1339. *Substituted Diphenylmethyl Protecting Groups in Peptide Synthesis*

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The value of three dimethoxydiphenylmethyl protecting groups in peptide synthesis has been investigated. Amines and thiols react readily with the respective aralkyl chlorides and the resulting *N*- and *S*-aralkyl derivatives are quantitatively cleaved by acidolysis. *S*-4,4′-Dimethoxydiphenylmethyl-L-cysteine has been used in the synthesis of peptides related to oxytocin.

Chemical and physico-chemical similarities between methoxy-substituted diphenylmethyl halides and trityl halides prompted us to investigate the use of substituted diphenylmethyl groups for the reversible protection of amine and thiol functions during peptide synthesis. Though widely used for this purpose, the trityl group suffers disadvantages because of its large size. The background to this investigation and some preliminary experimental results have been presented elsewhere.[1]

4,4′-Dimethoxydiphenylmethyl chloride,[2] 2,2′-dimethyl-4,4′-dimethoxydiphenylmethyl chloride, and 3,3′,4,4′-tetramethoxydiphenylmethyl chloride [3] were readily prepared by the action of thionyl chloride or hydrogen chloride on the respective alcohols. In the case of 2,2′,4,4′-tetramethoxydiphenylmethanol, only a high-melting, chlorine-free product could be obtained. A similar observation has been made in the veratroyl series.[3] Substituted *N*-diphenylmethylglycine and L-phenylalanine methyl esters were synthesised from the aralkyl chlorides by reacting them with the amino-acid esters in the presence of base in the usual way. The *N*-substituted amino-acid derivatives were isolated in good yields, but could not all be obtained in solid form. Similar attempts to prepare 2,2′,4,4′-tetramethoxydiphenylmethyl derivatives of amines without isolating the intermediate chloride were unsuccessful.

Optically active, *N*-trityl α-amino-acids which contain bulky substituents on the α-carbon atom can only be prepared in poor yields by the saponification of the corresponding esters, and the resistance of these esters to saponification is attributed to steric hindrance.[4] For the same reason, *N*-trityl amino-acid hydrazides cannot be conveniently synthesised by the hydrazinolysis of these esters.[5] Chromatographic evidence indicated that the substituted diphenylmethylphenylalanine esters were more readily saponified than their trityl counterparts and, except in the case of *N*-2,2′-dimethyl-4,4′-dimethoxydiphenylmethyl-L-phenylalanine, the free acids could be prepared in this way. Even so, the resistance of these esters to saponification was considerable. *N*-3,3′,4,4′-tetramethoxydiphenylmethyl-L-phenylalanine was also prepared, like the *N*-trityl derivative,[4] from diethylammonium phenylalaninate and the aralkyl chloride in solution in aqueous propan-2-ol, but the yield was low. *N*-4,4′-Dimethoxydiphenylmethylglycine hydrazide was prepared in excellent yield by the reaction of the methyl ester with hydrazine, but attempts to prepare the analogous phenylalanine derivative were unsuccessful.

It was not to be expected that the basicity of the amino-group in the *N*-diphenylmethyl amino-acid derivatives would be completely suppressed, since the introduction of the alkoxyl substituents, whilst increasing the lability of the diphenylmethyl moiety, would also increase the electron availability at the methyl carbon. Even in the trityl series the amino-group is still basic, as may be shown by the preparation of *N*-trityl amino-acid hydrochlorides.[4] However, *N*-trityl amino-acids, unlike the diphenylmethyl derivatives,

[1] H. D. Law and R. W. Hanson, Proc. Sixth European Peptide Symp., Athens, September 1963; Pergamon, in the press.
[2] D. Bethell and V. Gold, *J.*, 1958, 1905.
[3] J. Čtvrtník and J. Mayer, *Coll. Czech. Chem. Comm.*, 1959, **91**, 167.
[4] 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.
[5] A. Hillmann-Elies, G. Hillmann, and H. Jatzkewitz, *Z. Naturforsch.*, 1953, **8**b, 445.

are not zwitterions. The substituted *N*-diphenylmethyl amino-acids were finely crystalline compounds with high indefinite melting points and were generally insoluble in organic solvents other than acetone and water. The infrared spectrum (Nujol mull) showed no peak in the 1700—1750-cm.$^{-1}$ region, although treatment of *N*-4,4′-dimethoxydiphenylmethylglycine with an equivalent of hydrogen chloride yielded an oil with a strong peak at 1750 cm.$^{-1}$. Presumably, this substance was *N*-4,4′-dimethoxydiphenylmethylglycine hydrochloride. The resemblance of the *N*-diphenylmethyl amino-acids to *N*-benzyl and *N*-dibenzyl amino-acids is brought out by a comparison of the apparent dissociation constants of these compounds (pH measured at half-neutralisation in 1 : 1 aqueous dioxan; pK_a, pK_b): glycine, 3·19, 9·90; *N*-benzylglycine, 2·92, 9·22; *N*-4,4′-dimethoxydiphenylmethylglycine, 2·98, 8·20; *N*-2,2′-dimethyl-4,4′-dimethoxydiphenylmethylglycine, 2·89, 8·25; *NN*-dibenzylglycine, 2·70, 7·89; *N*-tritylglycine, 2·7—2·6, 6·4.

However, the substituted *N*-diphenylmethyl amino-acids are very similar to *N*-trityl amino-acids and totally unlike *N*-benzyl and *N*-dibenzyl amino-acids in the lability of the protecting group. The free amino-acids were liberated quantitatively from the *N*-4,4′-dimethoxydiphenylmethyl, *N*-2,2′-dimethyl-4,4′-dimethoxydiphenylmethyl, and *N*-3,3′,4,4′-tetramethoxydiphenylmethyl amino-acids by warming them gently on a water-bath in 50% aqueous acetic acid for 5 min. An intense colour was produced during this reaction, although the liberated amino-acid could be easily obtained pure. On thin-layer chromatograms exposed to hydrogen chloride vapour, *N*-4,4′-dimethoxydiphenylmethyl groups gave orange, *N*-2,2′-dimethyl-4,4′-dimethoxydiphenylmethyl groups, red, and *N*-3,3′,4,4′-tetramethoxydiphenylmethyl groups, magenta colours, which rendered the amino-acid derivatives easily detectable. We find that *N*-trityl amino-acids and peptides give a yellow colour under these conditions and although this is not as intense as the colours generated by the diphenylmethyl derivatives, pentapeptides, at least, are readily detected. Ninhydrin may be used before or after hydrogen chloride on these chromatograms.

Although the existence of the *N*-substituted amino-acids as zwitterions does not preclude their use in peptide synthesis (*NN*-dibenzyl amino-acids for example, may be coupled by the mixed anhydride procedure with amino-acid esters [6]), the steric and possible electronic interference of the substituted diphenylmethyl group with the carboxyl group makes this approach unattractive. Because of steric hindrance, the higher *N*-trityl amino-acids cannot be coupled by the mixed anhydride procedure.[4,7] Other possibilities, for example, that the substituted *N*-diphenylmethyl amino-acids, like *N*-trityl amino-acids,[8a] might form useful *N*-carboxyanhydrides, or that the substituted diphenylmethyl groups might be used for the protection of basic side-chains, remain to be investigated.

