New Methods in Peptide Synthesis. Part III. Protection of Carboxyl Group

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 by solutions of hydrogen chloride or hydrogen bromide in nitromethane, or 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-benzoxycarbonylamino-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,2 benzyl,4,5 or t-butyl6 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 amine 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 benzoylcarbonyl 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 method1,7 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,2,6 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,6 but unfortunately only the glycine derivative could be so obtained. This ester hydrochloride (I) is decomposed almost quantitatively in methanol or water-dioxan1 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°C is sufficient quantitatively to remove the ester group.

\[
\begin{align*}
\text{Ph}_2\text{C}-\text{NH}-\text{CH}_2\text{-CO}_2\text{H} & \quad \text{Ph}_2\text{C}-\text{NH}-\text{CH}_2\text{-CO}_2\text{Ph} \\
\text{H}_2\text{N}-\text{CH}_2\text{-CO}_2\text{H} & \quad \text{H}_2\text{N}-\text{CH}_2\text{-CO}_2\text{Ph} \\
\text{H}_2\text{O} & \quad \text{H}_2\text{O} \\
\text{Et}_3\text{N} & \quad \text{Et}_3\text{N} \\
\text{HCl} & \quad \text{HCl} \\
\text{acetone-water} & \quad \text{acetone-water} \\
\end{align*}
\]


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


Another mode of protection of the carboxyl group during peptide synthesis is offered by its transformation to the diphenylmethy] ester group,\textsuperscript{20-25} since these esters are easily hydrolysed to the free acids and diphenylmethane,\textsuperscript{20,25} as expected from the analogous behaviour of $O$-benzyl and $O$-trityl groups. Amino-acid diphenylmethyl esters have been prepared via $N$-protected amino-acids by esterification either indirectly through the corresponding silver salts,\textsuperscript{23} or directly with diphenylmethanol\textsuperscript{13} or diphenyldiazomethane.\textsuperscript{13} A simplified method for the preparation of diphenylmethyl esters of amino-acids and peptides has been published recently;\textsuperscript{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, \(\text{\textit{L}}\)-valine, \(\text{\textit{L}}\)-asparagine) are prepared either by the interaction of the silver salts of \(N\)-\(\text{o}\)-nitrophenylsulphenyl-\(L\)-,\textsuperscript{17} \(N\)-trityl-\(L\)-,\textsuperscript{16} or \(N\)-formyl-amino-acids with diphenylmethyl chloride,\textsuperscript{25} or by the action of diphenyldiazomethane on \(N\)-substituted amino-acids,\textsuperscript{118} followed (in each case) by the removal of the \(N\)-protecting group by an appropriate \(N\)-protecting diphenylmethyl group can subsequently be removed by hydrogenolysis.\textsuperscript{25,11-15} The diphenylmethyl esters can also be cleaved by hydrogen chloride,\textsuperscript{25,15} or by the action of approximately 0.15x-hydrogen bromide in nitromethane, ethyl acetate, etc.\textsuperscript{25} 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 \(N\)-benzoyloxy-carbonyl-\(L\)-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 \(N\)-benzoyloxy-carbonyl-dippeptide diphenylmethyl esters requires longer, so that the carbonyl group of \(N\)-benzoyloxy-carbonyl-\(L\)-valyl-\(L\)-valine diphenylmethyl ester is de-blocked 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,\textsuperscript{*} 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. \(N\)-Benzyloxy-carbonylaminio-acid or dipetide 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.\textsuperscript{26} The pH of these aqueous solutions drops slowly 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 \(N\)-,\textsuperscript{119} dicyclohexylcarbodi-imide method,\textsuperscript{20} the dipeptide diphenylmethyl esters of \(N\)-\(\text{o}\)-nitrophenylsulphenyl-\(L\)-valyl-\(L\)-valine (II) and \(N\)-benzoyloxy-carbonyl-\(L\)-valyl-\(L\)-valine (III) were obtained in good yield. Hydrogen chloride (2-3 equiv.) added to a solution of (II) splits

\footnotesize{
\textsuperscript{*} Nitromethane has a higher dielectric constant than ethyl acetate. To this physical property of nitromethane is also attributed the acceleration of the solvolysis of cyclohexylphenyl-carbonyl hydrogen phthalate by carboxylic acids.\textsuperscript{18}


\textsuperscript{15} Cf. ref. 7 in L. Zervas, A. Cosmatos, and P. Diamantis, \textit{Experientia}, \textit{1965}, 21, 5.


