# Amino-acids and Peptides. Part XXVI.[1] The Use of 1-Piperidyl Esters in Peptide Synthesis: Further Studies

By **J. H. Jones, B. Liberek,** and **G. T. Young,** The Dyson Perrins Laboratory, Oxford University

Further examples of the use of 1-piperidyl esters for the synthesis of protected dipeptides have given good yields of pure product except when two valine residues were involved (in which case the reaction of the analogous *p*-nitrophenyl ester was also very slow), or when one residue was α-aminoisobutyric acid. The reactions of benzyloxycarbonylamino-acid 1-piperidyl esters with the methyl esters of L-leucyl-L-leucylglycine, L-phenylalanyl-L-leucylglycine, and L-valyl-L-eucylglycine proceeded more slowly than with α-amino-esters but satisfactory yields were obtained except in one case, again involving valine.

PART XXIV [2] described the use of the acid-catalysed condensation of 1-piperidyl esters for the synthesis of pure protected dipeptides in high yield. Weygand and his co-workers [3] developed a convenient preparation of acyldipeptide 1-piperidyl esters, and gave many examples of their use in the racemisation-free synthesis of protected tripeptides. However, they found that the condensation of benzyloxycarbonyl-L-phenylalanyl-L-isoleucine 1-piperidyl ester with L-phenylalanyl-L-isoleucylglycine methyl ester and with L-prolyl-L-tyrosyl-L-isoleucine methyl ester gave low yields. We have continued our investigations and record here our further experience in the synthesis of a wide range of peptides by this method; some of the results have been briefly reported earlier.[4]

Table 1 extends the examples of couplings to form dipeptides; the results may be compared with those obtained by Method II in Part XXIV.[2] The sum of our experience is that in such cases good yields can be obtained in a reasonable reaction time at room temperature except when both components are severely hindered, as in the coupling of benzyloxycarbonyl-L-valine 1-piperidyl ester with L-valine methyl ester (Experiment 8); the use of 1,2,4-triazole as catalyst [5] in place of acetic acid in this reaction effected no improvement, and raising the temperature is not helpful because of decomposition of the 1-piperidyl ester. Steric hindrance is of course encountered with other modes of coupling, and the condensation of benzyloxycarbonyl-L-valine *p*-nitrophenyl ester with L-valine methyl ester was also still incomplete after 7 days at room temperature (Experiment 10), but steric factors do appear to be especially important in the case of 1-piperidyl esters. α-Amino-isobutyric acid (Experiment 9) presents of course an extreme and special case.[6]

To investigate the effect of the size of the amino-component, 1-piperidyl esters were condensed with L-leucyl-L-leucylglycine methyl ester, under the conditions (including the same reaction time) which gave satisfactory yields when the amino-component was L-leucine methyl ester. Table 2 shows that the retardation due to the increased length of chain is very marked. However, by raising the concentrations of the reactants and the proportion of the active ester, the rate can be increased considerably, and Table 3 gives the results obtained, with a reaction time of 72 hours at room temperature, in the synthesis of the following derivatives of L-leucyl-L-leucylglycine methyl ester: benzyloxycarbonyl-glycyl, -L-alanyl, -L-leucyl, -L-phenylalanyl, L-valyl, and -glycyl-L-phenylalanyl; the following derivatives of L-phenylalanyl-L-leucylglycine methyl ester: benzyloxycarbonyl-glycyl, -L-alanyl, -L-leucyl, and -L-phenylalanyl; and the following derivatives of L-valyl-L-leucylglycine methyl ester: benzyloxycarbonyl-L-alanyl and -L-phenylalanyl. All these products are new. The yields are satisfactory except, again, in a coupling of benzyloxycarbonyl-L-valine 1-piperidyl ester (Experiment 5). The high yield in the '2 + 3' synthesis of the pentapeptide (Experiment 6) is particularly noteworthy. We report here also the condensation of benzyloxycarbonylglycine

[1] Part XXV, I. Antonovics and G. T. Young, *J. Chem. Soc.* (*C*), 1967, 595.
[2] B. O. Handford, J. H. Jones, G. T. Young, and (in part) T. F. N. Johnson, *J. Chem. Soc.*, 1965, 6814.
[3] F. Weygand, W. König, E. Nintz, D. Hoffman, P. Huber, N. M. Khan, and W. Prinz, *Z. Naturforsch.*, 1966, **21***b*, 325.
[4] J. H. Jones, B. Liberek, and G. T. Young, 'Proceedings 8th European Peptide Symposium,' Noordwijk, 1966, ed. H. C. Beyerman, A. van der Linde, and W. Maassen van den Brink, North Holland, 1967, p. 15.
[5] H. C. Beyerman and W. Maassen van den Brink, *Proc. Chem. Soc.*, 1963, 266.
[6] M. T. Leplawy, D. S. Jones, G. W. Kenner, and R. C. Sheppard, *Tetrahedron*, 1960, **11**, 39.