The unsubstituted diphenylmethyl group has been used by Zervas and Photaki to protect thiol functions during peptide synthesis.[9] It was introduced by the reaction of cysteine toluene-*p*-sulphonate with diphenylmethyl chloride in solution in *NN*-dimethylformamide at 80—90° to give *S*-diphenylmethylcysteine in 53% yield. 4,4′-Dimethoxydiphenylmethyl chloride and 3,3′,4,4′-tetramethoxydiphenylmethyl chloride in solution in *NN*-dimethylformamide reacted quantitatively with cysteine tosylate at room temperature to give the *S*-substituted cysteine derivatives. For ease of manipulation, these derivatives were superior to the unsubstituted diphenylmethyl compound, but *S*-3,3′,4,4′-tetramethoxydiphenylmethylcysteine, which is not described here, formed gels extremely readily and seemed to offer no advantage. At room temperature, *S*-diphenylmethylcysteine was only obtained in 9% yield. In the readiness with which they are formed, *S*-4,4′-dimethoxydiphenylmethyl- and *S*-3,3′,4,4′-tetramethoxydiphenylmethylcysteine resemble *S*-tritylcysteine, but the cleavage of the *S*-dimethoxydiphenylmethyl derivatives

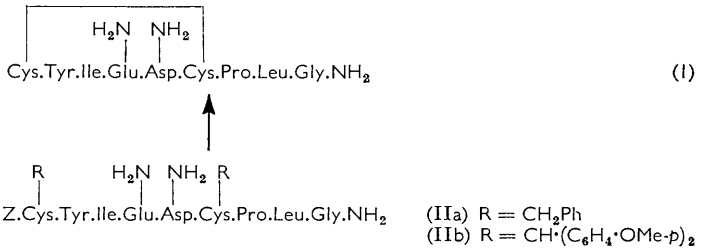
[6] L. Velluz, J. Anatol, and G. Amiard, *Bull. Soc. chim. France*, 1954, 1449.
[7] G. Amiard, R. Heymès, and L. Velluz, *Bull. Soc. chim. France*, 1955, 191.
[8] (*a*) H. Block and M. E. Cox, p. 83; (*b*) G. T. Young, Proc. Fifth European Peptide Symp., Oxford, September 1962; ed. G. T. Young, Pergamon, 1963, p. 261.
[9] L. Zervas and I. Photaki, *J. Amer. Chem. Soc.*, 1962, **84**, 3887.

is very similar to the cleavage of *S*-diphenylmethylcysteine. Only partial cleavage could be achieved by reaction with a solution of hydrogen bromide in glacial acetic acid at room temperature (0·25M-solutions, 10 equivalents of hydrogen bromide gave the following degrees of cleavage: *S*-diphenylmethylcysteine, 9%; *S*-4,4′-dimethoxydiphenylmethylcysteine, 12%; *S*-3,3′,4,4′-tetramethoxydiphenylmethylcysteine, 14%). As with the *S*-diphenylmethyl group, quantitative cleavage of the substituted diphenylmethyl compounds could be obtained by treating them with a solution of hydrogen bromide in acetic acid at 50° or by refluxing them in a solution of trifluoroacetic acid containing phenol.

Cleavage of the substituted *S*-diphenylmethyl groups with solutions of hydrogen bromide in acetic acid appears to reach an equilibrium very quickly. The cleavage of *S*-3,3′,4,4′-tetramethoxydiphenylmethylcysteine, for example, had already reached 14% after 1 min at room temperature and was not appreciably changed after 1 hr. According to Zervas and Photaki, *S*-diphenylmethylcysteine behaves similarly.[9] These results suggested that cysteine itself could be alkylated by the substituted diphenylmethyl chlorides in acid solution and, in confirmation of this, when excess of chloride was present during the cleavage reaction, the subsequent thiol titration was almost zero. Thus, in the case of *S*-3,3′,4,4′-tetramethoxydiphenylmethylcysteine, treated as before with a solution of hydrogen bromide in acetic acid, but this time in the presence of 5 equivalents of the chloride, the resulting thiol titration was reduced from 14 to 0·5%. The degree of cleavage of the *S*-diphenylmethyl derivative in hydrogen bromide in acetic acid solution could be similarly reduced at 50°, whereas the cleavage of *S*-trityl groups, which takes place very rapidly at room temperature in extremely dilute solutions of hydrogen bromide in acetic acid, was not reduced in the presence of 10 equivalents of trityl chloride. The possibility of inhibiting the cleavage of *S*-diphenylmethyl derivatives seems potentially important when the selective removal is desired of other *N*-protecting groups (for example, trityl, t-butyloxycarbonyl, benzyloxycarbonyl) susceptible to cleavage under acidic conditions. *S*-Diphenylmethylcysteine was prepared in 80% yield by the reaction of cysteine toluene-*p*-sulphonate in hydrogen bromide in acetic acid solution with diphenylmethyl chloride and, in our experience, this affords the most satisfactory preparation of this compound.

S-4,4′-Dimethoxydiphenylmethyl and *S*-3,3′,4,4′-tetramethoxydiphenylmethyl derivatives give the same colours as the *N*-substituted compounds on thin-layer chromatograms treated with hydrogen chloride vapour. The colours were very intense and could be detected when the concentration of the protecting group in the molecule was very small; 0·5 μg. of *S*-4,4′-dimethoxydiphenylmethylcysteine or *S*-3,3′,4,4′-tetramethoxydiphenylmethylcysteine was readily detected. *S*-Diphenylmethylcysteine gave no colours under these conditions.

To evaluate the usefulness of *S*-4,4′-dimethoxydiphenylmethylcysteine in peptide synthesis, we undertook the preparation of various peptides related to oxytocin (I). This hormone, which was first synthesised by du Vigneaud *et al.*,[10] has been synthesised by



Oxytocin (I) and the protected nonapeptide (II) used in its synthesis. The amino-acid residue abbreviations are those recommended by the Committee on Nomenclature which reported at the Fifth European Peptide Symposium.[8b]

[10] V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, P. G. Katsoyannis, and S. Gordon, *J. Amer. Chem. Soc.*, 1953, **75**, 4879; V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, and P. G. Katsoyannis, *ibid.*, 1954, **76**, 3115.

several different routes, most of which have employed benzyl groups for the protection of the thiol functions. The protecting groups have ultimately been removed from a nonapeptide derivative (*e.g.*, IIa) by reduction with sodium in liquid ammonia and, generally, oxytocin has been obtained from the resulting dithiol compound by aerial oxidation.[11] Since the *S*-4,4′-dimethoxydiphenylmethyl group is also susceptible to reduction, it seemed likely that the final stage of a synthesis of oxytocin employing this group could be accomplished by this well-known method and that this could serve as a control for the novel procedures to be investigated. Furthermore, oxytocin had previously been synthesised by the use of *S*-trityl groups,[12] so it was known that a final acidolytic stage was practicable. On the other hand, there is evidence that the terminal amido-group of the oxytocin molecule is essential for the manifestation of biological activity and that this group tends to be hydrolysed under acidic conditions.[11] It was therefore of interest to determine whether the more vigorous conditions required for the cleavage of the *S*-4,4′-dimethoxydiphenylmethyl group would lead to the formation of a biologically inactive product. Although the cleavage of *N*-benzyloxycarbonyl [13] and *S*-diphenylmethyl [9] groups with boiling trifluoroacetic acid has been reported, these conditions have not been used, so far as we are aware, in the synthesis of complex peptides.

The route employed for the preparation of the protected nonapeptide (II*b*) was adapted from a recent modification [14] of the "improved synthesis of oxytocin" described by Bodanszky and du Vigneaud.[15] *S*-4,4′-Dimethoxydiphenylmethyl-L-cysteine was converted in the usual way into the *N*-benzyloxycarbonyl derivative, isolated as the cyclohexylamine salt. The resulting *NS*-protected cysteine derivative was coupled by the *NN*′-dicyclohexylcarbodi-imide procedure [16] with methyl-L-tyrosinate to give the dipeptide derivative, methyl *N*-benzyloxycarbonyl-*S*-4,4′-dimethoxydiphenylmethyl-L-cysteinyl-L-tyrosinate. Saponification of the dipeptide ester derivative gave the free acid which was reacted by the mixed anhydride procedure [17] with L-isoleucyl-L-glutaminyl-L-asparagine to form the pentapeptide derivative, *N*-benzyloxycarbonyl-*S*-4,4′-dimethoxydiphenylmethyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparagine.

