazine* (0·39 g., 61%), needles m. p. 174—175°, $[\alpha]_D^{23}$ −59·5° (*c* 2·52 in acetic acid) (Found : C, 62·4; H, 5·8; N, 7·2. $C_{20}H_{22}O_2N_2S_2$ requires C, 62·3; H, 5·7; N, 7·25%).

L-*Cysteinyl*-L-*cysteine* (I; $n = 0$).—*S*-Benzyl-*N*-benzyloxycarbonyl-L-cysteinyl-*S*-benzyl-L-cysteine benzyl ester (5·7 g.), in liquid ammonia (250 ml.), was treated with sodium (1·9 g.). Ammonium chloride (5·5 g.) was added and the solution evaporated under nitrogen. The residue was extracted, in an atmosphere of nitrogen, with hot anhydrous ethanol (3 × 50 ml.). The extract was concentrated under reduced pressure in nitrogen and the granular hygroscopic *peptide* (1·0 g., 49%) filtered off, washed with dry ether and stored *in vacuo*; it had m. p. 150—152° (decomp.) (sealed tube) (Found : C, 31·6; H, 4·6. $C_6H_{12}O_3N_2S_2$ requires C, 32·1; H, 5·4%). Attempts to purify this compound further, by recrystallisation or by conversion into metal derivatives, failed.

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl*-S-*benzyl*-L-*cysteine Ethyl Ester* (II; $n = 0$, R = Et).—(*a*) *S*-Benzyl-*N*-benzyloxycarbonyl-L-cysteine hydrazide (1·8 g.) was converted into the azide and coupled with *S*-benzyl-L-cysteine ethyl ester hydrochloride (1·4 g.) as described above for the benzyl ester. Recrystallisation of the product from benzene–light petroleum (b. p. 60—80°) gave the ester (2·3 g., 82%), m. p. 103° (Found : N, 4·8. Calc. for $C_{30}H_{34}O_5N_2S_2$ : N, 4·95%) (Izumiya and Greenstein [3] give m. p. 105°).

(*b*) *S*-Benzyl-*N*-benzyloxycarbonyl-L-cysteine (3·45 g.) was coupled with *S*-benzyl-L-cysteine ethyl ester hydrochloride (2·75 g.) as described above for the benzyl ester, yielding the ethyl ester (2·3 g., 39%), m. p. 103—104°, $[\alpha]_D^{20}$ −66·5° (*c* 0·2 in EtOH).

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl*-S-*benzyl*-L-*cysteine* (II; $n = 0$, R = H) (with G. S. Heaton).—(*a*) *S*-Benzyl-*N*-benzyloxycarbonyl-L-cysteine (860 mg.), in tetrahydrofuran (5 ml.) containing triethylamine (252 mg.), was treated with ethyl chloroformate (271 mg.), and the mixture kept at 0° for 5 min. An ice-cold suspension of *S*-benzyl-L-cysteine (530 mg.) in 50% aqueous tetrahydrofuran (50 ml.) containing triethylamine (252 mg.) was then added. The mixture was kept at room temperature for 2 hr., then evaporated to dryness under reduced

pressure and the residue treated with 2N-hydrochloric acid and extracted with ethyl acetate. Evaporation of the dried extract and recrystallisation of the residue from aqueous ethanol and then from benzene containing a little ethanol afforded the peptide (690 mg., 51%), m. p. 145—146°.