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.

\[
\text{NPS-L-Val + L-Val.ODPM} \xrightarrow{\text{DCCI}} \text{NPS-L-Val-L-Val.ODPM} \xrightarrow{\text{HCl, ether}} \text{HCl-L-Val-L-Val.ODPM} \xrightarrow{\text{or H}_{2}\text{O}, 100^\circ} \text{H}_{2}\text{Pd} \xrightarrow{\text{Z-L-Val-L-Val.ODPM}} \text{CF}_{3}\text{CO}_{2}\text{H}, 20^\circ, 30 \text{~min. or } 0-2\text{o-HCl} \text{ in nitromethane, } 30^\circ, 14 \text{~hr.}
\]

On the other hand, the benzoxycarbonyl-dipeptide ester (III) can be converted into the N-protected-dipeptide by the action of either hydrogen chloride or high concentrations of these reagents. Therefore, phthalimidomethyl ester group, like the t-butyl and the diphenylmethyl ester group reported in this Paper to be sensitive to hydrogenolysis, as described in the Experimental section for the homogeneity of the salts of the amino-acid derivatives synthesised was provided by thin-layer chromatography \(^22\) on Kieselgel G, in the solvent system n-butanol-acetic acid-water-pyridine; \(^27\) single spots (revealed by ninhydrin) were observed.

\[
\begin{align*}
\text{Ph-CH}_2\text{OCO-NHCH}_2\text{CH}_2\text{CO}_2\text{H} & \xrightarrow{\text{Ph-HBr}} \text{Ph-CH}_2\text{OCO-NHCH}_2\text{CH}_2\text{CO}_2\text{H} \\
\text{Ph-CH}_2\text{OCO-NHCH}_2\text{CH}_2\text{CO}_2\text{H} & \xrightarrow{\text{Ph-HBr}} \text{Ph-CH}_2\text{OCO-NHCH}_2\text{CH}_2\text{CO}_2\text{H}
\end{align*}
\]

The use of the phenacyl esters in peptide syntheses can be illustrated by the synthesis of N-benzoxycarbonyl-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.

**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 \textit{in vacuo} at 35–40\(^{\circ}\) 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 \(^22\) on Kieselgel G, in the solvent system n-butanol-acetic acid–water–pyridine; \(^27\) single spots (revealed by ninhydrin) were observed.

**Tryglycine Trityl Ester (I).—**—To a suspension of trityl chloride (7-0 g, 0-025 mole) was added a solution of tritylglycine \(^22\) (8-2 g, 0-025 mole) and triethylamine (3-5 ml, 0-025 mole) in tetrahydrofuran (30 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\(^{\circ}\)) was added to the syrupy residue. After cooling, the crystalline \textit{trityl ester} (13-1 g, 93\%) was collected by filtration, m. p. 168–170\(^{\circ}\), raised to 175–176\(^{\circ}\) after recrystallisation from absolute ethyl acetate \(\text{lit.}^6\) (without elemental data), m. p. 136\(^{\circ}\) (Found: C, 85-7; H, 6-3; N, 2-7. \(C_{10}H_{9}NO_3\) requires 85-9; H, 5-9; N, 2-5%).

**Glycine Trityl Ester Hydrochloride (I).—**—To a suspension of tritylglycine \textit{trityl ester} (1-1 g, 0-002 mole) in absolute acetone (20 ml), 5\% hydrochloric acid (0-4 ml) was added. After shaking for 5 min., the clear solution obtained was allowed to stand at 18\(^{\circ}\) 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 \textit{glycine trityl ester hydrochloride} 0-35–0-50 g.