Two derivatives of the required *C*-terminal tetrapeptide amide, *N*-benzyloxycarbonyl-*S*-4,4′-dimethoxydiphenylmethyl-L-cysteinyl-L-prolyl-L-leucylglycine amide and the corresponding *N*-t-butyloxycarbonyl derivative, were prepared. For the synthesis of the *N*-benzyloxycarbonyl compound, *p*-nitrophenyl *N*-benzyloxycarbonyl-*S*-4,4′-dimethoxydiphenylmethyl-L-cysteinate, prepared from the protected amino-acid and *p*-nitrophenol by the *NN*′-dicyclohexylcarbodi-imide method, was reacted with the *C*-terminal tripeptide amide. *N*-t-Butyloxycarbonyl-*S*-4,4′-dimethoxydiphenylmethyl-L-cysteine was prepared from the *S*-protected cysteine by the standard procedure employing t-butyl *p*-nitrophenyl carbonate.[18] The free acid was converted into the *p*-nitrophenyl ester and coupled with the *C*-terminal tripeptide amide in the same way as the *N*-benzyloxycarbonyl derivative.

The *N*-t-butyloxycarbonyl group could be removed quantitatively from the protected tetrapeptide derivative, without cleavage of the *S*-4,4′-dimethoxydiphenylmethyl group, by heating it in glacial acetic acid containing excess 4,4′-dimethoxydiphenylmethyl chloride for 2 min. on a water-bath. Selective removal of the *N*-benzyloxycarbonyl group was less satisfactory, but, in the presence of excess of chloride, an ostensibly pure product could be obtained by dissolving the *N*-benzyloxycarbonyl derivative in a solution of hydrogen bromide in acetic acid at room temperature.

Preliminary experiments have now been carried out on the removal of the protecting

[11] H. D. Law, Review in *Progr. Medicinal Chem.*, ed. G. P. Ellis and G. B. West, 1965, **4**, 86.
[12] L. Velluz, G. Amiard, J. Bartos, B. Goffinet, and R. Heymès, *Bull. Soc. chim. France*, 1956, 1464.
[13] F. Weygand and W. Steglich, *Z. Naturforsch.*, 1959, **14**b, 472.
[14] A. P. Fosker and H. D. Law, *J.*, 1965, 4922.
[15] M. Bodanszky and V. du Vigneaud, *J. Amer. Chem. Soc.*, 1959, **81**, 2504.
[16] J. C. Sheehan and G. P. Hess, *J. Amer. Chem. Soc.*, 1955, **77**, 1067.
[17] J. R. Vaughan, jun., and J. A. Eichler, *J. Amer. Chem. Soc.*, 1953, **75**, 5556.
[18] G. W. Anderson and A. C. McGregor, *J. Amer. Chem. Soc.*, 1957, **79**, 6180.

groups from the nonapeptide derivative (IIb) formed by coupling (Reagent K procedure [19]) the pentapeptide and tetrapeptide components. Reduction of this compound by sodium in liquid ammonia, followed by oxidation in the usual way, gave a solution possessing biological activity in the avian depressor test [11] equivalent to 75 units of oxytocin per milligram of the protected nonapeptide.* On the other hand, when the protecting groups were cleaved under acidic conditions, much lower activities were recorded. Reaction with hydrogen bromide in acetic acid at room temperature followed by boiling phenol–trifluoroacetic acid solution gave ~30 units per milligram; treatment with boiling phenol–trifluoroacetic acid solution alone gave ~20 units per milligram; reaction with hydrogen bromide in acetic acid at 50° for an hour gave ~1 unit per milligram.

These results suggest that the 4,4′-dimethoxydiphenylmethyl group might prove useful as a chromogenic group removable by sodium–liquid ammonia reduction or by acidolysis, for the protection of thiol groups in peptide synthesis. The cleavage experiments involving peptides related to oxytocin indicate that the use in the synthesis of complex peptides of protecting groups which require boiling trifluoroacetic acid for their removal should be approached with caution.

Experimental

Melting points were measured with a Gallenkamp Electrothermal heated block apparatus and are uncorrected. Optical rotations were measured with a Bellingham and Stanley model A polarimeter. Microanalyses were carried out by H. Bieler, Organisch-Chemisches Institüt der Universität Wien. Reagent-K was purchased from K and K Laboratories Inc., Plainview, New York and 4,4′-dimethoxybenzophenone from Kodak Ltd., Kirkby, Lancashire. 4,4′-Dimethoxydiphenylmethyl chloride and 3,3′,4,4′-tetramethoxydiphenylmethyl chloride were prepared by the methods of Bethell and Gold [2] and Čtvrtník and Mayer,[3] respectively.

2,2′-Dimethyl-4,4′-dimethoxydiphenylmethane.—The method employed for the preparation of this compound was a modification, after Quelet,[21] of Blanc's procedure.[22] A mixture of *O*-methyl-*m*-cresol (132 g., 1 mole), formalin (36 g. of 40%), and zinc chloride (20 g.) was stirred vigorously and slowly saturated with hydrogen chloride (over 24 hr.). The supernatant liquid which separated after a short period at rest was poured into iced water (500 ml.). Ether (300 ml.) was added to the resulting emulsion and the aqueous layer was run off. The organic layer was washed and dried (Na_2SO_4) and the ether evaporated to leave an oil which was purified by distillation. Some *O*-methyl-*m*-cresol (21 g.; b. p. 71°/22 mm.) was recovered followed by a fraction (59·5 g.), b. p. 220—232°/22 mm. This material solidified readily and was recrystallised from hot ethanol to yield the required diphenylmethane derivative (40 g., 40%), m. p. 65—67° (Found: C, 79·8; H, 7·9. Calc. for $C_{17}H_{20}O_2$: C, 79·65; H, 7·9%) (lit.,[21] b. p. 215—216°/12 mm., m. p. 66°). Evaporation of the mother-liquors gave a yellow oil (14 g.) which was almost identical with the above material by infrared analyses but which could not be solidified.

2,2′-Dimethyl-4,4′-dimethoxybenzophenone.—A mixture of 2,2′-dimethyl-4,4′-dimethoxydiphenylmethane (30·5 g., 0·12 mole) and sodium dichromate dihydrate (35·5 g.) was refluxed in glacial acetic acid for 4 hr. The cooled reaction mixture was extracted with light petroleum (10 × 100 ml.) and the extract was washed with water (2 × 50 ml.). Dilution of the aqueous

* A sample of the dibenzylnonapeptide derivative (IIa) obtained by the Reagent-K procedure gave, after reduction and subsequent oxidation, a solution with avian depressor activity equivalent to 134 units of oxytocin per milligram of the protected nonapeptide.[14] At a similar stage of purification, the material prepared by Bodanszky and du Vigneaud [15] gave 140 units of oxytocic activity per milligram of the protected nonapeptide. The lower activity obtained in the present synthesis may possibly be accounted for by partial racemisation (this might have occurred, for example, during the preparation of the protected pentapeptide) or by the presence of a deamido-impurity. Such an impurity has already been reported in the preparation of a similar nonapeptide derivative.[14] After this Paper had been submitted for publication, another example of this type of impurity was reported in the vasopressin series.[20]

[19] R. B. Woodward, R. A. Olofson, and H. Mayer, *J. Amer. Chem. Soc.*, 1961, **83**, 1010.
[20] M. Zaoral and F. Šorm, *Coll. Czech. Chem. Comm.*, 1965, **30**, 611.
[21] R. Quelet, *Compt. rend.*, 1934, **198**, 103.
[22] G. Blanc, *Bull. Soc. chim. France*, 1923, **33—34**, 313.

phase with more water (500 ml.) resulted in the separation of some solid material which was extracted into light petroleum. The combined organic phases were washed with water (2 × 50 ml.), dried (Na_2SO_4), and evaporated to dryness under reduced pressure to give the required benzophenone derivative (22 g., 68·5%), m. p. 65—68°. A sample recrystallised from light petroleum had m. p. 68—71° (Found: C, 75·6; H, 6·8. Calc. for $C_{17}H_{18}O_3$: C, 75·5; H, 6·7%).

2,2′-Dimethyl-4,4′-dimethoxydiphenylmethanol.—Lithium aluminium hydride (4 g.) was stirred in dry ether (200 ml.) and a solution of the substituted benzophenone (14·5 g., 0·054 mole) in dry ether (140 ml.) was added. The resulting mixture was refluxed for 3 hr. Excess of lithium aluminium hydride was decomposed with wet ether and dilute hydrochloric acid was afterwards added to the cold mixture to pH 2. The ethereal layer was washed, dried (Na_2SO_4), and evaporated to give an oil which rapidly crystallised. Recrystallisation from light petroleum gave the *diphenylmethanol derivative* (11·13 g., 76·5%), m. p. 90·5—91·5° (Found: C, 74·8; H, 7·3. $C_{17}H_{20}O_3$ requires C, 75·0; H, 7·4%).