TABLE 1

The synthesis of protected dipeptides

| No. | Active ester [a] | Amino-component [a] | Time (hr.) | Yield (%) |
|---|---|---|---|---|
| 1 | Z.Gly.OPip | Gly.OMe | 1·2 | 76 [b] |
| 2 | ,, | Ala.OMe | 3·5 | 78 [c] |
| 3 | Z.Ala.OPip | Gly.OMe | 1·5 | 82 [d] |
| 4 | Z.Gly.OPip | Leu.OMe | 7·0 | 68 [e] |
| 5 | Z.Leu.OPip | Gly.OMe | 2·0 | 81 [f] |
| 6 | Z.Gly.OPip | Val.OMe | 9·0 | 75 [g] |
| 7 | Z.Val.OPip | Gly.OMe | 7·0 | 64 [h] |
| 8 | Z.Val.OPip | Val.OMe | — | — |
| 9 | Z.Gly.OPip | Aib.OMe | — | — |
| 10 | Z.Val.ONp | Val.OMe | — [i] | — |

[a] Abbreviations follow the Rules in 'Abbreviated Designation of Amino-Acid Derivatives and Polypeptides' (Information Bulletin No. 25, I.U.P.A.C.). Z = $PhCH_2OCO$; Pip = 1-piperidyl; ONp = $OC_6H_4{\cdot}NO_2$-*p*; Aib = α-aminoisobutyric acid. Optically active amino-acids were of the L-form. The conditions for the reactions are given in the Experimental section. The yields and constants in the Table refer to the crude, chromatographically pure product (without recrystallisation) unless otherwise stated. [b] M. p. 64—65°; N. F. Albertson and F. C. McKay (*J. Amer. Chem. Soc.*, 1953, **75**, 5323) give m. p. 63—65°. [c] New compound: see Experimental section. [d] M. p. 94—96°, $[\alpha]_D^{20}$ −26·6° (*c* 1·0 in MeOH); K. T. Poroshin, V. G. Debabov, V. A. Shibnev, and T. D. Kozarenko (*Zhur. Obshchei Khim.*, 1961, **31**, 3006; *Chem. Abs.*, 1962, **56**, 15,604), give m. p. 94—96°, $[\alpha]_D^{20}$ −25° (*c* 5 in MeOH). [e] Oil; E. L. Smith and N. B. Slonim (*J. Biol. Chem.*, 1948, **176**, 835) give m. p. 64—66°; H. J. Panneman, A. F. Marx, and J. F. Arens (*Rec. Trav. chim.*, 1959, **78**, 487) obtained an oil. [f] M. p. 90—92°, $[\alpha]_D^{20}$ −24·1° (*c* 1·0 in MeOH); R. W. Young, K. H. Wood, R. J. Joyce, and G. W. Anderson (*J. Amer. Chem. Soc.*, 1956, **78**, 2126) give m. p. 92—94°, $[\alpha]_D^{25}$ −26·9° (*c* 5 in MeOH). [g] Oil; P. G. Katsoyannis (*J. Amer. Chem. Soc.*, 1961, **83**, 4053) gives m. p. 78°, $[\alpha]_D^{28}$ −15·5° (*c* 1·5 in EtOH). [h] M. p. 157—160° (previous softening), $[\alpha]_D^{20}$ −22·0° (*c* 1·0 in EtOH). K. Hofmann, E. Stutz, G. Spühler, H. Yajima, and E. T. Schwartz (*J. Amer. Chem. Soc.*, 1960, **82**, 3727) give m. p. 160—161°, $[\alpha]_D^{27}$ −24·3° (*c* 1·6 in EtOH). [i] Free valine methyl ester used; intense spots due to unchanged active ester were still observed on t.l.c. plates of samples taken after a reaction time of 7 days. [j] After 7 days an intense spot due to unchanged 1-piperidyl ester was still found on the t.l.c. plate; the product was worked up to give (after recrystallisation from di-isopropyl ether) 6 mg. of a white crystalline solid, m. p. 107—110°; the infrared spectrum, and the peak of the highest *m/e* of the mass spectrum (308) indicated this to be methyl benzyloxycarbonylglycyl-α-aminoisobutyrate.