(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. C₂₃H₆₄NO₃HCl requires N, 3.95; Cl, 10.0%).

**Removal of the Trityl Ester Group.**—(a) A solution of fresh glycine tryl 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 tryglycine 16. (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 crystalline residue was triturated with cold methanol and recrystallised from ethyl acetate-light petroleum to give the crystalline residue was triturated with cold methanol and recrystallised from ethyl acetate-light petroleum to give the crystalline residue was triturated with cold methanol and recrystallised from ethyl acetate-light petroleum.

**N-Tritylglycylglycine diphenylmethyl ester** was prepared from tryglycylglycine 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. C₂₃H₆₄NO₃ requires C, 80-0; H, 6.0; N, 6.0%).

**N-Benzoxycarbonyl-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-benzoxycarbonyl-L-proline 29 dissolved in 15 ml. of 1N-NaOH) and diphenylmethylen chloride (2.0 g, 0.01 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-benzoxycarbonylaminoo acid ester (61%), m. p. 96—97°, [α]₂₀ = -54.9° (c 4 in chloroform) (Found: C, 75.4; H, 5.7; N, 3.4. C₂₃H₆₄NO₃ requires C, 75.2; H, 6.1; N, 3.4%).

(b) **To a solution of N-benzoxycarbonyl-L-proline**

28 G. Stadniov, Ber., 1924, 57, 5.
L-Asparagine Diphenylmethyl Ester Hydrochloride.—(a) To a suspension of N-o-nitrophenylsulphonyl-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 acid by the addition of a small amount of N-o-nitrophenylsulphonyl-L-asparagine. Upon addition of an aqueous solution of silver nitrate (3.73 g., 0.018 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% m. p. 155°-157°), 5% hydrochloric acid* (4.4 ml.) was added, and after 5 min. at 20° the solution was concentrated (at 25°-30°) to dryness. Upon addition 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 (K2CO3). Addition of ether containing hydrogen chloride to the ether solution of the ester, precipitated crystalline L-asparagine diphenylmethy1 ester hydrochloride (1.6 g., 30%), m. p. 155°, raised to 159°-160° after recrystallisation from methanol–ether; [a]D 18 +1.4° (c 6.4 in DMF) (Found: C, 60.4; H, 6.1; N, 8.35; Cl, 10.9). C13H17N2O5.HCl requires C, 61.0; H, 5.7; N, 8.4; Cl, 10.6%. 

(b) Diphenyldiazomethane was added to a suspension of N-o-nitrophenylsulphonyl-L-asparagine in acetone according to the procedure described above for the preparation of the diphenylmethyl ester of N-benzylxycarbonyl-L-proline (case b). 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-nitrophenylsulphonyl 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-Nitrophenylsulphonyl-L-valine dicyclohexylammonium salt 7 was transformed to the silver salt as in the preparation of the corresponding N-trityl-L-valine 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, the amount of 5N-hydrochloric acid used was doubled. 7 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.

N-o-nitrophenylsulphonyl-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 (1.28 g., 67% after the purification step), m. p. 156°-157°, raised to 159°-160° after recrystallisation from ethyl acetate (recovery 98%); [a]D 29 +3.1° (c 0.8 in tetrahydrofuran), [a]D 20 -26.3° (c 5 in DMF) (Found: C, 67.4; H, 7.0; N, 4.5; Cl, 10.9. C13H17N2O5.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 acid 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 (0.1 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 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 dicyclohexylammonium 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-benzylxycarbonyl-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-Nitrophenylsulphonyl-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-nitrophenylsulphonyl-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-nitrophenylsulphonyl 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-
dipeptide ester hydrochloride was purified as usual (as described above for the preparation of L-asparagine diphenylmethyl ester hydrochloride). 

Diphenylmethane (90 ml.) containing triethylamine (2.2 g., 0.015 mole) was added to the residue at the last stage of the reaction mixture. After 6 hr. at room temperature, the solvent was evaporated to dryness, and the residue was dissolved in ethyl acetate. 

