New Methods in Peptide Synthesis. Part III.[1] Protection of Carboxyl Group [2]

By G. C. Stelakatos, A. Paganou, and L. Zervas

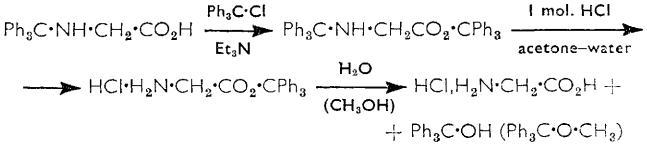
For the protection of the carboxyl group of amino-acids during peptide synthesis, the acid-labile diphenylmethyl ester group was used. *N*-Trityl- (*i.e.*, triphenylmethyl), *N*-formyl-, or *N*-*o*-nitrophenylsulphenyl-amino-acid diphenylmethyl esters were converted by known methods into the corresponding ester hydrochlorides. The deblocking of the carboxyl group was accomplished either by the action of dilute solutions of hydrogen chloride or hydrogen bromide in nitromethane, by trifluoroacetic acid, or by catalytic hydrogenolysis.

The acid-stable phenacyl ester group permitted the preparation of the corresponding amino-acid ester hydrobromides by treating *N*-benzyloxycarbonylamino-acid phenacyl esters with hydrogen bromide in acetic acid. Easy alkyl–oxygen ester fission was brought about by sodium thiophenoxide.

In continuation of our attempts to develop more refined methods for use in peptide synthesis, the problem of the protection of the carboxyl group of α-amino-acids has been investigated.

The protecting groups more commonly used so far have been limited to the methyl,[3] ethyl,[3] benzyl,[3,4] or t-butyl [5] ester groups. The use of methyl or ethyl esters has the advantage of permitting the lengthening of the peptide chain at the amino-end regardless of the method used for the temporary protection of the amino-group. Nevertheless, the main disadvantage of such a method is that hydrolysis of esters of long peptide chains, or of peptide esters bearing alkali-labile protecting groups in their side-chain is difficult. Benzyl esters are better because the carboxyl group can be unmasked by catalytic hydrogenolysis,[3,4] provided that the peptide chain is not too long, and does not include sulphur-containing amino-acids. In the latter case, t-butyl esters are more valuable, since the t-butyl group can be easily removed by hydrogen chloride in non-polar solvents, or by treatment for a few minutes with trifluoroacetic acid.[6] However, the use of t-butyl esters is restricted by the nature of the *N*-protecting group; since benzyloxycarbonyl or other groups removable by hydrogenolysis are usually employed to protect nitrogen, disadvantages similar to those described for the benzyl ester group are again encountered. However, after the introduction of the sulphenyl method [1,7] for the *N*-protection of amino-acids, the t-butyl esters became, in our opinion, more important, since a "step by step" lengthening of the polypeptide chain at the amino-end proceeds readily without any danger to the t-butyl ester group.

An ideal protection of the carboxyl group could be offered by the trityl (triphenylmethyl) group,[2a] because trityl esters are sensitive towards alcohols.[8] For this reason, we tried to prepare amino-acid trityl esters by removing selectively the *N*-trityl group from the corresponding *N*-tritylamino-acid trityl esters,* but unfortunately only the glycine derivative could be so obtained. This ester hydrochloride (I) is decomposed almost quantitatively in methanol or water–dioxan † solutions, after standing at room temperature for many hours; methyl triphenylmethyl ether and triphenylmethyl alcohol are formed, respectively, besides glycine hydrochloride. The rate of cleavage of the trityl ester group is accelerated by raising the temperature; heating for 1 min. at about 100° is sufficient quantitatively to remove the ester group.



* During the preparation of tritylamino-acids we have observed the formation of trityl esters, *e.g.*, trityl-L-alanine trityl ester; cf. ref. 2*a*. Similar observations have been reported by H. Block and M. E. Cox,[9] as well as by G. Amiard and R. Heymes.[10]

† A. Vollmar and M. S. Dunn (cf. ref. 5*b*) have reported that aqueous solutions of the hydrochlorides of amino-acid t-butyl esters become acid after several hours. Alkenes and t-butyl alcohol are among the products.

[1] Part II, L. Zervas and Ch. Hamalidis, *J. Amer. Chem. Soc.*, 1965, **87**, 99.

[2] Presented in part at the Fifth and Sixth European Peptide Symposia: (*a*) E. Gazis, B. Bezas, G. C. Stelakatos, and L. Zervas, "Peptides: Proceedings of the Fifth European Symposium," Oxford, 1962, ed. G. T. Young, Pergamon Press, Oxford, 1963, p. 17; and (*b*) E. Gazis, D. Borovas, Ch. Hamalidis, G. C. Stelakatos, and L. Zervas, "Peptides: Proceedings of the Sixth European Symposium," Athens, 1963, ed. L. Zervas, Pergamon Press, Oxford, 1966, p. 107; (*c*) cf. ref. 30*c* in the review by H. D. Law in "Progress in Medicinal Chemistry," ed. G. P. Ellis and G. B. West, Butterworths Scientific Publications, London, 1965, vol. IV, p. 86.

[3] A detailed account for the preparation and use of amino-acid esters is provided by J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," John Wiley and Sons, Inc., New York, 1961.

[4] M. Bergmann, L. Zervas, and L. Salzmann, *Ber.*, 1933, **66**, 1288.

[5] (*a*) R. W. Roeske, *Chem. and Ind.*, 1959, 1121; G. W. Anderson and F. M. Callahan, *J. Amer. Chem. Soc.*, 1960, **82**, 3359; R. Schwyzer and H. Kappeler, *Helv. Chim. Acta*, 1961, **44**, 1991; F. M. Callahan, G. W. Anderson, R. Paul, and J. E. Zimmerman, *J. Amer. Chem. Soc.*, 1963, **85**, 201; R. Roeske, *J. Org. Chem.*, 1963, **28**, 1251; (*b*) A. Vollmar and M. S. Dunn, *ibid.*, 1960, **25**, 387.

[6] H. Kappeler and R. Schwyzer, *Helv. Chim. Acta*, 1961, **44**, 1136.

[7] L. Zervas, D. Borovas, and E. Gazis, *J. Amer. Chem. Soc.*, 1963, **85**, 3660.

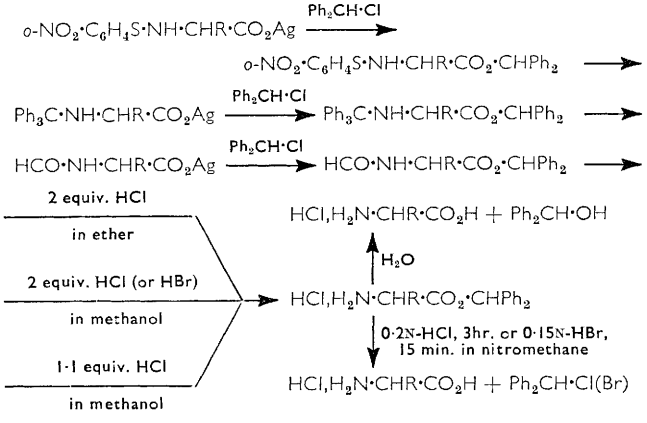
[8] M. Gomberg and G. T. Davis, *Ber.*, 1903, **36**, 3924; G. S. Hammond and J. T. Rudesill, *J. Amer. Chem. Soc.*, 1950, **72**, 2769; C. A. Bunton and A. Konasiewicz, *J. Chem. Soc.*, 1955, 1354; K. D. Berlin, L. H. Gower, J. W. White, D. E. Gibbs, and G. P. Sturm, *J. Org. Chem.*, 1962, **27**, 3595.

[9] H. Block and M. E. Cox, "Peptides: Proceedings of the Fifth European Symposium," Oxford, 1962, ed. G. T. Young, Pergamon Press, Oxford, 1963, p. 83.

[10] G. Amiard and R. Heymes, *Bull. Soc. chim. France*, 1957, 1373.

Another mode of protection of the carboxyl group during peptide synthesis is offered by its transformation to the diphenylmethyl ester group,[2b,c,11-14] since these esters are easily hydrogenolysed to the free acids and diphenylmethane [2b,11-15] as expected from the analogous behaviour of *O*-benzyl and *O*-tritryl groups. Amino-acid diphenylmethyl esters have been prepared *via* *N*-protected amino-acids by esterification either indirectly through the corresponding silver salts,[2b] or directly with diphenylmethanol [13] or diphenyldiazomethane.[13] A simplified method for the preparation of diphenylmethyl esters of amino-acids and peptides has been published recently;[14] aromatic sulphonic acid salts of amino-acids and peptides are easily esterified by diphenyldiazomethane.