2,2′-Dimethyl-4,4′-dimethoxydiphenylmethyl Chloride.—The diphenylmethanol derivative (16·9 g., 0·062 mole) was dissolved in ether (200 ml.) and hydrogen chloride was bubbled through the solution for 30 min. After the water layer which formed had been removed, the ether was evaporated to dryness leaving a crystalline, slightly discoloured residue. This was dissolved in ether and the solution dried (Na_2SO_4) and concentrated. The *chloride* separated out as colourless crystals (16·2 g., 88%), m. p. 74—78°. A sample recrystallised from light petroleum had m. p. 76—78° (Found: C, 70·5; H, 6·6. $C_{17}H_{19}ClO_2$ requires C, 70·3; H, 6·6%).

2,2′,4,4′-Tetramethoxydiphenylmethanol.—This compound was prepared by the reduction of the benzophenone derivative [23] with lithium aluminium hydride as described above. The *diphenylmethanol derivative*, recrystallised from light petroleum, had m. p. 90·5—92° (90% yield) (Found: C, 66·8; H, 6·5. $C_{17}H_{20}O_5$ requires C, 67·1; H, 6·6%).

Attempts to Prepare 2,2′,4,4′-Tetramethoxydiphenylmethyl Chloride.—(*a*) A small quantity of the carbinol derivative was dissolved in ether and hydrogen chloride bubbled through the solution in the usual way. The reaction mixture developed a brilliant purple colour and a solid material separated out. This material had m. p. ~200° and seemed to contain no halogen (Beilstein and silver nitrate). It was not investigated further.

(*b*) Oxalyl chloride (0·127 g., 1 mmole) dissolved in dry benzene (5 ml.) was added to a solution of the carbinol derivative (0·3 g., 1 mmole) in dry benzene (5 ml.). After being stirred for 1 hr. the pink solution was evaporated under reduced pressure to give a highly coloured, brown-purple residue. The residue was triturated with light petroleum to give a brown solid (156 mg.), m. p. >180°, which on infrared inspection seemed to be very similar to the material obtained above. It was not investigated further.

(*c*) A solution of thionyl chloride (0·119 g., 1·1 mmole) in ether (5 ml.) was added over 5 min. to a stirred solution of the substituted carbinol (0·3 g., 1 mmole) in ether (25 ml.) at −50°. The reaction mixture develped a pale yellow colour. Immediately after the addition of the thionyl chloride, light petroleum was added dropwise to the reaction mixture but no precipitation occurred. The mixture was evaporated at <10° but it became highly coloured during this time and a purple residue, m. p. >180°, was obtained.

In view of the pale coloration of the solutions in (*b*) and (*c*) above, attempts were made to prepare the *N*-substituted tetramethoxydiphenylmethylaniline derivative and the corresponding glycine methyl ester derivative without isolation of the chloride. These efforts were unsuccessful and no recognisable product could be obtained.

N-4,4′-Dimethoxydiphenylmethylglycine.—A stirred suspension of methyl glycinate hydrochloride (2·51 g., 20 mmoles), in dry, ice-cold chloroform (30 ml.) was treated with triethylamine (4·04 g., 5·4 ml., 40 mmoles), followed by 4,4′-dimethoxydiphenylmethyl chloride (5·24 g., 20 mmoles). The solution was allowed to warm slowly and was left at room temperature for 20 hr. Chloroform (30 ml.) was added to the mixture and the resulting solution was washed with water (3 × 15 ml.), dried and evaporated to leave a clear oil (6·19 g.; n_D^{18} 1·552). This oil (1·57 g.) was dissolved, by gentle warming, in N-ethanolic potassium hydroxide solution (10 ml.). After 1 hr., water (20 ml.) was added and the ice-cold mixture was acidified with acetic acid. An oil separated which solidified after trituration. The solid was collected, washed well with water and dilute ethanol, dried, and recrystallised from aqueous acetone to

[23] J. A. VanAllan, *J. Org. Chem.*, 1958, **23**, 1679.

give the N-*protected amino-acid* (1·03 g., 67%) in the form of long needles, m. p. 192—194° (decomp. gels 175—179°) (Found: C, 67·5; H, 6·3; N, 4·7. $C_{17}H_{19}NO_4$ requires C, 67·7; H, 6·3; N, 4·65%).

N-4,4′-*Dimethoxydiphenylmethylglycine Hydrazide.*—A solution of crude methyl *N*-4,4′-dimethoxydiphenylmethylglycinate (343 mg., 1 mmole) in dry dioxan (3 ml.) and methanol (3 ml.) containing hydrazine hydrate (1 ml.) was heated under reflux for 24 hr. The solvents were evaporated and the residue was triturated under water (10 ml.) to yield the *hydrazide* (330 mg., 96%), m. p. 109—110°. A sample crystallised from ethyl acetate–ether as large prisms, m. p. 113—114° (Found: C, 66·45; H, 7·3; N, 12·2. $C_{19}H_{25}N_3O_3$ requires C, 66·05; H, 7·2; N, 11·9%).

Attempted Preparation of N-4,4′-*Dimethoxydiphenylmethylglycine Hydrochloride.*—The *N*-substituted glyine derivative (0·06 g., 0·2 mmole) was dissolved in dry dioxan and a dry ethereal solution of hydrogen chloride (0·2 mmole HCl) was added. An oil started to precipitate and dry light petroleum was added to complete the precipitation. This oil (90% calculated for the hydrochloride) showed in the infrared a sharp absorption at 1750 $cm.^{-1}$.

Methyl N-4,4′-*Dimethoxydiphenylmethyl*-L-*phenylalaninate.*—A stirred suspension of methyl L-phenylalaninate hydrochloride (21·55 g., 0·1 mole) in dry, ice-cold chloroform (150 ml.) was treated with triethylamine (20·24 g., 27·80 ml., 0·2 mole), followed by 4,4′-dimethoxydiphenylmethyl chloride (26·25 g., 0·1 mole). The solution was allowed to attain room temperature and was left at room temperature for 20 hr. Chloroform (150 ml.) was added to the mixture and the solution was washed with water (3 × 50 ml.), dried, and evaporated to yield a thick oil which solidified after trituration. Recrystallisation of the solid (37·21 g.) from a mixture of ether and light petroleum gave the pure *amino-acid ester derivative* (33·6 g., 83%), m. p. 69—72°. A sample recrystallised from the same solvents separated as rhomboids, m. p. 70—72°, $[\alpha]_D^{20}$ −14·4° (*c* 2 in MeOH) (Found: C, 74·4; H, 6·9; N, 3·4. $C_{25}H_{27}NO_4$ requires C, 74·0; H, 6·4; N, 3·4%).

N-4,4′-*Dimethoxydiphenylmethyl*-L-*phenylalanine.*—Methyl *N*-4,4′-dimethoxydiphenylmethyl-L-phenylalaninate (2·03 g., 5·0 mmoles) was dissolved, by gentle warming, in N-ethanolic potassium hydroxide solution (10 ml.). After 2 hr. at room temperature the mixture was diluted with water (20 ml.), cooled to 0°, and acidified with acetic acid. The crude product (1·26 g.) was collected, washed with water and dilute ethanol, dried, and recrystallised from a mixture of acetonitrile, acetone, and water to yield the N-*protected amino-acid* (0·87 g., 46%) as rhomboids, m. p. 197° (decomp., gels 188—194°). A sample recrystallised from nitromethane had m. p. 198° (decomp., gels 188—192°), $[\alpha]_D^{20}$ +2·3° (*c* 0·25 in 0·1N-NaOH) (Found: C, 73·5; H, 6·25; N, 3·8. $C_{24}H_{25}NO_4$ requires C, 73·6; H, 6·4; N, 3·6%).