(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-benzylxoycarbonyl-L-valine 29 (4.1 g., 0.0165 mole) and NN'-dicyclohexylcarbodi-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., 66%), m. p. 136–137°, unchanged after a second recrystallisation from boiling tetrahydrofuran–ether (1:3 v/v); [α]D 20 −35°6 (c 5 in tetrahydrofuran) (Found: C, 72.2; H, 7.2; N, 5-4%). 

(b) N-Benzylxoycarbonyl-L-valyl-L-valine 29 was dissolved in acetone and the solution was treated with diphenyl-diazomethane as in the preparation of the diphenylmethanoyl ester of N-benzylxoycarbonyl-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-benzylxoycarbonyl-L-valyl-L-valine diphenylmethanoyl ester (71%), m. p. 136–137°. 

L-Valyl-L-valine Diphenylmethyl Ester Hydrochloride.—A mixture of N-o-nitrophenylsulphenyl-L-valine diclyclohexylammonium salt 7 (4.48 g., 0.01 mole) and L-valine diphenylmethanoyl 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 (60% v/v) were added, the precipitate (consisting of NN'-dicyclohexylurea contaminated with diclyclohexylamine hydrochloride) was filtered off, and the filtrate was worked up as described above for the preparation of the corresponding N-benzylxoycarbonyl dippeptide 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 diphenylmethanoyl ester hydrochloride (3.35 g., 80%), m. p. 180–181° after recrystallisation from tetrahydrofuran–ether; [α]D 20 −14°6 (c 8 in DMF) (Found: C, 55-9; H, 7-6; N, 6-7; Cl, 8.65. 

C29H29N3O7S.HCl requires C, 56-9; H, 7-5; N, 6-7; Cl, 8-5%). 

N-Benzylxoycarbonyl-L-proline 4:4'-dimethoxybenzylhydryl ester was prepared either (a) by the interaction of the corresponding silver salt with 4:4'-dimethoxydiphenylmethyl chloride as in the preparation of N-benzylxoycarbonyl-L-proline diphenylmethanoyl 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 both cases, 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-benzylxoycarbonyl-L-proline 4:4'-dimethoxydiphenylmethanoyl ester (75–80%), m. p. 80–81° and 83° after recrystallisation from ethyl acetate–light petroleum; [α]D 20 −53°6 (c 4-1 in chloroform) (Found: C, 71-0; H, 6-1; N, 2-7. C29H29N3O7S requires C, 70-7; H, 6-1; N, 2-9%). 

N-o-Nitrophenylsulphenyl-L-alanylglycine 4:4'-dimethoxydiphenylmethanoyl ester was prepared from the N-protected dipeptide 7 and 4:4'-dimethoxydiphenylmethyl chloride as in the preparation of the same ester of N-benzylxoycarbonyl-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; [α]D 20 −55°6 (c 5 in tetrahydrofuran) (Found: C, 59-1; H, 5-3; N, 7-95; S, 6-3. C29H29N3O7S 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% v/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 diphenylmethanoyl 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-benzylxoycarbonyl-L-valyl-L-valine diphenylmethanoyl 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 dippeptide was dissolved in hot water and the filtrate was concentrated to dryness to yield 0.18 g. (85%) of L-valyl-L-valine, [α]D 20 +10°8 (c 2 in water)
[lit. 25 \([\alpha]_D^{25} +10.8^\circ (c 2 \text{ in water})\)]. (ii) A solution of L-valyl-L-valine diphenylmethyl ester hydrochloride (0.41 g., 0.001 mole) in tetrahydrofuran (25 ml.) containing triethylamine (1 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%), \([\alpha]_D^{25} +10.0^\circ (c 2 \text{ in water})\) (lit. 26 \([\alpha]_D^{25} +10.8^\circ (c 2 \text{ in water})\)).