In our work, the diphenylmethyl esters of amino-acids (*e.g.*, of glycine, L-alanine, L-valine, L-asparagine) are prepared either by the interaction of the silver salts of *N*-*o*-nitrophenylsulphenyl-,[1,7] *N*-trityl-,[16] or *N*-formyl-amino-acids with diphenylmethyl chloride,[2b] or by the action of diphenyldiazomethane on *N*-substituted amino-acids,[11a] followed (in each case) by the



removal of the *N*-protecting group by an appropriate method, so that the ester group is left intact. The carboxyl-protecting diphenylmethyl group can subsequently be removed by hydrogenolysis.[2b,11-15] The diphenylmethyl esters can also be cleaved by hydrogen chloride,[2b,13,17] or by the action of approximately 0·15N-hydrogen bromide in nitromethane, ethyl acetate, etc.[2b] This cleavage by these halogen acids was attractive from the point of view of finding conditions for the selective removal of the diphenylmethyl group in the presence of t-butyl esters. In our experience, the diphenylmethyl esters of *N*-benzyloxycarbonyl-L-proline and -L-valine hydrochloride are cleaved almost quantitatively after a three-hour treatment at 30° with three equivalents of a very dilute solution (0·2N) of hydrogen chloride in nitromethane. The cleavage of *N*-benzyloxycarbonyl-dipeptide diphenylmethyl esters requires longer, so that the carboxyl group of *N*-benzyloxycarbonyl-L-valyl-L-valine diphenylmethyl ester is deblocked after a fourteen-hour treatment at 30° with 0·2N-hydrogen chloride solution in nitromethane. It is noteworthy that the cleavage with halogen acids proceeds much slower in ethyl acetate than in nitromethane,* as is also the case with t-butyl esters. The similarity between these two ester groups is further demonstrated by the sensitivity of diphenylmethyl esters towards trifluoroacetic acid. *N*-Benzyloxycarbonylamino-acid or dipeptide diphenylmethyl esters are converted into the corresponding carboxyl-free derivatives by the action of trifluoroacetic acid at 20° within 30 min. Therefore, the selective removal of one of these two ester groups in the presence of the other proved unfeasible in acid solutions.

Amino-acid diphenylmethyl ester hydrochlorides behave in water solutions similarly to glycine trityl ester hydrochloride, *i.e.*, they are hydrolysed almost quantitatively during several hours at room temperature.[2b] The pH of these aqueous solutions drops slowly [19] and diphenylmethanol soon starts to separate. The rate of hydrolysis is increased by raising the temperature, so that upon heating an aqueous solution of these ester hydrochlorides at about 100° for 3 min., diphenylmethanol is formed almost quantitatively.

The usefulness of diphenylmethyl esters in peptide chemistry can be illustrated by the following synthesis of a valine peptide. This example was chosen because the hydrolysis of valine esters presents some difficulties. Upon coupling L-valine diphenylmethyl ester with the appropriate *N*-protected valine derivatives by the *NN'*-dicyclohexylcarbodi-imide method,[20] the dipeptide diphenylmethyl esters of *N*-*o*-nitrophenylsulphenyl-L-valyl-L-valine (II) and *N*-benzyloxycarbonyl-L-valyl-L-valine (III) were obtained in good yield. Hydrogen chloride (2—3 equiv.) added to a solution of (II) splits

* Nitromethane has a higher dielectric constant than ethyl acetate. To this physical property of nitromethane is also attributed the acceleration of the solvolysis of cyclohexylphenylcarbinyl hydrogen phthalate by carboxylic acids.[18]

[11] (*a*) M. Bethell, D. B. Bigley, and G. W. Kenner, *Chem. and Ind.*, 1963, 653; (*b*) J. S. Morley, "Peptides: Proceedings of the Sixth European Symposium," Athens, 1963, ed. L. Zervas, Pergamon Press, Oxford, 1966, p. 351.

[12] Cf. ref. 7 in L. Zervas, A. Cosmatos, and P. Diamantis, *Experientia*, 1965, **21**, 5.

[13] R. G. Hiskey and J. B. Adams, jun., *J. Amer. Chem. Soc.*, 1965, **87**, 3969.

[14] A. A. Aboderin, G. R. Delpierre, and J. S. Fruton, *J. Amer. Chem. Soc.*, 1965, **87**, 5469.

[15] E. Hardegger, Z. E. Heweibi, and F. G. Robinet, *Helv. Chim. Acta*, 1948, **31**, 439; F. Bernoulli, H. Linde, and K. Meger, *ibid.*, 1962, **45**, 240; R. J. Morris and D. L. Tankersley, *J. Org. Chem.*, 1963, **28**, 240; E. Haslam, R. D. Haworth, and D. A. Lawton, *J. Chem. Soc.*, 1963, 2173.

[16] (*a*) 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; (*b*) cf. refs. 17—19 in L. Zervas, D. Borovas, and E. Gazis, *ibid.*, 1963, **85**, 3660.

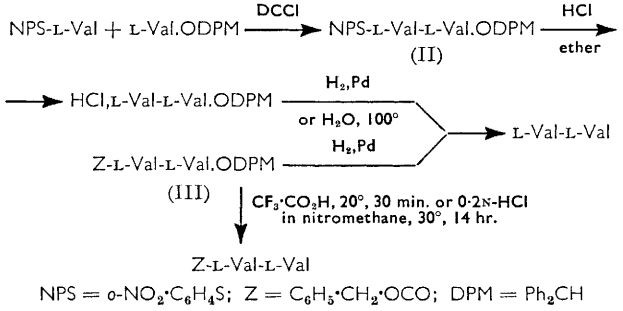
[17] A. C. Cope and W. R. Lyman, *J. Amer. Chem. Soc.*, 1953, **75**, 3312.

[18] M. P. Balfe, G. H. Beaven, and J. Kenyon, *J. Chem. Soc.*, 1951, 376.

[19] For the sensitivity of diphenylmethyl esters to hydrogen ions see G. J. Harvey and V. R. Stimson, *J. Chem. Soc.*, 1956, 3629; C. A. Bunton, J. N. E. Day, R. H. Flowers, P. Sheel, and J. L. Wood, *ibid.*, 1957, 963, as well as E. Haslam, R. D. Haworth, and G. K. Makinson, *ibid.*, 1961, 5153.

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

off almost immediately the nitrophenylsulphenyl group and L-valyl-L-valine diphenylmethyl ester hydrochloride is obtained in good yield; the lengthening of the peptide chain at the amino-end can subsequently be continued by the sulphenyl [1,7] or the trityl [16] method.

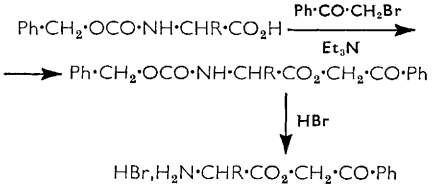


NPS = o-NO_2·C_6H_4S; Z = C_6H_5·CH_2·OCO; DPM = Ph_2CH

On the other hand, the benzyloxycarbonyl-dipeptide ester (III) can be converted into the *N*-protected-dipeptide by the action of either hydrogen chloride or trifluoroacetic acid.

4-Methoxybenzyl, [21] 4:4′-dimethoxydiphenylmethyl-,[22] and 4:4′:4″-trimethoxytrityl [23] *O*-, *S*-, or *N*-protecting groups are much more easily cleaved than the methoxy-free parent compounds. Therefore, it would be of interest to apply the conditions of cleavage of the diphenylmethyl ester group reported in this Paper to 4 : 4′-dimethoxydiphenylmethyl esters. It was found, however, that this ester group is so sensitive that it can be removed by the action of either water or alcohol. For this reason, it is extremely difficult to prepare amino-acid 4:4′-dimethoxydiphenylmethyl esters.

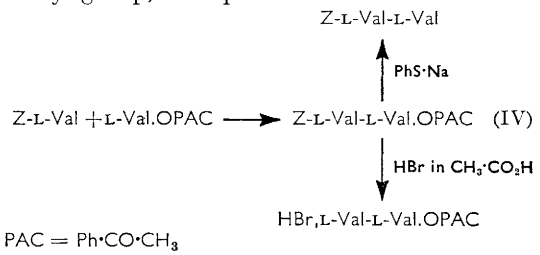
Other groups suitable for the temporary protection of carboxyl functions are phthalimidomethyl [24] and phenacyl; [25] alkyl–oxygen ester cleavage can be effected by sodium thiophenoxide [25a] in an inert solvent at or below room temperature.* However, whereas the phthalimidomethyl ester group, like the t-butyl and the diphenylmethyl ester groups, is sensitive to hydrogen chloride or hydrogen bromide, the phenacyl group, according to our experience, is stable even towards high concentrations of these reagents. Therefore, amino-acid (*e.g.*, glycine, alanine, valine, *S*-benzoylcysteine, etc.) phenacyl ester hydrobromides can be easily prepared by deblocking the amino-groups of the corresponding *N*-benzyloxycarbonyl compounds.



* The phenacyl ester group can also be subjected to catalytic hydrogenolysis, as described in the Experimental section for the case of glycine phenacyl ester hydrobromide.

[21] (*a*) F. C. Mackay and N. F. Albertson, *J. Amer. Chem. Soc.*, 1957, **79**, 4686; (*b*) F. Weygand and K. Hunger, *Chem. Ber.*, 1962, **95**, 1; (*c*) J. Rudinger, *Pure Appl. Chem.*, 1963, **7**, 335.