N-3,3′,4,4′-*Tetramethoxydiphenylmethyl*-L-*phenylalanine.*—(*a*) The *N*-substituted-L-phenylalanine methyl ester was prepared as an oil (quantitative) from the aralkyl chloride and methyl L-phenylalaninate in chloroform solution containing triethylamine as described above. This oil (1·39 g.) was dissolved in warm N-ethanolic potassium hydroxide solution (7·0 ml.) and the solution was kept for 3 hr. at room temperature. The ethanol was evaporated and the residue treated with ice (2 g.) and glacial acetic acid (0·34 ml.), followed immediately by water (10 ml.). The material which precipitated was removed, washed well with water, and triturated under ethanol (10 ml.). The insoluble residue (0·89 g.) m. p. 175—184° (decomp.) was recrystallised from aqueous acetone to yield the N-*protected amino-acid* in the form of needles (0·54 g., 40%), m. p. 190° (decomp., gels 174—185°) $[\alpha]_D^{18}$ −22·4° (*c* 0·4 in DMF) (Found: N, 2·95. $C_{26}H_{29}NO_6$ requires N, 3·1%).

(*b*) The diethylammonium salt of *N*-3,3′,4,4′-tetramethoxydiphenylmethyl-L-phenylalanine was prepared (21% yield) from the aralkyl chloride and diethylammonium L-phenylalaninate by the method previously reported for the preparation of *N*-trityl derivatives.[4] Acidification of the sodium salt in the usual way gave the *N*-protected acid (88%), m. p. 192—208° (decomp.) (Found: N, 2·9%). C and H analyses on this compound were variable.

Methyl N-4,4′-*Dimethoxy*-2,2′-*dimethyldiphenylmethylglycinate.*—The aralkyl chloride (5·8 g., 0·02 mole) was reacted with methyl glycinate (0·02 mole) in the manner described above to yield the N-*substituted amino-acid methyl ester* (91%), rhomboids from light petroleum, m. p. 62—64° (Found: C, 70·0; H, 7·3; N, 4·2. $C_{20}H_{25}NO_4$ requires C, 69·95; H, 7·3; N, 4·1%).

N-4,4′-*Dimethoxy*-2,2′-*dimethyldiphenylmethylglycine.*—Saponification of the above ester gave the N-*substituted amino-acid*, (82%), needles from ethanol, m. p. >160° (indefinite) (Found: N, 4·3. $C_{19}H_{23}NO_4$ requires N, 4·25%). C and H analyses on this compound were variable.

Attempted Preparation of N-4,4′-*Dimethoxy*-2,2′-*dimethyldiphenylmethyl*-L-*phenylalanine*.—The methyl ester derivative of this compound could be obtained in the usual way from the reaction of the aralkyl chloride with methyl-L-phenylalaninate, but even prolonged attempts to saponify the resulting oil were unsuccessful.

Action of Acetic Acid on Substituted N-*Diphenylmethyl Amino-acids*.—(*a*) N-4,4′-*Dimethoxydiphenylmethylglycine*. The *N*-protected amino-acid (0·9 g.) in a solution of 1 : 1 aqueous acetic acid (12 ml.) was heated on a boiling-water bath for 5 min. The cold solution was diluted with water (18 ml.), extracted with ether (2 × 15 ml.), and evaporated to dryness. The chromatographically pure residue (0·165 g., 73%) was filtered with the aid of a little ethanol and identified as glycine by comparison of its R_F values and infrared absorption spectrum with those of an authentic specimen. The ethanolic liquors later deposited more of the pure amino-acid (52 mg., 25%).

(*b*) N-4,4′-*Dimethoxydiphenylmethyl*-L-*phenylalanine*. The *N*-protected amino-acid (0·5 g.) after treatment with acetic acid, as described above, yielded chromatographically pure phenylalanine (0·18 g., 85%).

(*c*) N-3,3′,4,4′-*Tetramethoxydiphenylmethyl*-L-*phenylalanine*. This compound after treatment with acetic acid, as described above, gave phenylalanine in 63% yield.

S-4,4′-*Dimethoxydiphenylmethyl*-L-*cysteine*.—A suspension of L-cysteine toluene-*p*-sulphonate [9] (2·93 g., 10 mmoles) in dry *NN*-dimethylformamide (6 ml.) containing 4,4′-dimethoxydiphenylmethyl chloride (3·93 g., 15 mmoles) was shaken for 2·5 days at room temperature. The mixture was then poured into an aqueous solution of sodium acetate (50 ml., 10%). The insoluble material was removed, washed with water and acetone, and was then suspended for 5 min. in boiling acetone. The undissolved S-*protected cysteine* (3·34 g., 96%) had m. p. 208—209° (decomp.). A sample, recrystallised from aqueous *NN*-dimethylformamide, had m. p. 211° (decomp.) $[\alpha]_D^{20}$ +10·2° (*c* 2 in 0·1N-NaOH) (Found: N, 4·1; S, 9·4. $C_{18}H_{21}NO_4S$ requires N, 4·0; S, 9·2%).

S-3,3′,4,4′-*Tetramethoxydiphenylmethyl*-L-*cysteine*.—This compound, prepared in virtually quantitative yield from L-cysteine toluene-*p*-sulphonate and 3,3′,4,4′-tetramethoxydiphenylmethyl chloride by the method described above, had m. p. 207° (decomp.). After recrystallisation from aqueous *NN*-dimethylformamide, a sample had m. p. 208—210° (decomp.), $[\alpha]_D^{20}$+10·7° (*c* 2 in DMF) (Found: N, 3·55; S, 8·0. $C_{20}H_{25}NO_6S$ requires N, 3·4; S, 7·9%).

Removal of the S-*Protecting Groups from* S-*Diphenylmethyl*-L-*cysteine Derivatives*.—(*a*) *With hydrogen bromide in glacial acetic acid*. S-4,4′-Dimethoxydiphenylmethyl-L-cysteine (0·347 g., 1 mmole) was dissolved, by shaking, in a 5·5N-solution of hydrogen bromide in glacial acetic acid (4 ml.) maintained between 50 and 60°. After 30 min. the solution was added to saturated sodium acetate solution (20 ml.) and the insoluble material was removed and washed well with water. Titration of the combined filtrate and washings with 0·1N-iodine solution showed that quantitative cleavage of the dimethoxydiphenylmethyl group had occurred. When the reaction was repeated in the presence of 5 equivalents of 4,4′-dimethoxydiphenylmethyl chloride, the resulting thiol titration corresponded to 63% cleavage. In a similar experiment, the addition of 5 equivalents of 3,3′,4,4′-tetramethoxydiphenylmethyl chloride resulted in the thiol titration being reduced to 40%. At room temperature, with 10 equivalents of hydrogen bromide and a reaction time of 1 hr., 12% cleavage occurred.

S-3,3′,4,4′-Tetramethoxydiphenylmethyl-L-cysteine, in a series of experiments similar to those described above, gave the following degrees of cleavage: *ca.* 100% (30 min. at 50—60°), 44% (30 min. at 50—60° in the presence of 5 equivalents of 4,4′-dimethoxydiphenylmethyl chloride), 59% (30 min. at 50—60° in the presence of 3·7 equivalents of 3,3′,4,4′-tetramethoxydiphenylmethyl chloride), 14% (1 hr. at room temperature using 10 equivalents of hydrogen bromide). The latter value was also obtained after a reaction time of 1 min.; in both cases the presence of 5 equivalents of 3,3′,4,4′-tetramethoxydiphenylmethyl chloride reduced the values to <1%.

The *S*-diphenylmethyl group was cleaved from *S*-diphenylmethyl-L-cysteine to an extent of 9% using 10 equivalents of hydrogen bromide at room temperature for 1 hr.

(*b*) *With trifluoroacetic acid–phenol*. S-4,4′-Dimethoxydiphenylmethyl-L-cysteine (0·347 g., 1 mmole) and phenol (0·2 g.) were heated in refluxing trifluoroacetic acid (1·75 ml.) for 30 min. After the acid had been evaporated, the residue was treated with water (20 ml.) and the aqueous solution was extracted with ether (3 × 5 ml.). Titration of the aqueous solution with 0·1N-iodine showed that quantitative cleavage of the dimethoxydiphenylmethyl group had occurred.