(c) **By the action of hydrogen bromide.** (i) L-Valine diphenylmethyl ester hydrochloride (0.032 g., 0.001 mole) was dissolved in warm nitromethane (12.3 ml.). As soon as the temperature of the solution dropped to 30\(^\circ\), 1,16-diethylhydrochloride solution (2.6 ml., 0.003 mole) in nitromethane was added. After 3 hr. at 30\(^\circ\), the crystalline precipitate of L-valine hydrochloride was collected by filtration (0.13 g., 84%); m. p. 220–223\(^\circ\) (decomp.), \([\alpha]_D^{25} +10.1^\circ (c 3.7 \text{ in water})\) 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\(^\circ\) (decomp.), \([\alpha]_D^{25} +16.3^\circ (c 5 \text{ in water})\), and exhibited the same behaviour on thin-layer chromatograms.

(ii) To a solution of N-benzyloxycarbonyl-L-proline diphenylmethyl ester hydrochloride (0.41 g., 0.001 mole) in ethyl acetate (13.3 ml.), 1-8s-hydrogen chloride solution (1.6 ml., 0.003 mole) in ethyl acetate was added. After 3 hr. at 30\(^\circ\), 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 and yielded starting material (0.36 g., 90%), m. p. 93–95.5\(^\circ\). The yield of starting material did not decrease much (85%), m. p. 92.5–93.5\(^\circ\) if the solution of N-benzyloxycarbonyl-L-proline in 0.2 ml of hydrogen chloride in ethyl acetate was kept at 30 for 20 hr.

A similar experiment was carried out in 0.2 ml of hydrogen chloride solution in nitromethane instead of ethyl acetate. After 3 hr. at 30\(^\circ\), the solution was exchanged with ethyl acetate and the resulting solution was washed as usual. The potassium hydrogen carbonate extracts were combined, acidified with 5\% 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\(^\circ\) (lit. 27, 73–75\(^\circ\), and 77\(^\circ\) for the recrystallised compound).

(iii) N-Benzoxycarbonyl-L-valyl-L-valine diphenylacetate 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 0.2 ml of hydrogen chloride solution in nitromethane). After 14 hr. at 30\(^\circ\), the reaction mixture was worked up in the usual way to yield N-benzoxycarbonyl-L-valyl-L-valine (80%), m. p. 137–138\(^\circ\) (lit. 28 m. p. 139–140\(^\circ\)).

(d) **By the action of hydrogen bromide.** (i) L-Valine diphenylmethyl ester hydrobromide (0.036 g., 0.001 mole) was suspended in 0.15 ml of 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\(^\circ\), 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\(^\circ\) (decomp.) unchanged after recrystallisation from ethanol–ether; \([\alpha]_D^{25} +12.0^\circ (c 2.4 \text{ in water})\) (Found: N, 6.9; Br, 40.5. C\(_8\)H\(_{14}\)NO\(_3\)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\(^\circ\)) 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-Benzoxycarbonyl-L-proline diphenylmethyl ester (0.41 g., 0.001 mole) was dissolved in trifluoroacetic acid \(38 \text{ (1 ml.) containing freshly distilled phenol (0.2 g.)}\). After 30 min. at 20\(^\circ\), the solution was concentrated (within 5–7 min.) to 20\(^\circ\) 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\(^\circ\), \([\alpha]_D^{25} -40.7^\circ (c 2 \text{ in ethanol})\) (lit. 29 m. p. 73–74\(^\circ\), \([\alpha]_D^{20} -40.6^\circ (c 2 \text{ in ethanol})\).

(ii) N-Benzoxycarbonyl-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\(^\circ\), \([\alpha]_D^{25} -35.0^\circ (c 2.2 \text{ in 1N-KOH})\) (lit. 28 m. p. 139–140\(^\circ\), \([\alpha]_D^{20} -36.6^\circ (c 2.2 \text{ in 1N-KOH})\)). This compound was converted into the diphenylmethyl ester derivative by the action of diphenyl-diazomethane, as described above.

**Removal of the 4-4'-Dimethoxydiphenylmethyl Ester Group.—** (a) **In water-containing solution.** The N-benzoxycarbonyl-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\(^\circ\) 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\(^\circ\) was recovered from the organic layer, whereas N-benzyloxycarbonyl-L-proline (0.12 g., 49%), m. p. 73–74\(^\circ\) 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\(^\circ\). (N-Benzoxycarbonyl-L-proline diphenylmethyl ester was recovered in 73% yield, after refluxing for 4 hr. a solution of the ester in methanol.)