The use of the phenacyl esters in peptide syntheses can be illustrated by the synthesis of *N*-benzyloxycarbonyl-L-valyl-L-valine phenacyl ester (IV) and by its transformation to derivatives with either a free amino- or a free carboxyl group, as required.



PAC = Ph·CO·CH_3

EXPERIMENTAL

For the coupling reactions anhydrous reactants and dry solvents were used. Organic solvents used in the cleavage of esters were dry. Evaporations were carried out *in vacuo* at 35—40° unless otherwise stated. M. p.s were taken in capillary tubes and are not corrected. Before analysis the compounds were dried at room temperature under high vacuum over phosphoric oxide; microanalyses were by Dr. H. Mantzos, Analytical Laboratory of the Royal Hellenic Research Foundation. Further evidence for the homogeneity of the salts of the amino-acid derivatives synthesised was provided by thin-layer chromatography [26] on Kieselgel G, in the solvent system n-butanol–acetic acid–water–pyridine; [27] single spots (revealed by ninhydrin) were observed.

Tritylglycine Trityl Ester.—Trityl chloride (7·0 g., 0·025 mole) was added to a solution of tritylglycine [16a] (8·2 g., 0·025 mole) and triethylamine (3·5 ml., 0·025 mole) in tetrahydrofuran (50 ml.). After 3 days at room temperature, triethylamine hydrochloride was filtered off, the filtrate was concentrated to dryness, and light petroleum (b. p. 40—60°) was added to the syrupy residue. After cooling, the crystalline *trityl ester* (13·1 g., 93%) was collected by filtration, m. p. 168—170°, raised to 175—176° after recrystallisation from absolute ethyl acetate [lit.[9] (without elemental data), m. p. 136°] (Found: C, 85·7; H, 6·3; N, 2·7. $C_{40}H_{33}NO_2$ requires 85·9; H, 5·9; N, 2·5%).

Glycine Trityl Ester Hydrochloride (I).—To a suspension of tritylglycine trityl ester (1·1 g., 0·002 mole) in absolute acetone (20 ml.), 5N-hydrochloric acid (0·4 ml.) was added. After shaking for 5 min., the clear solution obtained was allowed to stand at 18° for 10 min. more. Upon addition of anhydrous ether (250 ml.) and cooling in the refrigerator for 15 min. with occasional scratching, the ester salt crystallised. It was filtered off, washed repeatedly with absolute ether, and dried in a desiccator over phosphoric oxide. Yield of *glycine trityl ester hydrochloride* 0·33—0·50 g.

[22] H. D. Law and R. W. Hanson, "Peptides: Proceedings of the Sixth European Symposium," Athens, 1963, ed. L. Zervas, Pergamon Press, Oxford, 1966, p. 39; R. W. Hanson and H. D. Law, *J. Chem. Soc.*, 1965, 7285.

[23] M. Smith, D. H. Rammler, I. H. Goldberg, and H. G. Khorana, *J. Amer. Chem. Soc.*, 1962, **84**, 430.

[24] G. H. L. Nefkens, G. I. Tesser, and R. J. F. Nivard, *Rec. Trav. chim.*, 1963, **82**, 941.

[25] (*a*) J. C. Sheehan and G. D. Daves, jun., *J. Org. Chem.*, 1964, **29**, 2006; (*b*) H. F. Duffie and D. E. Cooper, U.S.P. 2,650,218/1953 (*Chem. Abs.*, 1956, **50**, 410f).

[26] M. Brenner and A. Niederwieser, *Experientia*, 1960, **16**, 378; A. R. Fahmy, A. Niederwieser, G. Pataki, and M. Brenner, *Helv. Chim. Acta*, 1961, **44**, 2022.

[27] S. G. Waley and Watson, *Biochem. J.*, 1953, **55**, 328.

(47—70%), m. p. 134°, raised to 133—134° upon recrystallisation from tetrahydrofuran–*NN*-dimethylformamide (1 : 0·3 v/v) solution by the addition of ether (Found: N, 3·9; Cl, 10·1. $C_{21}H_{19}NO_2$,HCl requires N, 3·95; Cl, 10·0%).

Removal of the Trityl Ester Group.—(a) A solution of fresh glycine trityl ester hydrochloride (0·3 g., 0·0008 mole) in dioxan–water (1 : 1 v/v, 3 ml.) was allowed to stand at 18° for 5 hr., and the precipitate of triphenylmethanol was filtered off. Upon concentration of the filtrate to dryness and addition of ether to the residue, glycine hydrochloride (0·06., 72%) was obtained. Under these conditions, 0·12 g. (52%) of the methanol was formed (m. p. 160°, unchanged upon admixture with an authentic sample); if the solution was kept for 24 hr. at 18° or heated for 1 min. at 100°, the yield of the methanol was raised to 98%.

(*b*) A solution of fresh ester hydrochloride (10% w/v) in methanol was kept at 18° for 24 hr. After evaporation of the solvent and addition of ice-cold water to the syrupy residue, crystals of methyl triphenylmethyl ether were obtained on scratching (95%), m. p. 79—80° (lit.,[28] m. p. 83—84°).

N-*Tritylglycine Diphenylmethyl Ester.*—To a solution of tritylglycine [16a] (10·2 g., 0·032 mole) in N-NaOH (30 ml.), an aqueous solution of silver nitrate (5·6 g., 0·03 mole) was added. The silver salt formed was filtered off, washed with cold water, and dried in a desiccator over phosphoric oxide. The total yield of salt was suspended in chloroform (100 ml.) and diphenylmethyl chloride (6·1 g., 0·03 mole) was added. The mixture was shaken at room temperature for about 20 hr. and then it was heated under reflux for 1 hr. The silver chloride formed was removed by filtration through Celite, and the filtrate was evaporated to dryness. The crystalline residue was triturated with cold methanol and recrystallised from ethyl acetate, giving the N-*protected amino-acid ester* (7·2 g., 50%), m. p. 139—140° (Found: C, 84·7; H, 6·3; N, 3·2. $C_{34}H_{29}NO_2$ requires C, 84·45; H, 6·0; N, 3·0%).

N-*Tritylglycylglycine diphenylmethyl ester* was prepared from tritylglycylglycine [16a] according to the procedure described above for the glycine derivative. The crude product was recrystallised from ethyl acetate–methanol, giving the N-*protected dipeptide ester* (35%), m. p. 146° (Found: C, 79·8; H, 6·1; N, 5·3. $C_{36}H_{32}N_2O_3$ requires C, 80·0; H, 6·0; N, 6·0%).

N-*Benzyloxycarbonyl*-L-*proline diphenylmethyl ester* was prepared (*a*) by the interaction of the corresponding silver salt (3·9 g., 0·011 mole, prepared as usual from 2·8 g., 0·016 mole of silver nitrate and 4·2 g., 0·016 mole of *N*-benzyloxycarbonyl-L-proline [29] dissolved in 15 ml. of 1N-NaOH) and diphenylmethyl chloride (2·0 g., 0·010 mole) in chloroform (50 ml.). After shaking for 20 hr. at room temperature and heating under reflux for 1 hr., the mixture was filtered through Celite and the filtrate was washed with aqueous potassium hydrogen carbonate and with water, dried, and evaporated to dryness. The crude product was triturated with light petroleum (b. p. 40—60°), filtered off, and recrystallised from ethyl acetate–light petroleum to give the N-*benzyloxycarbonylamino-acid ester* (61%), m. p. 96—97°, $[\alpha]_D^{20}$ −54·9° (*c* 4 in chloroform) (Found: C, 75·4; H, 5·7; N, 3·4. $C_{26}H_{25}NO_4$ requires C, 75·2; H, 6·1; N, 3·4%).

(*b*) To a solution of *N*-benzyloxycarbonyl-L-proline (3·72 g., 0·015 mole) in acetone (25 ml.), diphenyldiazomethane [30] (3·2 g., 0·00165 mole) was added during 30 min. with stirring and occasional cooling in an ice-bath. After 4 hr. at room temperature, the yellowish solution was concentrated to dryness. Ethyl acetate was added to the residue and the solution was filtered to remove insoluble material. The filtrate was washed successively with aqueous potassium hydrogen carbonate and with water, dried, and evaporated to dryness. The residue crystallised upon addition of light petroleum (b. p. 40—60°) giving 4·5 g. of *N*-protected amino-acid ester (72%), m. p. 93—94°, raised to 96—97° after recrystallisation from ethyl acetate–light petroleum.