(*b*) Ethyl chloroformate (0·84 ml.) was added to a cooled solution of *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine (3 g.) in chloroform (15 ml.) containing triethylamine (1·25 ml.). After 15 min., thiophenol (0·93 ml.) was added and the mixture kept at room temperature for an hour and then warmed to 50° for 30 min. The solvent was then removed under reduced pressure and the residue triturated with water and with light petroleum (b. p. 40—60°); recrystallisation of the solid product from ethanol afforded S-*benzyl*-N-*benzyloxycarbonyl*-L-*cysteine phenyl thiolester* (VI; R = SPh) (3·0 g., 79%), m. p. 100° (Found: C, 65·4; H, 5·6; N, 3·3. $C_{24}H_{23}O_3NS_2$ requires C, 65·9; H, 5·3; N, 3·2%). This ester (1·4 g.), in tetrahydrofuran (20 ml.), was added to *S*-benzyl-L-cysteine (0·8 g.) in N-sodium hydroxide (5 ml.). A few drops of methanol were added to homogenise the mixture, which was then boiled under reflux for 10 hr., after which the organic solvents were removed under reduced pressure. The residue was extracted with ether, and the residual aqueous layer acidified and extracted with warm ethyl acetate. Evaporation afforded the peptide (1·72 g., 100%), m. p. 141°; recrystallisation from ethanol raised the m. p. to 147°, $[\alpha]_D^{19}$ −39·0° (*c* 0·20 in EtOH) (Found: N, 5·1. Calc. for $C_{28}H_{30}O_5N_2S_2$: N, 5·2%) (Izumiya and Greenstein [3] give m. p. 152°).

NN′-*Dibenzyloxycarbonyl*-L-*cystinylbis*-(S-*benzyl*-L-*cysteine*) (V).—*NN′*-Dibenzyloxycarbonyl-L-cystine (Bergmann and Zervas[16]) (3 g.), in warm benzonitrile (5 ml.) containing 1-ethylpiperidine (1·65 ml.), was treated slowly with benzoyl chloride (1·4 ml.), and the mixture kept at 0° for 15 min. and at room temperature for 20 min. A solution of *S*-benzyl-L-cysteine (2·52 g.) in N-sodium hydroxide (12 ml.) was then added with shaking; more N-sodium hydroxide (12 ml.) was then added in portions, with shaking, and the mixture then shaken overnight. The suspension was filtered and the solid extracted with ether in a Soxhlet apparatus. The residual solid was dissolved in the minimum volume of ethanol and treated with dilute hydrochloric acid; dilution with water gave a flocculent precipitate which was thrice precipitated from acetic acid with water; the *peptide* (2·5 g., 47%) had m. p. 120°, $[\alpha]_D^{20}$ −53·0° (*c* 0·13 in EtOH) (Found: C, 56·4; H, 5·4. $C_{42}H_{46}O_{10}N_4S_4$ requires C, 56·4; H, 5·2%).

*Derivatives of Cysteinylglycylcysteine.*

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl*-*glycine Ethyl Ester* (VII; *n* = 0).—*S*-Benzyl-*N*-benzyloxycarbonyl-L-cysteine (1·0 g.), in chloroform (10 ml.) containing triethylamine (0·5 ml.), was cooled and treated with ethyl chloroformate (0·4 ml.), immediately followed by glycine ethyl ester [from the hydrochloride (0·4 g.) and triethylamine (0·5 ml.)] in chloroform (20 ml.). The mixture was kept at room temperature for 1 hr. and then at 60° for 15 min. The cooled solution was then washed successively with 2N-hydrochloric acid, saturated sodium hydrogen carbonate solution, and water, dried, and evaporated. Recrystallisation from aqueous ethanol afforded the *ester* (0·9 g., 72%), m. p. 94°, $[\alpha]_D^{20}$ −39·6° (*c* 4·32 in dioxan) (Found: C, 61·6; H, 6·1; N, 6·4. $C_{22}H_{26}O_5N_2S$ requires C, 61·4; H, 6·1; N, 6·5%).

An attempt to prepare this compound by the usual azide procedure from *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine hydrazide (3·6 g.) and glycine ethyl ester hydrochloride (1·4 g.) yielded L-N-(2-*benzylthio*-1-*benzyloxycarbonylaminoethyl*)*carbamoylglycine ethyl ester* (IV) (2·0 g., 46%), m. p. 157° after recrystallisation from ethanol (Found: C, 59·2; H, 6·1; N, 9·6. $C_{22}H_{27}O_5N_3S$ requires C, 59·3; H, 6·1; N, 9·7%).