TABLE 2

Comparisons of couplings with an amino-ester and a tripeptide ester

| No. | Active ester [a] | Amino-component [a] | Time (hr.) | Yield (%) |
|---|---|---|---|---|
| 1 | Z.Gly.OPip | Leu.OMe | 48 | 72 [b] |
| 2 | ,, | Leu.Leu.Gly.OMe | 48 | 54·5 [c] |
| 3 | Z.Leu.OPip | Leu.OMe | 75 | 75 [d] |
| 4 | ,, | Leu.Leu.Gly.OMe | 75 | 32 [e] |
| 5 | Z.Phe.OPip | Leu.OMe | 70 | 70 [f] |
| 6 | ,, | Leu.Leu.Gly.OMe | 70 | 36 [g] |

[a] See footnote *a* of Table 1. [b] See footnote *e* of Table 1. [c] M. p. 131—134°, with some melting and recrystallisation at *ca.* 111°; see Table 3, Experiment 1. [d] M. p. 97—98°; G. W. Anderson, J. Blodinger, R. W. Young, and A. D. Welcher (*J. Amer. Chem. Soc.*, 1952, **74**, 5304) give m. p. 93—95°; M. A. Nyman and R. M. Herbst (*J. Org. Chem.*, 1950, **15**, 108) give m. p. 97—98°. [e] M. p. 162—170° (with some change at *ca.* 155°); see Table 3, Experiment 3. [f] M. p. 107—110°; R. B. Woodward, R. A. Olofson, and H. Mayer (*J. Amer. Chem. Soc.*, 1961, **83**, 1010) give m. p. 109—109·5°. [g] M. p. 183—187°; see Table 3, Experiment 4.

1-piperidyl ester with L-leucylglycine methyl ester and with L-leucyl-L-leucine methyl ester; both proceeded satisfactorily.

Of the intermediates prepared for this work, benzyloxycarbonyl-L-valyl-L-leucylglycine methyl ester, and the methyl ester hydrochlorides of L-phenylalanyl-L-leucylglycine, of L-leucyl-L-leucylglycine, and of L-valyl-L-leucylglycine, are new. For the preparation of methyl α-aminoisobutyrate hydrochloride we found Brenner and Huber's method [7] using thionyl chloride more satisfactory than the usual Fischer esterification.

## EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus, and optical rotations on a Perkin-Elmer 141 automatic polarimeter with solutions in a 10 cm. cell. The nuclear magnetic resonance spectrum was measured (by Mrs. E. E. Richards) on a Perkin-Elmer R10 spectrometer with tetramethylsilane as internal standard and deuteriochloroform as solvent. The mass spectra were obtained (by Dr. R. T. Aplin) on an A.E.I. M.S.9 Mass spectrometer, using a direct inlet system at 150—200°. Thin-layer chromatography was on Kieselgel G (unbaked), using ether as solvent and iodine vapour for detection unless otherwise stated. Evaporation was by rotary evaporator and solutions in organic solvents were dried over magnesium sulphate. Samples for analysis were dried at 70°/0·1 mm. We thank Mr. W. Sabel (Oxford College of Technology) for supplies of 1-hydroxypiperidine.

*Synthesis of Protected Dipeptides* (*Table* 1).—The amino-ester hydrochloride (1·2 mmoles) was added to a suspension of finely powdered sodium acetate trihydrate (0·163 g., 1·2 mmoles) in a solution of the 1-piperidyl ester [2] (1·0 mmole) in dioxan (2 ml.). The mixture was stirred and the formation of 1-hydroxypiperidine and consumption of active ester were followed by t.l.c. When no ester remained, the solvent was evaporated and the residue was distributed between ethyl acetate and water. The organic phase was washed with 2N-hydrochloric acid, N-sodium hydrogen carbonate, and water and was then dried. Evaporation left chromatographically pure protected dipeptide. For Experiments 8 and 10 free amino-ester was used instead of the amino-ester hydrochloride with sodium acetate; in Experiment 10 the *p*-nitrophenyl ester was used in place of the 1-piperidyl ester. The results are shown in Table 1. The following compound is new.