N-*Trityl*-L-*alanine Diphenylmethyl Ester.*—The silver salt of *N*-trityl-L-alanine was prepared by the addition of a concentrated aqueous solution of silver nitrate (2·7 g., 0·0165 mole) to a hot solution of *N*-trityl-L-alanine diethylammonium salt [16a] (6·06 g., 0·015 mole) in aqueous methanol (85% v/v, 140 ml.). The silver salt thus obtained (4·8 g., 0·011 mole) was treated with diphenylmethyl chloride (2·02 g., 0·01 mole) in chloroform (50 ml.) as in the preparation of the corresponding *N*-benzyloxycarbonyl-L-proline ester. The mixture was filtered through Celite and the clear filtrate was washed repeatedly with 1N-NaOH, then with water, dried, and evaporated to dryness. The residue crystallised upon addition of methanol, yielding the N-*protected amino-acid ester* (2·8 g., 58%), m. p. 102—103°, after recrystallisation from methanol; $[\alpha]_D^{22}$ +3·5° (*c* 5 in chloroform) (Found: C, 84·6; H, 6·5; N, 2·95. $C_{35}H_{31}NO_2$ requires C, 84·5; H, 6·3; N, 2·8%).

Glycine Diphenylmethyl Ester Hydrochloride.—The corresponding *N*-trityl derivative (4·8 g., 0·01 mole) was dissolved in 0·2N-hydrogen chloride in methanol [16a] (50 ml.) with occasional shaking. After 4 hr. at room temperature, the solution was evaporated to dryness. Upon addition of ether to the residue, the hydrochloride crystallised. An ethyl acetate suspension of the product was heated under reflux for 3–4 min. and filtered, giving 2·2 g. of *ester hydrochloride* (81%), m. p. 134—135° (Found: N, 5·1; Cl, 12·7. $C_{15}H_{15}NO_2$,HCl requires N, 5·0; Cl, 12·8%).

Glycylglycine diphenylmethyl ester hydrochloride was prepared by detritylation of the corresponding *N*-trityl derivative as in the preparation of glycine diphenylmethyl ester hydrochloride. Trituration of the crude product with ethyl acetate and cooling for 2 days in the refrigerator resulted in the precipitation of the *dipeptide ester hydrochloride* (74%), m. p. 138—142° (Found: C, 61·05; H, 5·7; N, 8·7; Cl, 10·6. $C_{17}H_{18}N_2O_3$,HCl requires C, 61·0; H, 5·7; N, 8·7; Cl, 10·6%).

L-*Alanine diphenylmethyl ester hydrochloride* was prepared by detritylation of the corresponding *N*-trityl compound in the manner described for the glycine derivative. Recrystallisation of the crude product (95%, m. p. 176—178°) from chloroform–ethyl acetate gave the *amino-acid ester hydrochloride*, m. p. 178°, $[\alpha]_D^{23}$ −14·4° (*c* 10 in DMF) (Found: C, 66·0; H, 6·3; N, 4·7; Cl, 12·1. $C_{16}H_{17}NO_2$,HCl requires C, 65·9; H, 6·2; N, 4·8; Cl, 12·15%).

Upon evaporation of the crude-product's filtrate to dryness and addition of a few ml. of methanol to the residue, methyl triphenylmethyl ether was obtained (80%), m. p. 79—81° (lit.,[28] m. p. 83—84°).

[28] G. Stadnikoff, *Ber.*, 1924, **57**, 5.
[29] W. Grassmann and E. Wünsch, *Chem. Ber.*, 1958, **91**, 462.
[30] L. I. Smith and K. L. Howard, *Org. Synth.*, 1944, **24**, 53. A simplified procedure affording crystalline diphenyldiazomethane has been reported by J. B. Miller, *J. Org. Chem.*, 1959, **24**, 560.

L-*Asparagine Diphenylmethyl Ester Hydrochloride.*—(*a*) To a suspension of *N*-*o*-nitrophenylsulphenyl-L-asparagine [7] (5·7 g., 0·02 mole) in aqueous methanol (20% v/v, 25 ml.), diethylamine (2 ml.) was added. After gentle heating, the clear solution was made slightly acidic by the addition of a small amount of *N*-*o*-nitrophenylsulphenyl-L-asparagine. Upon addition of an aqueous solution of silver nitrate (3·73 g., 0·022 mole), the corresponding silver salt precipitated as a gum which quickly solidified; it was filtered off, washed with methanol, and dried in a desiccator over phosphoric oxide. To a suspension of the silver salt thus obtained (7·4 g., 0·018 mole, 94%) in chloroform (100 ml.), diphenylmethyl chloride (3·2 g., 0·016 mole) was added. After shaking for 24 hr. at room temperature, the mixture was heated under reflux for 1 hr. The silver chloride formed was removed by suction through Celite and the filtrate was concentrated to dryness. The oily residue was taken up in ether and the resulting solution was repeatedly washed with concentrated aqueous sodium carbonate. The ether layer was then washed with water, dried, and evaporated to dryness. After the syrupy residue had been kept in the refrigerator for many days under light petroleum (b. p. 40—60°), *N*-*o*-nitrophenylsulphenyl-L-asparagine diphenylmethyl ester was obtained (4·3 g., 50%) as an amorphous powder. This product was dissolved in acetone (30 ml.), 5N hydrochloric acid* (4·4 ml.) was added, and after 5 min. at 20° the solution was concentrated (at 25—30°) to dryness.† Upon trituration of the oily residue with ether, an amorphous precipitate of the ester hydrochloride was formed. The crude ester salt was suspended in a small amount of water, the mixture was filtered, and saturated aqueous sodium carbonate was added to the filtrate. The free ester liberated was taken up in ether and the organic layer was dried (K_2CO_3). Addition of ether containing hydrogen chloride to the ether solution of the ester, precipitated crystalline L-*asparagine diphenylmethyl ester hydrochloride* (1·6 g., 30%), m. p. 155°, raised to 159—160° after recrystallisation from methanol–ether; $[\alpha]_D^{18}$ +1·4° (*c* 6·4 in DMF) (Found: C, 60·4; H, 6·1; N, 8·35; Cl, 10·9. $C_{17}H_{18}N_2O_3$,HCl requires C, 61·0; H, 5·7; N, 8·4; Cl, 10·6%).

(*b*) Diphenyldiazomethane was added to a suspension of *N*-*o*-nitrophenylsulphenyl-L-asparagine in acetone according to the procedure described above for the preparation of the diphenylmethyl ester of *N*-benzyloxycarbonyl-L-proline. The mixture was stirred at room temperature for 9 hr. (no cooling was applied). The turbid yellow solution thus obtained was filtered, and the filtrate concentrated to small volume. Removal of the *N*-*o*-nitrophenylsulphenyl group by treatment with 5N-hydrochloric acid in the manner described above (case *a*) yielded the amino-acid ester hydrochloride (10%), m. p. 155°.

L-*Valine Diphenylmethyl Ester Hydrochloride.*—(*a*) *N*-*o*-Nitrophenylsulphenyl-L-valine dicyclohexylammonium salt [7] was transformed to the silver salt as in the preparation of the corresponding *N*-trityl-L-alanine salt. By the interaction of the silver salt thus obtained (7·5 g., 0·02 mole) with diphenylmethyl chloride (3·64 g., 0·018 mole) in chloroform (30 ml.) and after working up the mixture as in the preparation of the corresponding L-asparagine derivative, *N*-*o*-nitrophenylsulphenyl-L-valine diphenylmethyl ester (7·4 g., 85%) was obtained as an oil. The crude *N*-protected amino-acid ester was treated with 5N-hydrochloric acid in the usual manner to yield L-*valine diphenylmethyl ester hydrochloride* (4·28 g., 67% after the purification step), m. p. 156—157°, raised to 159·5—160° after recrystallisation from ethyl acetate (recovery 98%); $[\alpha]_D^{30}$ −35·0° (*c* 0·8 in tetrahydrofuran), $[\alpha]_D$ −26·3° (*c* 5 in DMF) (Found: C, 67·4; H, 7·0; N, 4·5; Cl, 10·9. $C_{18}H_{21}NO_2$,HCl requires C, 67·6; H, 6·9; N, 4·4; Cl, 11·0%).

(*b*) A suspension of *N*-formyl-L-valine [31] (5·8 g., 0·04 mole) in aqueous acetone (15% v/v, 40 ml.) was treated with diethylamine (4 ml.). After gentle heating, the clear solution was made slightly acidic by the addition of a small amount of *N*-formyl-L-valine. Upon addition of a concentrated aqueous solution of silver nitrate (7·47 g., 0·044 mole), the silver salt of *N*-formyl-L-valine was precipitated (9·1 g., 90%). By the interaction of this silver salt (5 g., 0·02 mole) with diphenylmethyl chloride (3·6 g., 0·018 mole) in chloroform suspension as described above, *N*-formyl-L-valine diphenylmethyl ester (4·4 g., 70%) was obtained as an oil. To a solution of this oil (3·1 g.,) in methanol (30 ml.), 1N-methanolic hydrochloric acid [32] (3·3 ml.) was added. After 48 hr. at room temperature, the solution was concentrated (at 25—30°) almost to dryness. Ether was added to the residue and the mixture was concentrated again to dryness. Upon addition of a fresh portion of ether, the residue formed a jelly-like precipitate; it was filtered off and washed on the filter many times with dry ether. The product was purified as described for the corresponding L-asparagine derivative to yield L-valine diphenylmethyl ester hydrochloride (0·75 g., 24%), m. p. 156—157°.