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl*-*glycine Hydrazide* (VIII; *n* = 0, R = NH·$NH_2$).—The ethyl ester (VII; *n* = 0) (2·15 g.) was refluxed in ethanol (5 ml.) with hydrazine hydrate (0·25 g.) for 5 hr. The solid which separated on cooling was the *hydrazide* (1·7 g., 80%) which, recrystallised from ethanol, had m. p. 142°, $[\alpha]_D^{24}$ −18·1° (*c* 0·17 in EtOH) (Found: N, 13·2. $C_{20}H_{24}O_4N_4S$ requires N, 13·5%).

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl*-*glycyl*-S-*benzyl*-L-*cysteine Benzyl Ester* (II; *n* = 1, R = $CH_2$Ph).—*S*-Benzyl-*N*-benzyloxycarbonyl-L-cysteinylglycine hydrazide (5·0 g.), in 50% acetic acid (60 ml.) containing concentrated hydrochloric acid (5 ml.), was cooled to 0° and treated with 10% sodium nitrite solution (10 ml.). The precipitated azide was extracted with ether, and the extract thrice washed with 10% sodium hydrogen carbonate solution and dried. *S*-Benzyl-L-cysteine benzyl ester hydrochloride (4·2 g.) was suspended in water (10 ml.) and ether (50 ml.), cooled to 0°, and treated with triethylamine (2 ml.) with vigorous shaking. The ether layer was separated, dried, and added to the ethereal solution of the azide. Next day the

precipitate was collected by filtration and recrystallised from ethanol; the *ester* (8·0 g., 99%) had m. p. 161°, $[\alpha]_D^{20}$ −34·7° (*c* 2·58 in dioxan) (Found: C, 64·7; H, 5·9. $C_{37}H_{39}O_6N_3S_2$ requires C, 64·8; H, 5·7%).

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl-glycyl*-S-*benzyl*-L-*cysteine ethyl ester* (II; $n = 1$, R = Et), similarly prepared in 81% yield from the same hydrazide (1·04 g.) and *S*-benzyl-L-cysteine ethyl ester hydrochloride (0·7 g.), had m. p. 125—126° (Found: C, 61·8; H, 6·2. $C_{32}H_{37}O_6N_3S_2$ requires C, 61·6; H, 6·0%).

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl-glycine* (VIII; $n = 0$, R = OH).—*S*-Benzyl-*N*-benzyloxycarbonyl-L-cysteine phenyl thiolester (5 g.), in tetrahydrofuran (50 ml.), was treated with glycine (0·85 g.) in N-sodium hydroxide (11·5 ml.), and the mixture refluxed for 6 hr. after being made homogeneous with a few drops of methanol. Tetrahydrofuran was then removed under reduced pressure and the residue extracted with ether; acidification of the aqueous residue, followed by extraction with ethyl acetate and evaporation, yielded the *peptide* (3·2 g., 69%) which, recrystallised from ethanol, had m. p. 153—154°, $[\alpha]_D^{20}$ −11·4° (*c* 2·6 in EtOH) (Found: N, 6·7. $C_{20}H_{22}O_5N_2S$ requires N, 7·0%).

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl*-S-*benzylglycyl*-L-*cysteine* (II; $n = 1$, R = H).—*S*-Benzyl-*N*-benzyloxycarbonyl-L-cysteinylglycine (600 mg.) was converted as usual (cf. p. 3153) into the *phenyl thiolester* (500 mg., 68%), m. p. 102° (from ethanol), $[\alpha]_D^{20}$ −14·5° (*c* 0·21 in EtOH) (Found: N, 5·7. $C_{26}H_{26}O_4N_2S_2$ requires N, 5·7%), which was coupled by the usual procedure (cf. p. 3153) with *S*-benzyl-L-cysteine (250 mg.). Recrystallisation of the product from ethanol–light petroleum (b. p. 60—80°) afforded the *peptide* (185 mg., 21%), m. p. 148—150° (Found: N, 6·9. $C_{30}H_{33}O_6N_3S_2$ requires N, 7·1%).