S-3,3′,4,4′-Tetramethoxydiphenylmethyl-L-cysteine was also cleaved quantitatively in this way.

Alkylation of L-Cysteine with 4,4′-Dimethoxydiphenylmethyl Chloride in the Presence of Hydrogen Bromide.—L-Cysteine toluene-*p*-sulphonate (1·46 g., 5 mmoles) and 4,4′-dimethoxydiphenylmethyl chloride (6·56 g., 25 mmoles) were shaken with a 2·75N-solution of hydrogen bromide in glacial acetic acid (40 ml.) at 50° for 30 min. The crude *S*-4,4′-dimethoxydiphenylmethyl-L-cysteine (0·505 g., 29%), m. p. 208—210° (decomp.), was isolated as described earlier. The aqueous filtrate and washings, after removal of the initial crude product, consumed 18·7 ml. of 0·1N-iodine solution, indicating the presence of 37·6% unreacted cysteine.

S-*Diphenylmethyl-L-cysteine.*—(*a*) L-Cysteine toluene-*p*-sulphonate (1·46 g., 5 mmoles) and diphenylmethyl chloride (5·13 g., 25 mmoles) were stirred with a 5·5N-solution of hydrogen bromide in glacial acetic acid (20 ml.) for 2·5 days at room temperature. The solution was then poured into saturated sodium acetate solution (230 ml.), the precipitate was collected, washed with water and with acetone, and was finally suspended for 5 min. in boiling acetone. The insoluble, chromatographically pure cysteine derivative (1·15 g., 80%) had m. p. 203—204° (decomp.) (lit.,[9] 202—203°).

(*b*) The above reaction was repeated at 50° for a period of 1 hr. The crude product (1·27 g., 88%), m. p. 190—192° (decomp.) was dissolved in hot aqueous acetonitrile (2 : 8, 20 ml.) by the gradual addition of concentrated hydrochloric acid. The addition of pyridine then caused the pure compound (0·83 g., 58%) to separate in the form of micro-needles, m. p. 210° (decomp.).

(*c*) The reaction was repeated as in (*a*) using a 1·4N-solution of hydrogen bromide in glacial acetic acid (10 ml.) at 50° and a reaction time of 2·5 hr. The crude product was purified as described in (*b*), above, to give material (1·10 g., 77%), m. p. 205—206° (decomp.).

(*d*) A preparation similar to that described for *S*-4,4′-dimethoxydiphenylmethyl-L-cysteine using L-cysteine toluene-*p*-sulphonate and diphenylmethyl chloride in suspension in *NN*-dimethylformamide at room temperature gave a crude product, m. p. 204—206°, in 9% yield.

N-*Benzyloxycarbonyl*-S-4,4′-*dimethoxydiphenylmethyl-L-cysteine.*—*S*-4,4′-Dimethoxydiphenylmethyl-L-cysteine (6·9 g., 20 mmoles) was dissolved in 0·5N-sodium hydroxide (40 ml.) and the solution was stirred at 2—5° whilst benzyloxycarbonyl chloride (4 ml., 30 mmoles) and N-sodium hydroxide (30 ml.) were added simultaneously in 4 batches over 20 min. Despite efficient stirring (Vibromix), the mixture became very thick and, after the last addition, water (160 ml.) was added to keep it mobile. After 30 min. at room temperature, the reaction mixture was extracted with ether (2 × 25 ml.) and the aqueous phase acidified to pH 2 with dilute sulphuric acid. The product was extracted into ether (2 × 40 ml.) and the extract washed with aqueous sodium acetate (5%; 1 × 10 ml.) and water (2 × 10 ml.). Addition of cyclohexylamine to the dried (Na_2SO_4) ethereal solution resulted in the immediate deposition of crystals. After 20 min. these were filtered off, washed with ether, and dried to give the NS-*protected cysteine cyclohexylamine salt* (9·5 g., 83·5%), m. p. 141—144·5°.

For analysis, a small sample of this compound was recrystallised from ethanol with 88% recovery, m. p. 141—145°, $[\alpha]_D^{17}$ −6·47° (*c* 0·6 in MeOH). Further recrystallisation from warm chloroform–ether gave no change in properties although the appearance (fine needles) of the compound was improved (Found: C, 65·9; H, 6·9; N, 4·5. $C_{32}H_{40}N_2O_6S$ requires C, 66·2; H, 6·9; N, 4·8%).

The dicyclohexylamine salt of *N*-benzyloxycarbonyl-*S*-4,4′-dimethoxydiphenylmethyl-L-cysteine and the free acid (see below) could only be obtained as oils.

p-*Nitrophenyl* N-*Benzyloxycarbonyl*-S-4,4′-*dimethoxydiphenylmethyl*-L-*cysteinate.*—Cyclohexylammonium *N*-benzyloxycarbonyl-*S*-4,4′-dimethoxydiphenylmethyl-L-cysteinate (18 g., 31 mmoles) was dissolved in warm aqueous methanol and N-hydrochloric acid (35 ml.) was added. Evaporation of the methanol under reduced pressure resulted in the precipitation of an oil. This was extracted into ether and the ethereal solution washed with water and dried (Na_2SO_4) to give oily *N*-benzyloxycarbonyl-*S*-4,4′-dimethoxydiphenylmethyl-L-cysteine (14·6 g., 97%).

The oil was dissolved in ethyl acetate (150 ml.) and *p*-nitrophenol (5·15 g., 37 mmoles) was added, followed, after the reaction mixture had been cooled to 0°, by *NN*′-dicyclohexylcarbodiimide (6·3 g., 30 mmoles). After being stirred at 0° for several hours, the mixture was left at room temperature overnight. The precipitate of *NN*′-dicyclohexylurea was filtered off and washed with ethyl acetate, and the combined ethyl acetate fractions were evaporated under

reduced pressure. An oily product was formed but, when this was dissolved in boiling ethanol, it started to crystallise almost immediately. The crystals were filtered off after 3 hr. at 0°, washed with cold ethanol, and dried to give the NS-*diprotected cysteine* p-*nitrophenyl ester* (16·7 g., 93%), m. p. 121—124°.

A small sample recrystallised from absolute ethanol (75% recovery) had m. p. 126—127·5°, $[\alpha]_D^{19}$ −15·0° (*c* 0·9 in DMF) (Found: C, 64·0; H, 5·1; N, 4·4. $C_{32}H_{30}N_2O_8S$ requires C, 63·8; H, 5·0; N, 4·7%).

N-*t-Butyloxycarbonyl*-S-4,4′-*dimethoxydiphenylmethyl*-L-*cysteine*.—A mixture of *S*-4,4′-dimethoxydiphenylmethyl-L-cysteine (6·94 g., 20 mmoles), t-butyl *p*-nitrophenyl carbonate (7·18 g., 30 mmoles), sodium carbonate (5·3 g., 50 mmoles), water (20 ml.), and t-butyl alcohol (30 ml.) was refluxed on a steam-bath for 30 min. Nitrogen was bubbled through the warm solution to remove t-butyl alcohol and the cooled solution was afterwards diluted to a total volume of 150 ml. A layer of ether was added and the mixture was acidified to pH 5·8 with saturated aqueous citric acid and extracted thoroughly with more ether (4 × 10 ml.). The combined ethereal fractions were dried and evaporated and the residual oil was dissolved in dilute aqueous sodium carbonate (500 ml.). Saturated aqueous citric acid was added to adjust the solution to pH 7·5 and the mixture was extracted thoroughly with ether (4 × 25 ml.). Finally, the aqueous phase was adjusted to pH 2 by the addition of more citric acid and the product was extracted into ether (4 × 25 ml.). The washed ether was dried (Na_2SO_4), concentrated to 25 ml., and cyclohexylamine was added dropwise. Crystals began to form almost immediately and were removed by filtration in two batches, washed with ether, and air-dried. The combined colourless material (6·66 g., 61%) had m. p. 156—158°, $[\alpha]_D^{19}$ +12·3° (*c* 0·29 in DMF). A third batch (1·7 g.), obtained when cyclohexylamine was present in excess, was yellow and had m. p. 85—105°. This material was not processed further.