(*c*) Diphenyldiazomethane was added to a suspension of *N*-formyl-L-valine in acetone according to the procedure described for the preparation of the diphenylmethyl ester of *N*-benzyloxycarbonyl-L-proline (case *b*). After stirring at room temperature for 30 min., the evolution of nitrogen almost ceased. The yellowish solution was kept at room temperature for 1 hr. more, filtered, and the filtrate was concentrated to dryness. The residue was dissolved in ethyl acetate, the solution washed successively with aqueous potassium hydrogen carbonate and with water, dried, and evaporated to dryness. The residue was treated with methanolic hydrogen chloride to split off the formyl group as described above (case *b*) giving the amino-acid ester hydrochloride (27%), m. p. 152—153°, after recrystallisation from ethyl acetate.

(*d*) *N*-*o*-Nitrophenylsulphenyl-L-valine dicyclohexylammonium salt was treated with 0·2N-sulphuric acid according to a procedure previously reported [7] for the corresponding L-isoleucine derivative. To the acetone-suspension of the *N*-*o*-nitrophenylsulphenyl-L-valine thus obtained, diphenyldiazomethane was added as described above (case *c*). The solvent was evaporated to about half the original volume and 5N-hydrochloric acid was added to split off the *N*-*o*-nitrophenylsulphenyl group, as in case (*a*), yielding the ester hydrochloride (37%), m. p. 151—152°, raised to 153—155° after the purification step.

L-*Valine Diphenylmethyl Ester Hydrobromide.*—A solution of L-valine diphenylmethyl ester hydrochloride (1·6 g., 0·005 mole) in water (15 ml.) was treated with a concen-

* The yield of cleaved product was substantially unchanged, if the amount of 5N-hydrochloric acid used was doubled.

† Di-*o*-nitrophenyl disulphide sometimes crystallised out. It was either filtered off at this stage, or removed by filtration together with water-insoluble material at the next stage.

[31] E. Fischer, *Ber.*, 1906, **39**, 2320.

[32] J. C. Sheehan and D.-D. H. Yang, *J. Amer. Chem. Soc.*, 1958, **80**, 1154.

trated (ice-cold) sodium carbonate solution. The free ester thus generated was taken up in ether, the organic layer was dried (K_2CO_3), and treated with a solution (1·37 g., 30% w/v, 0·0055 mole) of hydrogen bromide in glacial acetic acid; the crystalline precipitate was filtered off and washed many times with dry ether yielding the *amino-acid diphenylmethyl ester hydrobromide* (1·6 g., 87%), m. p. 141—142°, raised to 148—149° after recrystallisation from ethyl acetate; $[\alpha]_D^{21}$ −24·8° (*c* 5 in dimethylformamide) (Found: C, 59·8; H, 6·1; N, 3·8; Br, 21·9. $C_{18}H_{21}NO_2$,HBr requires C, 59·3; H, 6·1; N, 3·8; Br, 21·95%).

N-*Benzyloxycarbonyl*-L-*valyl*-L-*valine Diphenylmethyl Ester* (III).—(*a*) To a solution of L-valine diphenylmethyl ester hydrochloride (4·8 g., 0·015 mole) in chloroform (60 ml.) containing triethylamine (2·2 ml., 0·015 mole), *N*-benzyloxycarbonyl-L-valine [29] (4·1 g., 0·0165 mole) and *NN'*-dicyclohexylacarbodi-imide [20] (3·1 g., 0·015 mole) were added. After 6 hr. at room temperature, a few drops of aqueous acetic acid (50% v/v) were added and the insoluble precipitate of *NN'*-dicyclohexylurea (3·2 g., 94%) was removed by filtration. The filtrate was washed successively with water, dilute sulphuric acid, water, aqueous potassium hydrogen carbonate, again with water, dried, and evaporated to dryness. Ethyl acetate was added to the residue, traces of *NN'*-dicyclohexylurea were filtered off, and the filtrate was evaporated again to dryness. The crystalline residue was recrystallised from a boiling mixture of ethyl acetate–ether (1 : 1 v/v, 60 ml.) to yield the N-*protected dipeptide ester* (5 g., 64%), m. p. 136–137°, unchanged after a second recrystallisation from boiling tetrahydrofuran–ether (1:3 v/v); $[\alpha]_D^{21}$ −35·6° (*c* 5 in tetrahydrofuran) (Found: C, 72·2; H, 7·2; N, 5·4. $C_{31}H_{36}N_2O_5$ requires C, 72·1; H, 7·0; N, 5·4%).

(*b*) *N*-Benzyloxycarbonyl-L-valyl-L-valine [33] was dissolved in acetone and the solution was treated with diphenyldiazomethane as in the preparation of the diphenylmethyl ester of *N*-benzyloxycarbonyl-L-proline (case *b*); after 5 hr. at room temperature, the solvent was evaporated to dryness, the residue was dissolved in chloroform, and the solution was washed as described in case (*b*). Light petroleum (b. p. 40—60°) was added to the residue at the last stage of the isolation of the compound to give crystalline *N*-benzyloxycarbonyl-L-valyl-L-valine diphenylmethyl ester (71%), m. p. 136—137°.

L-*Valyl*-L-*valine Diphenylmethyl Ester Hydrochloride*.—A mixture of *N*-*o*-nitrophenylsulphenyl-L-valine dicyclohexylammonium salt [7] (4·48 g., 0·01 mole) and L-valine diphenylmethyl ester hydrochloride (3·2 g., 0·01 mole) was suspended in chloroform (50 ml.) and stirred at 7—8° for a few minutes. *NN'*-Dicyclohexylcarbodi-imide [20] (2·06 g., 0·01 mole) was added to the clear solution, and the resulting mixture was stirred at room temperature overnight. A few drops of aqueous acetic acid (50% v/v) were added, the precipitate (consisting of *NN'*-dicyclohexylurea contaminated with dicyclohexylamine hydrochloride) was filtered off, and the filtrate was worked up as described above for the preparation of the corresponding *N*-benzyloxycarbonyl dipeptide ester. The product was obtained as a sticky glass and was treated with acid to split off the *N*-*o*-nitrophenylsulphenyl group as described above for the preparation of L-asparagine diphenylmethyl ester hydrochloride (case *a*). The crude dipeptide ester hydrochloride was purified as usual (generation of the free ester and re-precipitation of the ester hydrochloride) to yield L-*valyl*-L-*valine diphenylmethyl ester hydrochloride* (3·35 g., 80%), m. p. 180—181° after recrystallisation from tetrahydrofuran–ether; $[\alpha]_D^{20}$ −14·9° (*c* 8 in DMF) (Found: C, 65·9; H, 7·6; N, 6·7; Cl, 8·65. $C_{23}H_{30}N_2O_3$,HCl requires C, 65·9; H, 7·5; N, 6·7; Cl, 8·5%).

N-*Benzyloxycarbonyl*-L-*proline* 4:4'-*dimethoxybenzhydryl ester* was prepared either (*a*) by the interaction of the corresponding silver salt with 4:4'-dimethoxydiphenylmethyl chloride [34] as in the preparation of *N*-benzyloxycarbonyl-L-proline diphenylmethyl ester, or (*b*) directly from the *N*-protected amino-acid and the chloride in the presence of triethylamine in the manner described for the preparation of tritylglycine trityl ester. In the second case, the reaction mixture was kept at room temperature for 20 hr., triethylamine hydrochloride was filtered off, the filtrate was concentrated to dryness, and the residue was taken up in ethyl acetate. The resulting solution was washed with ice-cold water, aqueous potassium hydrogen carbonate, again with water, dried, and evaporated to dryness. In both cases, the syrupy residue obtained after evaporation of the solvent crystallised upon trituration with light petroleum (b. p. 40—60°) and cooling in the refrigerator to yield N-*benzyloxycarbonyl*-L-*proline* 4:4'-*dimethoxydiphenylmethyl ester* (75—80%), m. p. 80—81° and 83° after recrystallisation from ethyl acetate–light petroleum; $[\alpha]_D^{19}$ −53·1° (*c* 4·1 in chloroform) (Found: C, 71·0; H, 6·1; N, 2·7. $C_{28}H_{29}NO_6$ requires C, 70·7; H, 6·1; N, 2·9%).

N-*o*-*Nitrophenylsulphenyl*-L-*alanylglycine* 4:4'-*dimethoxydiphenylmethyl ester* was prepared from the *N*-protected dipeptide [7] and 4:4'-dimethoxydiphenylmethyl chloride as in the preparation of the same ester of *N*-benzyloxycarbonyl-L-proline (case b). After evaporation of the solvent, the residue crystallised to yield the N-*protected dipeptide ester* (75%), m. p. 130—133°, raised to 138° after recrystallisation from ethyl acetate; $[\alpha]_D^{20}$ −55·6° (*c* 5 in tetrahydrofuran) (Found: C, 59·1; H, 5·3; N, 7·95; S, 6·3. $C_{26}H_{27}N_3O_7S$ requires C, 59·4; H, 5·2; N, 8·0; S, 6·1%).