**N-Benzoxycarbonylglycine Phenacyl Ester.—** To a solution of N-benzyloxycarbonylglycine \(37 \text{ (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 acid and water.**


M. Bergmann and L. Zervas, Ber., 1932, 65, 1192.

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

4 K
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, 5-56; N, 4-4. C₁₈H₁₅NO₄ requires C, 66-05; H, 5-2; N, 4-3%).

N-Benzylxycarbonyl-L-alanine Phenacyl Ester.—(a) A solution of N-benzylxycarbonyl-L-alanine 28,27 (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-benzylxycarbonyl-L-alanine phenacyl ester was 2-9 g. (89%), m. p. 104-105° unchanged after recrystallisation from ethanol; [α]D²⁰ + 26-5° (c 2 in chloroform) (Found: C, 66-9; H, 5-65; N, 4-1. C₁₈H₁₅NO₄ requires C, 66-85; H, 5-6; N, 4-1%).

(b) To a solution of N-benzylxycarbonyl-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-Benzylxycarbonyl-L-valine phenacyl ester was prepared from N-benzylxycarbonyl-L-valine 28 and phenacyl bromide in the way described for the corresponding glycine ester. Upon addition of ether-light petroleum the product crystallised (88%), m. p. 61-62°, [α]D²⁰ + 14-0° (c 2 in chloroform) (Found: C, 68-5; H, 6-3; N, 3-9. C₂₉H₂₉NO₄ requires C, 68-3; H, 6-3; N, 3-8%).

N-Benzylxycarbonyl-L-phenylalanine phenacyl ester was prepared from N-benzylxycarbonyl-L-phenylalanine 29 and phenacyl bromide in the described way for the corresponding glycine derivative; the yield was 77% after recrystallisation from ethanol, m. p. 100°, [α]D²⁰ + 3-5° (c 4 in chloroform) (Found: C, 72-0; H, 5-7; N, 3-1. C₂₉H₂₉NO₄ requires C, 71-95; H, 5-5; N, 3-3%).

N-Benzylxycarbonyl-L-leucine phenacyl ester was prepared from N-benzylxycarbonyl-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°), [α]D²⁰ + 25-5° (c 4 in chloroform) (Found: C, 69-1; H, 6-5; N, 3-5. C₂₉H₂₉NO₄ requires C, 68-9; H, 6-6; N, 3-6%).

N-Benzylxycarbonyl-L-aspartic acid Phenacyl Di-ester.—To a cold solution of N-benzylxycarbonyl-L-aspartic acid 27 (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; [α]D²⁰ + 1-5° (c 2 in chloroform) (Found: C, 66-8; H, 4-9; N, 2-6. C₂₉H₂₉NO₄ requires C, 66-8; H, 5-0; N, 2-8%).

N-Benzylxycarbonyl-S-benzoyl-L-cysteine phenacyl ester was prepared from N-benzylxycarbonyl-S-benzoyl-L-cysteine 28 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; [α]D²⁰ - 8-5° (c 2 in chloroform) (Found: C, 65-4; H, 5-05; N, 2-9; S, 6-6. C₂₉H₂₉NO₄S requires C, 65-4; H, 4-85; N, 2-9; S, 6-7%).

Glycine Phenacyl Ester Hydrobromide.—To a solution of N-benzylxycarbonylglycine phenacyl ester (1 g., 0-003 mole) in ethyl acetate (10 ml.), 3-5% hydrogen bromide solution (7 ml.) in acetic acid was added. After 45 min. at room temperature, ether was added to precipitate the amine-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. C₁₆H₁¹NO₅HBr requires Br, 29-15; N, 5-1%).