The material from the first two batches was recrystallised from absolute ethanol (85% recovery) and finally from chloroform–ether (80% recovery) to give *cyclohexylammonium* N-*t-butyloxycarbonyl*-S-4,4′-*dimethoxydiphenylmethyl*-L-*cysteinate*, m. p. 157—159°, $[\alpha]_D^{19}$ +16·3° (*c* 1·8 in DMF) (Found: C, 63·6; H, 7·9; N, 5·0. $C_{23}H_{29}NO_6S,C_6H_{13}N$ requires C, 63·7; H, 7·7; N, 5·1%).

p-*Nitrophenyl* N-*t-Butyloxycarbonyl*-S-4,4′-*dimethoxydiphenylmethyl*-L-*cysteinate*.—Cyclohexylammonium *N*-t-butyloxycarbonyl-S-4,4′-dimethoxydiphenylmethyl-L-cysteinate (1·09 g., 2 mmoles) was dissolved in 66% aqueous ethanol (15 ml.) and 2N-hydrochloric acid (0·95 ml.) was added followed by water (30 ml.). The mixture was extracted with ether (3 × 30 ml.) and the ethereal extracts were dried (Na_2SO_4) and evaporated under reduced pressure. Ethyl acetate (15 ml.) was added to the resulting oil followed by *p*-nitrophenol (0·306 g., 2·2 mmoles) and, after the clear solution had cooled to 0°, by *NN*′-dicyclohexylcarbodi-imide (0·412 g., 2 mmoles). The reaction mixture was allowed to warm slowly to room temperature and was stirred at room temperature overnight. *NN*′-Dicyclohexylurea was filtered off and washed with ethyl acetate (5 ml.) and the combined filtrates were evaporated under reduced pressure. The residual oil was dissolved in boiling absolute ethanol (10 ml.) and crystals started to appear almost immediately. After 30 min. at room temperature, the crystals were filtered off, washed with a small amount of ethanol, and air-dried to give the NS-*protected* p-*nitrophenyl cysteinate* (0·82 g., 72%), m. p. 107—110°.

A sample recrystallised from ethanol had m. p. 111—112·5°, $[\alpha]_D^{19}$ −21·85° (*c* 0·9 in DMF) (Found: C, 61·2; H, 5·6; N, 4·8. $C_{29}H_{32}N_2O_8S$ requires C, 61·3; H, 5·7; N, 4·9%).

Methyl N-*Benzyloxycarbonyl*-S-4,4′-*dimethoxydiphenylmethyl*-L-*cysteinyl*-L-*tyrosinate*.—Cyclohexylammonium *N*-benzyloxycarbonyl-*S*-4,4′-dimethoxydiphenylmethyl-L-cysteinate (18 g., 31 mmoles) was converted into the free acid (15 g., 100·5%) in the form of an oil as described above. This oil was dissolved in *NN*-dimethylformamide (40 ml.) and crystalline methyl L-tyrosinate (5·66 g., 29 mmoles) was added, followed, when the mixture had been cooled to 0°, by *NN*′-dicyclohexylcarbodi-imide (6·38 g., 31 mmoles).

The reaction mixture was stirred at 0° overnight and a few drops of acetic acid were then added followed by ether (200 ml.). *NN*′-Dicyclohexylurea was filtered off and washed with ether (20 ml.) and the combined ether fractions were washed with aqueous sodium carbonate (2 × 20 ml.; 5%), dilute acetic acid (2 × 20 ml.; 10%), and water (2 × 20 ml.), then dried (Na_2SO_4), and evaporated. More *NN*′-dicyclohexylurea separated out and was removed by filtration from the resultant oil after it had been diluted with ether. Addition of n-hexane to the stirred ethereal solution resulted in the separation of an oil which, after prolonged trituration

with n-hexane, gave the *protected dipeptide ester* as an amorphous, white solid (17·5 g., 91·5%), m. p. 82—84° (decomp.), $[\alpha]_D^{17.5}$ −15·5° (*c* 0·8 in MeOH).

A sample reprecipitated from ether-n-hexane had m. p. 81—83° (decomp.), $[\alpha]_D^{17.5}$ −12·45° (*c* 0·6 in MeOH) (Found: C, 65·9; H, 5·8; N, 4·1. $C_{36}H_{38}N_2O_8S$ requires C, 65·6; H, 5·8; N, 4·3%).

N-*Benzyloxycarbonyl*-S-4,4′-*dimethoxydiphenylmethyl*-L-*cysteinyl*-L-*tyrosine*. — Methyl *N*-benzyloxycarbonyl-*S*-4,4′-dimethoxydiphenylmethyl-L-cysteinyl-L-tyrosinate (10 g., 15·2 mmoles) was dissolved in acetone (250 ml.) and N-sodium hydroxide (22·8 ml.) was added slowly to the vigorously stirred solution. Stirring was continued for 5 hr. at room temperature prior to the addition of 2N-hydrochloric acid (12 ml.). The solvent was evaporated under reduced pressure and the residual oil was dissolved in ethyl acetate (200 ml.). The water-washed (2 × 20 ml.) and dried (Na_2SO_4) solution was poured slowly into light petroleum (500 ml.) with efficient stirring and the amorphous precipitate which formed was filtered off and dried in air (8·6 g., 88%), m. p. 144—147° (shrinks 132°).

A sample reprecipitated from ethyl acetate–light petroleum had m. p. 149—150°, $[\alpha]_D^{18.5}$ −8·25° (*c* 0·49 in MeOH) (Found: C, 65·4; H, 5·55; N, 4·2. $C_{35}H_{36}N_2O_8S$ requires C, 65·2; H, 5·6; N, 4·35%).

N-*Benzyloxycarbonyl*-S-4,4′-*dimethoxydiphenylmethyl*-L-*cysteinyl*-L-*tyrosyl*-L-*isoleucyl*-L-*glutaminyl*-L-*asparagine*.—*N*-Benzyloxycarbonyl-*S*-4,4′-dimethoxydiphenylmethyl-L-cysteinyl-L-tyrosine (2·7 g., 4·2 mmoles) was dissolved in dry tetrahydrofuran and to the stirred solution at −15°, triethylamine (0·57 ml., 4·1 mmoles) was added, followed by isobutyl chloroformate (0·536 ml., 4·1 mmoles). The reaction mixture was allowed to warm to −5° (bath) over 20 min. and was then cooled to −15° prior to the addition of a solution of L-isoleucyl-L-glutaminyl-L-asparagine (1·6 g., 4·1 mmoles) in water (10 ml.) containing triethylamine (0·57 ml., 4·1 mmoles). After 1 hr. at 0°, the reaction mixture was allowed to warm slowly to room temperature and was stirred at room temperature overnight.

The reaction mixture was finally acidified (pH 1) with 3N-hydrochloric acid and poured into water (300 ml.). Evaporation of the tetrahydrofuran resulted in the formation of a semi-gelatinous precipitate which was filtered off, washed with water (until washings pH 4), and dried *in vacuo* over P_2O_5 to give an amorphous, off-white solid (3·5 g.). Repeated boiling with ethyl acetate served to remove the dipeptide impurity. The *pentapeptide derivative* which remained (2·66 g., 65%) had m. p. 238—240° (decomp.) $[\alpha]_D^{20}$ −23·69° (*c* 0·8 in DMF).

A sample of this material was dissolved in boiling *NN*-dimethylformamide and n-hexane was added to the filtered solution, followed by ethyl acetate. The oily precipitate which resulted was rubbed until a mainly particulate, though gelatinous, precipitate formed which could be filtered easily (addition of ethyl acetate directly to the *NN*-dimethylformamide solution resulted in the formation of an unmanageable gel). The reprecipitated material had m. p. 240—244° (decomp.), $[\alpha]_D^{17}$ −21·5° (*c* 0·72 in DMF) (Found: C, 59·8; H, 6·3; N, 9·65. $C_{50}H_{61}O_{13}N_7S$ requires C, 60·1; H, 6·1; N, 9·8%).