Selective removal of the *N*-*o*-nitrophenylsulphenyl group (without affecting the ester group) was not achieved.

Removal of the Diphenylmethyl Ester Group.—(a) *By hydrolysis*. Upon keeping aqueous solutions (5% w/v) of the ester hydrochlorides of glycine, L-alanine, and L-valine at room temperature for 20 hr. (initial pH 3·4), diphenylmethanol (46—50%, m. p. 64—65°) crystallised (final pH 1·8). The yield of diphenylmethanol was raised (95—97%, m. p. 63—64°) upon heating these solutions to 100° for 3 min. The diphenylmethyl ester hydrochlorides of glycylglycine and L-valyl-L-valine required a longer time for hydrolysis at 100°, *i.e.*, 6 min. (diphenylmethanol isolated: 80%) and 30 min. (60%), respectively.

(b) *By hydrogenolysis*. (i) A solution of *N*-benzyloxycarbonyl-L-valyl-L-valine diphenylmethyl ester (0·52 g., 0·001 mole) in methanol–tetrahydrofuran (3 : 1 v/v, 20 ml.) was hydrogenated over palladium-black catalyst. After 3 hr., the free peptide formed and the catalyst were filtered off and washed with a small amount of methanol–tetrahydrofuran. The dipeptide was dissolved in hot water and the filtrate was concentrated to dryness to yield 0·18 g. (85%) of L-valyl-L-valine, $[\alpha]_D^{26}$ +10·8° (*c* 2 in water)

[33] M. A. Nyman and R. M. Herbst, *J. Org. Chem.*, 1950, **15**, 108.

[34] D. Bethell and V. Gold, *J. Chem. Soc.*, 1958, 1905; D. Bethell, V. Gold, and D. P. N. Satchell, *ibid.*, p. 1918.

[lit.,[35] $[\alpha]_D^{25}$ +10·8° (*c* 2 in water)]. (ii) A solution of L-valyl-L-valine diphenylmethyl ester hydrochloride (0·41 g., 0·001 mole) in tetrahydrofuran (25 ml.) containing *NN*-dimethylformamide (1·5 ml.) was hydrogenated over palladium–black catalyst. After 3 hr., the theoretical amount of hydrogen had been consumed; the mixture was filtered and the filtrate was concentrated to dryness. The residue was dissolved in a small amount of ethanol and pyridine (0·8 ml., 0·001 mole) was added to give L-valyl-L-valine (0·19 g., 90%), $[\alpha]_D^{18}$ +10·9° (*c* 2 in water) {lit.,[35] $[\alpha]_D^{25}$ +10·8° (*c* 2 in water)}.

(c) *By the action of hydrogen chloride.* (i) L-Valine diphenylmethyl ester hydrochloride (0·32 g., 0·001 mole) was dissolved in warm nitromethane (12·3 ml.). As soon as the temperature of the solution dropped to 30°, 1·16N-hydrogen chloride solution (2·6 ml., 0·003 mole) in nitromethane was added. After 3 hr. at 30°, the crystalline precipitate of L-valine hydrochloride was collected by filtration (0·13 g., 84%); m. p. 220—223° (decomp.), $[\alpha]_D^{25}$ +16·1° (*c* 3·7 in water) after recrystallisation from methanol–ether. For comparison, L-valine hydrochloride was prepared from L-valine and hydrochloric acid; this product had m. p. 220—223° (decomp.), $[\alpha]_D^{25}$ +16·3° (*c* 5 in water), and exhibited the same behaviour on thin-layer chromatograms.

(ii) To a solution of *N*-benzyloxycarbonyl-L-proline diphenylmethyl ester (0·41 g., 0·001 mole) in ethyl acetate (13·3 ml.), 1·8N-hydrogen chloride solution (1·6 ml., 0·003 mole) in ethyl acetate was added. After 3 hr. at 30°, the solution was diluted with ethyl acetate and washed with ice-cold aqueous potassium hydrogen carbonate and water. The ethyl acetate solution was dried and concentrated to dryness to yield starting material (0·36 g., 90%), m. p. 93—95·5°. The yield of starting material did not decrease much (85%, m. p. 92·5—93·5°) if the solution of *N*-benzyloxycarbonyl-L-proline in 0·2N-hydrogen chloride in ethyl acetate was kept at 30 for 20 hr.

A similar experiment was carried out in 0·2N-hydrogen chloride solution in nitromethane instead of ethyl acetate. After 3 hr. at 30°, the solvent was exchanged with ethyl acetate and the resulting solution was washed as usual. The potassium hydrogen carbonate extracts were combined, acidified with 5N-hydrochloric acid, and extracted with ethyl acetate. The organic layer was washed repeatedly with water to remove traces of the mineral acid, dried, and concentrated to dryness. The residue was triturated with light petroleum to yield *N*-benzyloxycarbonyl-L-proline (0·32 g., 86%), m. p. 73—74° (lit.,[29] 73—75°, and 77° for the recrystallised compound).

(iii) *N*-Benzyloxycarbonyl-L-valyl-L-valine diphenylmethyl ester was treated with hydrogen chloride in nitromethane as described above for the corresponding L-proline derivative (2·5% solution of the ester, w/v, in 0·2N-hydrogen chloride solution in nitromethane). After 14 hr. at 30°, the reaction mixture was worked up in the usual way to yield *N*-benzyloxycarbonyl-L-valyl-L-valine (80%), m. p. 137—138° (lit.,[33] m. p. 139·5—140°).

(d) *By the action of hydrogen bromide.* (i) L-Valine diphenylmethyl ester hydrobromide (0·36 g., 0·001 mole) was suspended in 0·15N-hydrogen bromide solution (20 ml., 0·003 mole) in nitromethane. Part of the starting material did not dissolve immediately, whereas crystals of the cleavage product started separating. After the mixture had been shaken for 15 min. at 20°, the crystalline material was filtered off and washed with nitromethane and with ether, yielding *L-valine hydrobromide* (0·17 g., 90%), m. p. 208—210° (decomp.) unchanged after recrystallisation from ethanol–ether; $[\alpha]_D^{28}$ +12·0° (*c* 2·4 in water) (Found: N, 6·9; Br, 40·5. $C_5H_{11}NO_2$,HBr requires N, 7·1; Br, 40·2%).

The same experiment was repeated using ethyl acetate instead of nitromethane. Starting material (0·32 g., 88%, m. p. 141—142°) contaminated with cleaved product was recovered after evaporation of the solvent and addition of ether to the jelly residue.

(e) *By the action of trifluoroacetic acid.* (i) *N*-Benzyloxycarbonyl-L-proline diphenylmethyl ester (0·41 g., 0·001 mole) was dissolved in trifluoracetic acid [36] (1 ml.) containing freshly distilled phenol (0·2 g.). After 30 min. at 20°, the solution was concentrated (within 5—7 min.) at 20° to dryness. The residue was taken up in ethyl acetate and the solution was worked up as usual. The product isolated from the alkaline extracts was washed many times with light petroleum (to remove traces of phenol) yielding 0·2 g. of *N*-benzyloxycarbonyl-L-proline (82%), m. p. 73—74°, $[\alpha]_D^{21}$ −40·7° (*c* 2 in ethanol) {lit.,[29] m. p. 73—74°, $[\alpha]_D^{20}$ −40·6 ±0·5° (*c* 2 in ethanol)}.

(ii) *N*-Benzyloxycarbonyl-L-valyl-L-valine diphenylmethyl ester was treated with trifluoroacetic acid containing phenol, as described above, to yield the *N*-protected dipeptide (95%), m. p. 138—139°, $[\alpha]_D^{25}$ −35·0° (*c* 3·2 in 1N-KOH) {lit.,[33] m. p. 139·5—140°, $[\alpha]_D^{20}$ −36·6° (*c* 3·2 in 1N-KOH)}. This compound was converted into the diphenylmethyl ester derivative by the action of diphenyldiazomethane, as described above.

Removal of the 4 : 4′-Dimethoxydiphenylmethyl Ester Group.—(a)*In water-containing solution.* The *N*-benzyloxycarbonyl-L-proline ester (0·47 g., 0·001 mole) was dissolved in dioxan (5 ml.). Water (2 ml.) was added and the solution kept at room temperature. After 20 hr., the solution was concentrated (within 10 min.) at 30° to dryness. The residue was taken up in ethyl acetate and the resulting solution was washed repeatedly with aqueous potassium hydrogen carbonate and with water. Starting material (0·21 g., 44%, m. p. 77—79°) was recovered from the organic layer, whereas *N*-benzyloxycarbonyl-L-proline (0·12 g., 49%, m. p. 73—74°) was isolated from the alkaline extracts after acidification and extraction as usual.