N-*Benzyloxycarbonylglycyl*-S-*benzyl*-L-*cysteine* (IX; R = H), prepared in 75% yield, as described for its isomeride, from *N*-benzyloxycarbonylglycine phenyl thiolester [13] and *S*-benzyl-L-cysteine and recrystallised from benzene, had m. p. 117°, $[\alpha]_D^{19}$ −65·0° (*c* 0·08 in EtOH) (Found: C, 60·0; H, 5·6. $C_{20}H_{22}O_5N_2S$ requires C, 59·7; H, 5·5%). The ethyl ester, prepared in 54% yield from *N*-benzyloxycarbonylglycine and *S*-benzyl-L-cysteine ethyl ester by the Boissonnas procedure described above (p. 3153) for its isomeride, and recrystallised from ether, had m. p. 80°, $[\alpha]_D^{19}$ −34·5° (*c* 0·43 in EtOH) (Found: N, 6·2. Calc. for $C_{22}H_{26}O_5N_2S$: N, 6·5%) (Goldschmidt and Wick [24] give m. p. 80°); the *benzyl ester*, similarly prepared in 53% yield, had m. p. 97°, $[\alpha]_D^{20}$ −22·0° (*c* 0·18 in EtOH) (Found: C, 65·9; H, 6·0. $C_{27}H_{28}O_5N_2S$ requires C, 65·9; H, 5·7%).

N-*Phthaloylglycyl*-S-*benzyl*-L-*cysteine ethyl ester* was prepared in 67% yield by coupling phthaloylglycine (2·22 g.) and *S*-benzyl-L-cysteine (3·0 g.), by the usual Boissonnas procedure, and recrystallised from ethanol; it had m. p. 165°, $[\alpha]_D^{20}$ −29·0° (*c* 0·10 in EtOH) (Found: C, 61·8; H, 5·4. $C_{22}H_{22}O_5N_2S$ requires C, 62·0; H, 5·1%).

*Derivatives of Cysteinyldiglycylcysteine.*

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl-glycylglycine Ethyl Ester* (VII; $n = 1$).—(*a*) *S*-Benzyl-*N*-benzyloxycarbonyl-L-cysteine (3·6 g.) was dissolved in chloroform (35 ml.) containing triethylamine (1·8 ml.). Ethyl chloroformate (1·2 ml.) was added, with shaking and cooling, followed by glycylglycine ethyl ester hydrochloride [25] (1·2 g.) in chloroform (25 ml.) containing triethylamine (1·8 ml.). The mixture was kept at room temperature for 15 min. and then at 60° for 1 hr. Working up as usual, followed by recrystallisation from aqueous ethanol, afforded the *ester* (4·1 g., 86%), m. p. 114°, $[\alpha]_D^{20}$ −12·9° (*c* 3·21 in EtOH) (Found: C, 58·9; H, 6·2; N, 8·5. $C_{24}H_{29}O_6N_3S$ requires C, 59·1; H, 6·0; N, 8·6%).

(*b*) *S*-Benzyl-*N*-benzyloxycarbonyl-L-cysteine hydrazide (1·0 g.), in 50% acetic acid (20 ml.) containing concentrated hydrochloric acid (2 ml.), was cooled to 0° and treated with 2% sodium nitrite solution (10 ml.). The azide was extracted with chloroform, and the extract thrice washed with 10% sodium hydrogen carbonate solution and dried. Glycylglycine ethyl ester hydrochloride (0·6 g.), in water (2 ml.), was treated, with vigorous shaking, with triethylamine (0·5 ml.) in chloroform (20 ml.); the aqueous layer was saturated with potassium carbonate, and the organic layer separated and dried. This solution was added to the azide solution and the mixture kept overnight and worked up as usual. Recrystallisation from aqueous ethanol gave the ester (1·1 g., 81%), m. p. 115°.