L-Alanine phenacyl ester hydrobromide was prepared in 96% yield from the corresponding N-benzylxycarbonyl 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; [α]D²⁰ + 1-5° (c 4 in methanol) (Found: C, 45-7; H, 4-95; Br, 27-95; N, 4-75. C₁₆H₁¹NO₅HBr requires C, 45-85; H, 4-9; Br, 27-7; N, 4-8%).

L-Valine Phenacyl Ester Hydrobromide.—To a solution of N-benzylxycarbonyl-L-valine phenacyl ester (3-7 g., 0-01 mole) in ether (35 ml.), 3-4% 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; [α]D²⁰ + 12-0° (c 2 in methanol) (Found: C, 49-5; H, 5-85; Br, 25-45; N, 4-4. C₂₉H₂₉NO₄HBr 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-benzylxycarbonyl derivative in the way described for glycine phenacyl ester hydrobromide; m. p. 158° after recrystallisation from methanol-ether; [α]D²⁰ + 1-1° (c 4 in methanol) (Found: C, 56-3; H, 5-3; Br, 22-3; N, 3-6. C₁₆H₁¹NO₅HBr 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-benzylxycarbonyl derivative as described for the glycine phenacyl ester salt; m. p. 159° after recrystallisation from methanol-ether; [α]D²⁰ + 15-7° (c 2 in methanol) (Found: C, 51-1; H, 6-3; Br, 24-3; N, 4-2. C₁₆H₁¹NO₅HBr 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-benzylxycarbonyl 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; [α]D²⁰ + 10-3° (c 2 in methanol) (Found: C, 53-3; H, 4-8; Br, 18-0; N, 3-0. C₂₉H₂₉NO₅HBr requires C, 53-3; H, 4-5; Br, 17-7; N, 3-1%).

S-Benzoyl-L-cysteine phenacyl ester hydrobromide was prepared.
prepared in 94% yield from the corresponding N-benzoyloxy-carbonyl 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; $[\alpha]_D^{15} = -38.0^\circ$ (c 2 in dimethyl-formamide) (Found: Br, 18.9; N, 3.2; S, 7.35. C$_{19}$H$_{17}$NO$_4$S.HBr requires Br, 18.8; N, 3.3; S, 7.55%).

**N-Benzoyloxy-carbonyl-L-alanyl-L-glycine 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-benzoyloxy-carbonyl-L-alanine (1.15 g., 0.005 mole) was added, followed by the immediate addition of NN'-dicyclohexylcarbodi-imide (1.1 g., 0.0033 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, diluted 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 required dipeptide ester hydrobromide (80%), m. p. 120°.

Removal of the Phenacyl Ester Group. —(a) By sodium thiophenoxide. (i) A solution of N-benzoyloxy-carbonyl-L-cysteine phenacyl ester (0.65 g., 0.002 mole) and sodium thiophenoxide (0.52 g., 0.004 mole) in NN-dimethyl-formamide (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-benzoyloxy-carbonylglycine (0.32 g., 80%, m. p. 137°) was obtained.

(ii) To a solution of N-benzoyloxy-carbonyl-L-valyl-L-valine phenacyl ester hydrobromide (80% v/v, 25 ml.) was added water (15 ml.) 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-benzoyloxy-carbonyl-L-valyl-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-benzoyloxy-carbonyl-L-valyl-L-valine phenacyl ester (0.71 g., 0.0015 mole) in NN-dimethyl-formamide (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 repeated extracted with ether. The aqueous layer was acidified with 5N-sulphuric acid, after removing in vacuo traces of ether; upon cooling, the N-benzoyloxy-carbonyl dipeptide crystallised. The yield was 0.38 g. (72%), m. p. 137–138°, $[\alpha]_D^{15} = -36.8^\circ$ (c 3.2 in 1N KOH) {lit. $^{33}$ m. p. 139–140°, $[\alpha]_D^{15} = -36.6^\circ$ (c 3.2 in 1N KOH)}.

(b) By hydrolysis. A solution of glycine phenacyl ester hydrobromide (1.4 g., 0.005 mole) in aqueous methanol (50% 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$_{14}$H$_{14}$N$_2$O$_4$ 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.** [6/907 Received, January 24th, 1966]