(b) *In alcohol solution.* A solution of the *N*-benzyloxycarbonyl-L-proline ester (2·5% w/v) in absolute ethanol was refluxed for 15 min. The solvent was evaporated to dryness, the residue was taken up in ethyl acetate, and the resulting solution was worked up as usual to yield *N*-benzyloxycarbonyl-L-proline (83%), m. p. 73—74°. (*N*-Benzyloxycarbonyl-L-proline diphenylmethyl ester was recovered in 73% yield, after refluxing for 4 hr. a solution of the ester in methanol.)

N-*Benzyloxycarbonylglycine Phenacyl Ester.*—To a solution of *N*-benzyloxycarbonylglycine [37] (2·1 g., 0·01 mole) in ethyl acetate (20 ml.), triethylamine (1·4 ml., 0·01 mole) and phenacyl bromide (2 g., 0·01 mole) were added. After the solution had stood at room temperature overnight, the precipitate of triethylamine hydrobromide was filtered off, and the filtrate was washed with water, dilute sulphuric

[35] T. Sugimura and P. W. Paik, unpublished data; cf. ref. 4, p. 1229.

[36] F. Weygand and W. Steglich, *Z. Naturforsch.*, 1959, **14**b, 472.

[37] M. Bergmann and L. Zervas, *Ber.*, 1932, **65**, 1192.

acid, aqueous potassium hydrogen carbonate, again with water, dried, and evaporated to dryness. The crystalline residue, was triturated with light petroleum (b. p. 40—60°), filtered off, and recrystallised from isopropyl alcohol to yield the required *phenacyl ester* (2·7 g., 83%), m. p. 103° (Found: C, 66·3; H, 5·35; N, 4·4. $C_{18}H_{17}NO_5$ requires C, 66·05; H, 5·2; N, 4·3%).

N-*Benzyloxycarbonyl*-L-*alanine Phenacyl Ester*.—(*a*) A solution of *N*-benzyloxycarbonyl-L-alanine [29,37] (2·3 g., 0·01 mole), triethylamine (1·4 ml., 0·01 mole), and phenacyl bromide (2 g., 0·01 mole) in ethyl acetate (20 ml.) was kept at room temperature overnight. The phenacyl ester and triethylamine hydrobromide formed a precipitate; it was collected by filtration and washed with ethyl acetate. Upon treatment of the dried precipitate with water, the amine hydrobromide dissolved and pure phenacyl ester of the *N*-protected amino-acid was obtained. A second crop of the compound was secured upon working up the ethyl acetate filtrate in the way described for the corresponding glycine ester. The total yield of *N*-benzyloxycarbonyl-L-alanine phenacyl ester was 2·9 g. (86%), m. p. 154—155°, unchanged after recrystallisation from ethanol; $[\alpha]_D^{18}$ −26·5° (*c* 2 in chloroform) (Found: C, 66·9; H, 5·65; N, 4·1. $C_{19}H_{19}NO_5$ requires C, 66·85; H, 5·6; N, 4·1%).

(*b*) To a solution of *N*-benzyloxycarbonyl-L-alanine (2·3 g., 0·01 mole) in cold absolute ethanol (20 ml.), triethylamine (1·4 ml., 0·01 mole) and phenacyl bromide (2 g., 0·01 mole) were added. The mixture was allowed to stand at room temperature for 5 hr., and then it was placed in the refrigerator for 10 hr. more. Crude reaction product precipitated; it was filtered off and washed with cold ethanol to yield the required ester (70%), m. p. 155°, unchanged after recrystallisation from ethanol.

N-*Benzyloxycarbonyl*-L-*valine phenacyl ester* was prepared from *N*-benzyloxycarbonyl-L-valine [29] and phenacyl bromide in the way described for the corresponding glycine derivative. Upon addition of ether–light petroleum the product crystallised (88%), m. p. 61—62°, $[\alpha]_D^{18}$ −14·0° (*c* 2 in chloroform) (Found: C, 68·5; H, 6·3; N, 3·9. $C_{21}H_{23}NO_5$ requires C, 68·3; H, 6·3; N, 3·8%).

N-*Benzyloxycarbonyl*-L-*phenylalanine phenacyl ester* was prepared from *N*-benzyloxycarbonyl-L-phenylalanine [29] and phenacyl bromide in the way described for the corresponding glycine derivative; the yield was 77% after recrystallisation from ethanol, m. p. 100°, $[\alpha]_D^{18}$ −7·5° (*c* 4 in chloroform) (Found: C, 72·0; H, 5·7; N, 3·1. $C_{25}H_{23}NO_5$ requires C, 71·95; H, 5·5; N, 3·3%).

N-*Benzyloxycarbonyl*-L-*leucine phenacyl ester* was prepared from *N*-benzyloxycarbonyl-L-leucine [29] and phenacyl bromide, as described for the corresponding glycine derivative; the yield was 77%, m. p. 54—55° after recrystallisation from ether–light petroleum (b. p. 40—60°), $[\alpha]_D^{18}$ −25·5° (*c* 4 in chloroform) (Found: C, 69·1; H, 6·5; N, 3·5. $C_{22}H_{25}NO_5$ requires C, 68·9; H, 6·6; N, 3·65%).

N-Benzyloxycarbonyl-L-*aspartic acid Phenacyl Di-ester*.—To a cold solution of *N*-benzyloxycarbonyl-L-aspartic acid [37] (1·34 g., 0·005 mole) in tetrahydrofuran (15 ml.), triethylamine (1·4 ml., 0·01 mole) and phenacyl bromide (2 g., 0·01 mole) were added. The mixture was stirred overnight at room temperature, the amine hydrobromide was filtered off, and the filtrate was concentrated to dryness. The residue was dissolved in chloroform and the solution was worked up as usual to give the *di-ester* (2·4 g., 96%), m. p. 105—107° after recrystallisation from ethanol; $[\alpha]_D^{18}$ +1·5° (*c* 2 in chloroform) (Found: C, 66·8; H, 4·9; N, 2·6. $C_{28}H_{25}NO_8$ required C, 66·8; H, 5·0; N, 2·8%).

N-*Benzyloxycarbonyl*-S-*benzoyl*-L-*cysteine phenacyl ester* was prepared from *N*-benzyloxycarbonyl-*S*-benzoyl-L-cysteine [38] and phenacyl bromide, as described for the corresponding glycine derivative, in 80% yield; the m. p. was 111—112°, unchanged after recrystallisation from isopropyl alcohol; $[\alpha]_D^{18}$ −8·5° (*c* 2 in chloroform) (Found: C, 65·4; H, 5·05; N, 2·9; S, 6·6. $C_{26}H_{23}NO_6S$ requires C, 65·4; H, 4·85; N, 2·9; S, 6·7%).

Glycine Phenacyl Ester Hydrobromide.—To a solution of *N*-benzyloxycarbonylglycine phenacyl ester (1 g., 0·003 mole) in ethyl acetate (10 ml.), 3·5N-hydrogen bromide solution (7 ml.) in acetic acid was added. After 45 min. at room temperature, ether was added to precipitate the *amino-acid phenacyl ester hydrobromide* (0·8 g., 98%), m. p. 171—172°, unchanged after recrystallisation from isopropyl alcohol (Found: Br, 29·3; N, 5·0. $C_{10}H_{11}NO_3$,HBr requires Br, 29·15; N, 5·1%).

L-*Alanine phenacyl ester hydrobromide* was prepared in 90% yield from the corresponding *N*-benzyloxycarbonyl compound by removing the *N*-protecting group with hydrogen bromide, as described above for the glycine derivative; m. p. 174—175°, unchanged after recrystallisation from methanol–ethyl acetate; $[\alpha]_D^{15}$ +1·5° (*c* 4 in methanol) (Found: C, 45·7; H, 4·95; Br, 27·95; N, 4·75. $C_{11}H_{13}NO_3$,HBr requires C, 45·85; H, 4·9; Br, 27·7; N, 4·85%).

L-*Valine Phenacyl Ester Hydrobromide*.—To a solution of *N*-benzyloxycarbonyl-L-valine phenacyl ester (3·7 g., 0·01 mole) in ether (35 ml.), 3·4N-hydrogen bromide solution (35 ml.) in acetic acid was added. After 40 min. at room temperature, the precipitate was filtered off and washed with ether to give the required *amino-acid phenacyl ester hydrobromide* (3 g., 95%), m. p. 186—187°, unchanged after recrystallisation from methanol–ethyl acetate; $[\alpha]_D^{15}$ +12·0° (*c* 2 in methanol) (Found: C, 49·5; H, 5·85; Br, 25·45; N, 4·4. $C_{13}H_{17}NO_3$,HBr requires C, 49·4; H, 5·7; Br, 25·3; N, 4·4%).

L-*Phenylalanine phenacyl ester hydrobromide* was prepared in 83% yield from the corresponding *N*-benzyloxycarbonyl derivative in the way described for glycine phenacyl ester hydrobromide; m. p. 158° after recrystallisation from methanol–ether; $[\alpha]_D^{15}$ +1·1° (*c* 4 in methanol) (Found: C, 56·3; H, 5·3; Br, 22·3; N, 3·6. $C_{17}H_{17}NO_3$,HBr requires C 56·1; H, 5·0; Br, 21·9; N, 3·8%).