(*c*) Glycylglycine ethyl ester hydrochloride (0·7 g.) was suspended in pyridine (30 ml.) containing phosphorus trichloride (0·3 ml.), and the mixture kept at room temperature for

[24] Goldschmidt and Wick, *Annalen*, 1952, **575**, 217.
[25] Schott, Larkin, Rockland, and Dunn, *J. Org. Chem.*, 1947, **12**, 490.

30 min. *S*-Benzyl-*N*-benzyloxycarbonyl-L-cysteine (1·1 g.), in pyridine (20 ml.), was added and the mixture refluxed for 3 hr., cooled, filtered, and evaporated to dryness under reduced pressure. The residue was taken up in ethyl acetate and washed with 2N-hydrochloric acid, 10% sodium hydrogen carbonate solution, and water; evaporation of the dried extract, followed by recrystallisation from aqueous ethanol, gave the ester (1·0 g., 64%), m. p. 114—115°.

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl-glycylglycine methyl ester*, prepared in 21% yield by the Boissonnas procedure, (*a*), described for the ethyl ester and recrystallised from benzene–ether, had m. p. 110° (Found: C, 58·3; H, 5·6. $C_{23}H_{27}O_6N_3S$ requires C, 58·3; H, 5·8%).

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl-glycylglycine* (VIII; $n = 1$, R = OH), prepared in 31% yield by the usual procedure (p. 3153) from *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine phenyl thiolester (1 g.) and glycylglycine hydrochloride [25] (0·4 g.), and recrystallised from ethanol–light petroleum (b. p. 60—80°), had m. p. 129—130°, $[\alpha]_D^{20}$ −9·4° (*c* 0·21 in EtOH) (Found: N, 9·0. $C_{22}H_{25}O_6N_3S$ requires N, 9·1%). The *phenyl thiolester* (VIII; $n = 1$, R = SPh), prepared in the usual manner (72% yield) and recrystallised from ethanol, had m. p. 105—106°, $[\alpha]_D^{20}$ −5·8° (*c* 0·51 in EtOH) (Found: N, 7·3. $C_{28}H_{29}O_5N_3S_2$ requires N, 7·6%).

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl-glycylglycine Hydrazide* (VIII; $n = 1$, R = $NH{\cdot}NH_2$).—The corresponding ethyl ester (500 mg.) was kept at room temperature overnight with hydrazine hydrate (0·2 ml.) in ethanol (2 ml.). Recrystallisation of the precipitate from aqueous ethanol gave the *hydrazide* (400 mg., 82%), m. p. 164° (Found: N, 14·6. $C_{22}H_{27}O_5N_5S$ requires N, 14·8%).

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl-diglycyl*-S-*benzyl*-L-*cysteine benzyl ester* (II; $n = 2$, R = $CH_2Ph$), prepared in 73% yield by the procedure described on p. 3151 from the above hydrazide (2·5 g.) and *S*-benzyl-L-cysteine benzyl ester hydrochloride (2·2 g.) and recrystallised from acetone–light petroleum (b. p. 60—80°), had m. p. 175°, $[\alpha]_D^{20}$ −29·6° (*c* 2·73 in pyridine) (Found: C, 63·3; H, 5·9. $C_{39}H_{42}O_7N_4S_2$ requires C, 63·1; H, 5·7%). The *ethyl ester* (II; $n = 2$, R = Et), prepared similarly in 47% yield and recrystallised from ethanol–light petroleum (b. p. 60—80°), had m. p. 135° (Found: C, 60·6; H, 5·9. $C_{34}H_{40}O_7N_4S_2$ requires C, 60·0; H, 6·0%).