N-*Benzyloxycarbonyl*-S-4,4′-*dimethoxydiphenylmethyl*-L-*cysteinyl*-L-*prolyl*-L-*leucylglycine Amide*.—L-Prolyl-L-leucylglycine amide was obtained as an oil by the hydrogenolysis of the *N*-benzyloxycarbonyl tripeptide amide (2·09 g., 5 mmoles) over 10% palladised charcoal. The oil was dissolved in *NN*-dimethylformamide (2·5 ml.) and *p*-nitrophenyl *N*-benzyloxycarbonyl-*S*-4,4′-dimethoxydiphenylmethyl-L-cysteinate (2·95 g., 4·9 mmoles) was added to the clear solution. After 2 days, ethyl acetate (4 ml.) was added to the thick slurry which had formed and the mixture was stored at 0° for 1 day. The filtered precipitate was washed with more ethyl acetate (12 ml.) and dried *in vacuo* to give the *tetrapeptide derivative* (2·4 g., 64%), m. p. 156—158°. Evaporation of the ethyl acetate from the filtrate followed by the addition of ether to the *NN*-dimethylformamide solution resulted in the formation of a further quantity of solid matter (0·92 g., 24%), m. p. 144—146°. The combined material, recrystallised from methanol with the aid of decolorising charcoal, had m. p. 154—157° (2·45 g., 65·6%), $[\alpha]_D^{20}$ −37·25° (*c* 0·49 in DMF). A sample recrystallised several times in the same manner had m. p. 156—158°, $[\alpha]_D^{19}$ −37·89° (*c* 0·8 in DMF) (Found: C, 62·4; H, 6·4; N, 9·3. $C_{39}H_{49}N_5O_8S$ requires C, 62·6; H, 6·6; N, 9·4%).

N-*t*-*Butyloxycarbonyl*-S-4,4′-*dimethoxydiphenylmethyl*-L-*cysteinyl*-L-*prolyl*-L-*leucylglycine Amide*.—*N*-Benzyloxycarbonyl-L-prolyl-L-leucylglycine amide (0·209 g., 0·5 mmole) was converted into the free base by hydrogenolysis and the resultant oil was dissolved in *NN*-dimethylformamide (2 ml.). *p*-Nitrophenyl *N*-t-butyloxycarbonyl-*S*-4,4′-dimethoxydiphenylmethyl-L-cysteinate (0·284 g., 0·5 mmole) was added and the reaction mixture was stirred at room

temperature for 2 days. After the addition of ether (40 ml.) the mixture was left at 0° for 1 day. The precipitate was subsequently filtered off, washed with ether, and air-dried to yield an amorphous solid (0·27 g., 76%), m. p. 134—139°. This material was recrystallised from aqueous methanol with the aid of decolorising charcoal to yield the *protected tetrapeptide* (1·94 g., 54%), m. p. 167—169°, $[\alpha]_D^{19}$ −35·7° (*c* 0·53 in DMF) (Found: C, 60·3; H, 7·5; N, 9·8. $C_{36}H_{51}N_5O_8S$ requires C, 60·6; H, 7·2; N, 9·8%).

Removal of the N-*Protecting Group from the Tetrapeptide Derivatives.*—(*a*) N-*Benzyloxycarbonyl*-S-4,4′-*dimethoxydiphenylmethyl*-L-*cysteinyl*-L-*prolyl*-L-*leucylglycine amide.* (i) The protected tetrapeptide (1 g., 1·34 mmoles) was stirred in a solution of hydrogen bromide in glacial acetic acid (5 ml., 45% HBr, w/v) at room temperature for 1 hr. Dry ether (100 ml.) was added to the mixture and the supernatant liquid was poured off the oil which precipitated. The oil was triturated several times with ether and was finally dissolved in ethanol (10 ml.). A small amount of insoluble matter was removed by filtration and the filtrate was passed quickly through a column (4½ × ½ in.) of Amberlite IRA-410 in the hydroxyl cycle. The tetrapeptide free base was eluted with more ethanol (200 ml.) and was obtained as an oil (0·74 g., 90%) by evaporation of the solvent. Thin-layer chromatography revealed that this substance was predominantly one component which was ninhydrin-positive and which gave a pink colour on exposure to hydrogen chloride vapour. However, other components which did not fulfil one or other of these criteria were also present.

(ii) A reaction carried out as above, but in the presence of 4,4′-dimethoxydiphenylmethyl chloride (2·62 g., 10 mmoles), gave a similar product. In this case, the other components of the mixture were still present, but in considerably reduced amounts.

(*b*) N-*t*-*Butyloxycarbonyl*-S-4,4′-*dimethoxydiphenylmethyl*-L-*cysteinyl*-L-*prolyl*-L-*leucylglycine amide.* The protected tetrapeptide (0·1 g., 0·14 mmole) and 4,4′-dimethoxydiphenylmethyl chloride (0·8 g., 0·34 mmole) were dissolved in glacial acetic acid (2 ml.) and the solution was heated in a boiling-water bath for 2 min. Evaporation of the acetic acid under reduced pressure (to *ca.* 0·2 ml.) was followed by the addition of dry ether. The resulting precipitate was collected by centrifugation and converted to the free base (90%) as described above. This preparation was virtually homogeneous as determined by thin-layer chromatography.

Formation of the Protected Nonapeptide and Removal of the Protecting Groups.—The *NS*-protected pentapeptide (0·1 g., 0·1 mmole) was dissolved in *NN*-dimethylformamide (0·5 ml.) containing triethylamine (0·014 ml., 0·1 mmole) and to the stirred solution at 0° was added Reagent K [19] (0·025 g., 0·1 mmole). After 2 hr., a solution of the tetrapeptide free base (from 0·1 g. *N*-t-butoxycarbonyl derivative) in *NN*-dimethylformamide (1 ml.) was added and the reaction mixture was allowed to warm over several hours to room temperature. After 2 days, the mixture was poured into water (200 ml.) and the product separated by centrifugation. It was washed well with water and dried *in vacuo* over calcium chloride to give a fine powder, homogeneous by thin-layer chromatography (0·12 g.), m. p. (vague) fused 180—200°. The protecting groups were removed from this product as described below:

(*a*) A sample (30 mg.) was reduced by sodium in liquid ammonia and the resulting dithiol was oxidised in the usual way. The avian depressor activity of the resulting solution was equivalent to 75 units of oxytocin/mg. of the protected nonapeptide.

(*b*) A sample (19 mg.) was dissolved in acetic acid (0·5 ml.) and a solution of hydrogen bromide in acetic acid (0·3 ml. of 45% w/v) was added. After 25 min., the solvent was evaporated at room temperature and the residue triturated with dioxan. The dioxan was evaporated at room temperature and this treatment was repeated several times to remove traces of hydrogen bromide. Finally, the residue was dissolved in trifluoroacetic acid (0·8 ml.) containing phenol (0·2 g.) and the solution was refluxed for 20 min. Evaporation of the trifluoroacetic acid and further treatment with dioxan gave a coloured residue which was almost entirely soluble in water (50 ml.). Aeration in the usual way gave a solution possessing avian depressor activity equivalent to ~30 units of oxytocin/mg. of the protected nonapeptide.

(*c*) A sample (10 mg.) in solution in trifluoroacetic acid (1 ml.) containing phenol (0·2 g.) was refluxed for 30 min. Following the removal of the trifluoroacetic acid, the residue was dissolved in water and aerated at neutral pH in the usual way. The resulting solution possessed avian depressor activity equivalent to ~20 units of oxytocin/mg. of the protected nonapeptide.

(*d*) A sample (11 mg.) was dissolved in acetic acid and a solution of hydrogen bromide in acetic acid was added as described above. The resulting mixture was heated at 50° for 1 hr. and the hydrogen bromide and acetic acid removed in the usual way. A coloured residue

resulted which was dissolved in water (50 ml.). After aeration, the avian depressor activity of this solution was equivalent to 2 units of oxytocin/mg. of the protected nonapeptide.

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