L-*Leucine phenacyl ester hydrobromide* was prepared in 87% yield from the corresponding *N*-benzyloxycarbonyl derivative as described for the glycine phenacyl ester salt; m. p. 159° after recrystallisation from methanol–ether; $[\alpha]_D^{15}$ +15·7° (*c* 2 in methanol) (Found: C, 51·1; H, 6·3; Br, 24·3; N, 4·2. $C_{14}H_{19}NO_3$,HBr requires C, 50·9; H, 6·1; Br, 24·1; N, 4·2%).

L-*Aspartic acid phenacyl di-ester hydrobromide* was prepared in 72% yield from the corresponding *N*-benzyloxycarbonyl compound by removing the *N*-protecting group with hydrogen bromide, as described for the glycine derivative; m. p. 150—151° after recrystallisation from methanol–ether; $[\alpha]_D^{15}$ +10·3° (*c* 2 in methanol) (Found: C, 53·3; H, 4·8; Br, 18·0; N, 3·0. $C_{20}H_{19}NO_6$,HBr requires C, 53·3; H, 4·5; Br, 17·7; N, 3·1%).

S-*Benzoyl*-L-*cysteine phenacyl ester hydrobromide* was

[38] L. Zervas, I. Photaki, and N. Ghelis, *J. Amer. Chem. Soc.*, 1963, **85**, 1337.

prepared in 94% yield from the corresponding *N*-benzyloxycarbonyl compound by removing the *N*-protecting group with hydrogen bromide, as described for the glycine derivative; m. p. 181—182°, unchanged after recrystallisation from methanol–ethyl acetate; $[\alpha]_D^{15}$ −38·0° (*c* 2 in dimethylformamide) (Found: Br, 18·9; N, 3·2; S, 7·35. $C_{18}H_{17}NO_4S$,HBr requires Br, 18·8; N, 3·3; S, 7·55%).

N-*Benzyloxycarbonyl*-L-*alanylglycine Phenacyl Ester*.—To a cold solution of glycine phenacyl ester hydrobromide (1·4 g., 0·005 mole) and triethylamine (0·7 ml., 0·005 mole) in chloroform (15 ml.), *N*-benzyloxycarbonyl-L-alanine (1·15 g., 0·005 mole) was added, followed by the immediate addition of *NN'*-dicyclohexylcarbodi-imide (1·1 g., 0·0053 mole). After the mixture had stood at room temperature overnight, the insoluble precipitate of *NN'*-dicyclohexylurea was filtered off and the filtrate was washed with water, dilute hydrochloric acid, water, aqueous potassium hydrogen carbonate, again with water, dried, and evaporated to dryness. The crystalline residue was triturated with ether and collected by filtration to give the N-*protected dipeptide ester* (1·4 g., 70%), m. p. 153—155°, unchanged after recrystallisation from ethanol; $[\alpha]_D^{18}$ −7·5° (*c* 2 in chloroform) (Found: C, 63·25; H, 5·6; N, 7·1. $C_{21}H_{22}N_2O_6$ requires C, 63·3; H, 5·6; N, 7·0%).

N-*Benzyloxycarbonyl*-L-*valyl*-L-*valine phenacyl ester* (IV) was prepared from *N*-benzyloxycarbonyl-L-valine and L-valine phenacyl ester by coupling with *NN'*-dicyclohexylcarbodi-imide, as described for the L-alanylglycine derivative. The crude product was recrystallised from ethanol to give the required *compound* (64%), m. p. 162—163°; $[\alpha]_D^{18}$ −22·0° (*c* 2 in chloroform) (Found: C, 66·9; H, 7·0; N, 5·9. $C_{26}H_{32}N_2O_6$ requires C, 66·6; H, 6·9; N, 6·0%).

L-*Alanylglycine Phenacyl Ester Hydrochloride*.—A solution of *N*-benzyloxycarbonyl-L-alanylglycine phenacyl ester (1·6 g., 0·004 mole) in trifluoroacetic acid [36] (8 ml.) containing phenol (0·8 g.) was heated under reflux for 30 min. The solution was concentrated to dryness and the residue was dissolved in ethyl acetate. Upon addition of ether saturated with hydrogen chloride, the dipeptide ester hydrochloride separated out. The crystalline precipitate was filtered off, washed with ether, and recrystallised from methanol–ethyl acetate to give the required *compound* (1·1 g., 90%), m. p. 171°; $[\alpha]_D^{15}$ −2·5° (*c* 2 in methanol) (Found: C, 52·1; H, 5·7; Cl, 12·05; N, 9·2. $C_{13}H_{16}N_2O_4$,HCl requires C, 51·9; H, 5·7; Cl, 11·8; N, 9·3%).

L-*Valyl*-L-*valine Phenacyl Ester Hydrohalide Salts*.—(*a*) The *hydrochloride* of this dipeptide ester was prepared in 86% yield after removing the *N*-protecting group from the corresponding *N*-benzyloxycarbonyl derivative with trifluoroacetic acid and adding ether saturated with hydrogen chloride to the reaction mixture, as described for L-alanylglycine phenacyl ester hydrochloride; m. p. 198°, $[\alpha]_D^{18}$ −22·0° (*c* 2 in methanol) (Found: C, 58·3; H, 7·5; Cl, 9·6; N, 7·3. $C_{18}H_{26}N_2O_4$,HCl requires C, 58·3; H, 7·3; Cl, 9·55; N, 7·55%).

(*b*) Upon addition of hydrogen bromide, instead of hydrogen chloride, to the product obtained after cleavage of the *N*-benzyloxycarbonyl group with trifluoroacetic acid, the required *dipeptide ester hydrobromide* was obtained in 70% yield; m. p. 196—197°, $[\alpha]_D^{18}$ −18·5° (*c* 2 in methanol) (Found: C, 52·1; H, 6·65; Br, 19·55; N, 6·5. $C_{18}H_{26}N_2O_4$,HBr requires C, 52·05; H, 6·55; Br, 19·2; N, 6·7%).

Removal of the Phenacyl Ester Group.—(*a*) *By sodium thiophenoxide*. (i) A solution of *N*-benzyloxycarbonylglycine phenacyl ester (0·65 g., 0·002 mole) and sodium thiophenoxide [25a] (0·52 g., 0·004 mole) in *NN*-dimethylformamide (2 ml.) was kept at room temperature for 30 min. The solution was diluted with a large volume of ethyl acetate and washed repeatedly with dilute hydrochloric acid, as well as with aqueous potassium hydrogen carbonate. Upon acidification of the alkaline extracts with hydrochloric acid, *N*-benzyloxycarbonylglycine (0·32 g., 80%, m. p. 120°) was obtained.

(ii) To a solution of *N*-benzyloxycarbonyl-*S*-benzoyl-L-cysteine phenacyl ester (0·95 g., 0·002 mole) in *NN*-dimethylformamide (5 ml.), sodium thiophenoxide (0·52 g., 0·004 mole) was added and the mixture was kept at room temperature for 30 min. Upon addition of dilute hydrochloric acid and concentration of the solution to about half of its volume, *N*-benzyloxycarbonyl-*S*-benzoyl-L-cysteine was precipitated. It was filtered off, washed with water, and then with ether; the yield was 0·7 g. (71%), m. p. 136—137°, undepressed upon admixture with an authentic sample.

(iii) To a solution of *N*-benzyloxycarbonyl-L-valyl-L-valine phenacyl ester (0·71 g., 0·0015 mole) in *NN*-dimethylformamide (4 ml.), sodium thiophenoxide (0·39 g., 0·003 mole) was added and the solution was kept at room temperature for 30 min. Water (15 ml.) was then added and the mixture was repeatedly extracted with ether. The aqueous layer was acidified with 5N-sulphuric acid, after removing *in vacuo* traces of ether; upon cooling, the *N*-benzyloxycarbonyl dipeptide crystallised. The yield was 0·38 g. (72%), m. p. 137—138°, $[\alpha]_D^{15}$ −36·8° (*c* 3·2 in 1N KOH) {lit.,[33] m. p. 139·5—140°, $[\alpha]_D$ −36·6° (*c* 3·2 in 1N KOH)}.

(*b*) *By hydrogenolysis*. A solution of glycine phenacyl ester hydrobromide (1·4 g., 0·005 mole) in aqueous methanol (80% v/v, 25 ml.) was hydrogenated over palladium-black catalyst. The hydrogenation was complete in 1 hr., 345 ml. of hydrogen (757 mm. Hg, 12°) being consumed. After removal of the catalyst by filtration and evaporating to dryness the filtrate, the residue was dissolved in absolute ethanol. Upon addition of a small amount of pyridine, glycine (0·27 g., 72%) was obtained (Found: N, 18·4. $C_2H_5NO_2$ requires N, 18·7%).

This investigation was supported by the Royal Hellenic Research Foundation. We thank Dr. D. Borovas and Dr. Ch. Hamalidis for assistance.

Laboratory of Organic Chemistry,
University of Athens,
Athens, Greece. [6/097 *Received, January 24th*, 1966]