*Derivatives of Cysteinyltriglycylcysteine.*

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl-diglycylglycine Ethyl Ester* (VII; $n = 2$).—(*a*) Ethyl chloroformate (0·5 ml.) was added, with shaking and cooling, to *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine (1·7 g.), in chloroform (20 ml.) containing triethylamine (0·7 ml.), and immediately followed by a solution of diglycylglycine ethyl ester hydrochloride [17] (1·3 g.) in chloroform (20 ml.) containing triethylamine (0·7 ml.). The mixture was kept at room temperature for 15 min. and at 50° for 1 hr. and then worked up in the usual manner. Recrystallisation of the product from chloroform–ether gave the *ester* (2·0 g., 75%), m. p. 120°, $[\alpha]_D^{20}$ −2·2° (*c* 4·60 in dioxan) (Found: C, 57·0; H, 5·9. $C_{26}H_{32}O_7N_4S$ requires C, 57·3; H, 5·9%).

(*b*) The same product, m. p. 122—123°, was obtained in 45% yield by coupling, in the usual manner, the azide from *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine hydrazide with diglycylglycine ethyl ester.

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl-diglycylglycine Hydrazide* (VIII; $n = 2$, R = $NH{\cdot}NH_2$).—The above ester (1·0 g.) was kept overnight with hydrazine hydrate (0·4 ml.) in ethanol (15 ml.). Recrystallisation of the precipitated solid from aqueous ethanol gave the hydrazide (0·8 g., 82%), m. p. 196° (Found: N, 16·1. $C_{24}H_{30}O_6N_6S$ requires N, 15·85%).

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl-triglycyl*-S-*benzyl*-L-*cysteine Benzyl Ester* (II; $n = 3$, R = $CH_2Ph$).—The azide from the above hydrazide (2·0 g.) was coupled in the usual manner, in ethyl acetate solution, with the free ester from *S*-benzyl-L-cysteine benzyl ester hydrochloride (1·4 g.). Recrystallisation of the product from benzene–light petroleum (b. p. 60—80°) gave the *ester* (1·6 g., 53%), m. p. 157—158°, $[\alpha]_D^{20}$ −30·6° (*c* 2·89 in dioxan) (Found: C, 61·2; H, 5·9; N, 9·0. $C_{41}H_{45}O_8N_5S_2$ requires C, 61·6; H, 5·6; N, 8·8%).

*Derivatives of Cysteinyltetraglycylcysteine.*

N-*Benzyloxycarbonyldiglycyl*-S-*benzyl*-L-*cysteine Benzyl Ester* (X; R = $OCH_2Ph$).—(*a*) *S*-Benzyl-L-cysteine benzyl ester toluene-*p*-sulphonate (2·4 g.), in pyridine (20 ml.) containing phosphorus trichloride (0·5 ml.), was treated with *N*-benzyloxycarbonylglycyl-glycine [16] (1·4 g.), in pyridine (20 ml.), and the mixture refluxed for 2 hr. Working up in the usual manner and recrystallisation from ethanol–light petroleum ether (b. p. 60—80°) gave the ester (1·1 g., 38%), m. p. 100°.

(*b*) *N*-Benzyloxycarbonylglycyl-glycine hydrazide [26] (2·8 g.) was converted into the azide and coupled, as usual, in chloroform with the free ester from *S*-benzyl-L-cysteine benzyl ester hydrochloride (3·4 g.); recrystallisation of the product as before gave the *ester* (1·0 g., 18%), m. p. 100—101° (Found: C, 64·0; H, 5·7; N, 7·8. $C_{29}H_{31}O_6N_3S$ requires C, 63·4; H, 5·6; N, 7·65%).

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl*-*tetraglycyl*-S-*benzyl*-L-*cysteine Benzyl Ester* (II; $n = 4$, R = $CH_2Ph$).—Hydrogen bromide was passed through a solution of the above ester (700 mg.) in acetic acid (28 ml.) for 30 min. Addition of ether (280 ml.) precipitated diglycyl-*S*-benzyl-L-cysteine benzyl ester hydrobromide (750 mg.). The free ester was liberated as usual and coupled, in the usual manner, with the azide from *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteinyl-glycylglycine hydrazide (700 mg.). Recrystallisation of the product from aqueous ethanol gave the *benzyl ester* (500 mg., 39%), m. p. 205—206°, $[\alpha]_D^{20}$ −29·7° (*c* 1·10 in pyridine) (Found: C, 59·5; H, 5·4; N, 9·5. $C_{43}H_{48}O_9N_6S_2$ requires C, 60·2; H, 5·6; N, 9·8%).

N-*Benzyloxycarbonyldiglycyl*-S-*benzyl*-L-*cysteine* (X; R = OH).—*N*-Benzyloxycarbonyl-glycyl-glycine phenyl thiolester [27] (3 g.), in tetrahydrofuran (50 ml.), was added to *S*-benzyl-L-cysteine (1·7 g.) in N-sodium hydroxide (8 ml.). After treatment with a few drops of methanol, the homogeneous solution was heated at 60° for 4 hr. Tetrahydrofuran was removed under reduced pressure and thiophenol by extraction with ether. Acidification precipitated an oil and a solid; the former was taken up in ethyl acetate, and the latter collected by filtration. Evaporation of the ethyl acetate and trituration of the oily residue with water gave a solid which was added to that directly precipitated. Recrystallisation from aqueous ethanol gave the *peptide* (3·0 g., 78%), m. p. 161—162° (Found: C, 57·3; H, 5·3; N, 9·3. $C_{22}H_{25}O_6N_3S$ requires C, 57·5; H, 5·4; N, 9·15%).

*Derivatives of Cysteinyltetraglycylglycine.*

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl*-*tetraglycylglycine Benzyl Ester*.—*N*-Benzyloxycarbonylglycyl-glycine (1·3 g.) was coupled, by the usual Boissonnas procedure, with glycine benzyl ester (from the hydrochloride, 1·0 g.). Recrystallisation of the product from aqueous ethanol yielded N-*benzyloxycarbonyldiglycyl*-*glycine benzyl ester* (1·2 g., 59%), m. p. 148° (Found: C, 61·1; H, 5·6; N, 10·5. $C_{21}H_{23}O_6N_3$ requires C, 61·3; H, 5·6; N, 10·2%). Hydrogen bromide was passed through a suspension of this compound (1·0 g.) in acetic acid (15 ml.) for 30 min. Ether (50 ml.) was added and the solution kept at 0° for 2 hr. The precipitated hydrobromide was filtered off and dissolved in water (5 ml.); triethylamine (0·5 ml.) was added and the free ester extracted into ethyl acetate. *S*-Benzyl-*N*-benzyloxycarbonyl-L-cysteinylglycylglycine hydrazide (1·2 g.) was converted into the azide in the usual manner; the ethyl acetate solution of the azide was mixed with the ethyl acetate solution of diglycylglycine benzyl ester. Next day the mixture was worked up as usual and the product recrystallised from aqueous Cellosolve; the *benzyl ester* (0·3 g., 16·5%) had m. p. 207° (Found: C, 58·1; H, 5·5; N, 11·8. $C_{35}H_{40}O_9N_6S$ requires C, 58·3; H, 5·6; N, 11·7%).

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteinyl*-*tetraglycylglycine Ethyl Ester*.—*S*-Benzyl-*N*-benzyloxycarbonyl-L-cysteinyl-glycylglycine hydrazide (4·5 g.) was coupled with diglycylglycine ethyl ester [17] (2·0 g.) in chloroform by the usual azide procedure. Recrystallisation afforded the *ester* (4·1 g., 66%), m. p. 198—199° (Found: C, 55·4; H, 5·7; N, 12·5. $C_{30}H_{38}O_9N_6S$ requires C, 54·7; H, 5·8; N, 12·75%).

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