*Benzyloxycarbonylglycyl-L-alanine Methyl Ester*.—The oil which remained after the evaporation of the solvent (see the above general procedure) was triturated with light petroleum; after 14 days at −5° (with occasional scratching) the white solid, m. p. 58—60° (0·241 g., 78%) was collected and recrystallised from ethyl acetate–light petroleum to give *protected dipeptide*, m. p. 62—64°; $[\alpha]_D^{20}$ −8·1° (*c* 1·0 in EtOAc); $\nu_{max.}$ ($CHCl_3$) 1740 (broad) and 1680 cm.$^{-1}$; τ 2·70 (5H, singlet, aromatic *H*); 3·0—3·7 (1H, broad, peptide CON*H*); 4·2—4·7 (1H, broad, urethane CON*H*); 4·90 (2H, singlet, $PhCH_2$); 5·1—5·7 [1H, complex, $NH{\cdot}CH(CH_3){\cdot}CO$]; 6·14 (2H, doublet $J$ = 6 c./sec., $NH{\cdot}CH_2{\cdot}CO$); 6·30 (3H, singlet, $OCH_3$); 8·64 [3H, doublet $J$ = 7 c./sec., $CH(CH_3)$]; *m/e* 294 (molecular ion) (Found: C, 57·0; H, 6·1; N, 9·2. $C_{14}H_{18}N_2O_5$ requires C, 57·1; H, 6·1; N, 9·5%).

[7] M. Brenner and W. Huber, *Helv. Chim. Acta*, 1953, **36**, 1109.

TABLE 3

Synthesis of protected tetra- and penta-peptides

| No. | Active ester [a] | Amino-component [a] | Crude product M. p. | Crude product Yield (%) | Recrystallised product M. p. | Recrystallised product $[\alpha]_D^{20}$ | Found (%) C | Found (%) H | Found (%) N | Formula | Required (%) C | Required (%) H | Required (%) N |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Z.Gly.OPip | Leu.Leu.Gly.OMe | 132—135° [b] | 80 | 135° | −45·2° [c] | 59·2 | 7·4 | 11·3 | $C_{25}H_{38}N_4O_7$ | 59·3 | 7·6 | 11·1 |
| 2 | Z.Ala.OPip | ,, | 176—178 [d] | 84 | 178 | −68·9 [c] | 59·9 | 7·8 | 11·3 | $C_{26}H_{40}N_4O_7$ | 60·0 | 7·7 | 10·8 |
| 3 | Z.Leu.OPip | ,, | 167—171 [e] | 62 | 169—171 | −66·0 [c] | 61·8 | 8·3 | 9·5 | $C_{29}H_{46}N_4O_7$ | 61·9 | 8·2 | 10·0 |
| 4 | Z.Phe.OPip | ,, | 183—185 [f] | 76·5 | 188—189 | −49·2 [g] | 64·4 | 7·4 | 9·0 | $C_{32}H_{44}N_4O_7$ | 64·4 | 7·4 | 9·4 |
| 5 | Z.Val.OPip | ,, | 200—204 [h] | 18 | 204—207 | −64·7 [i] | 61·7 | 8·3 | 10·9 | $C_{28}H_{44}N_4O_7$ | 61·3 | 8·1 | 10·2 |
| 6 | Z.Gly.Phe.OPip [j] | ,, | 164—172 [k] | 87 | 188 | −32·0 [c] | 62·85 | 7·25 | 10·6 | $C_{34}H_{47}N_5O_8$ | 62·5 | 7·25 | 10·7 |
| 7 | Z.Gly.OPip | Phe.Leu.Gly.OMe | 154—155 [d] | 80 | 155 | −25·5 [c] | 62·0 | 6·7 | 10·5 | $C_{28}H_{36}N_4O_7$ | 62·2 | 6·7 | 10·4 |
| 8 | Z.Ala.OPip | ,, | 186—187 [d, l] | 88 | 186—187 | −52·6 [c] | 62·2 | 6·7 | 9·7 | $C_{29}H_{38}N_4O_7$ | 62·8 | 6·9 | 10·1 |
| 9 | Z.Leu.OPip | ,, | 171—174 [h, m] | 70 | 173—174 | −53·8 [c] | 64·0 | 7·3 | 9·75 | $C_{32}H_{44}N_4O_7$ | 64·4 | 7·4 | 9·4 |
| 10 | Z.Phe.OPip | ,, | 176—179 [n] | 91 | 181—182 | −30·0 [o] | 66·3 | 6·7 | 9·25 | $C_{35}H_{42}N_4O_7$ | 66·65 | 6·7 | 8·9 |
| 11 | Z.Ala.OPip | Val.Leu.Gly.OMe | 197—199 [p] | 64 | 206—209 [p] | −21·0 [o] | 58·6 | 7·2 | 11·2 | $C_{25}H_{38}N_4O_7$ | 59·3 | 7·6 | 11·1 |
| 12 | Z.Phe.OPip | ,, | 202—204 [d] | 57 | 203—205 | −17·2 [q] | 63·0 | 7·2 | 9·6 | $C_{31}H_{42}N_4O_7$ | 63·9 | 7·3 | 9·6 |

[a] See footnote *a* of Table 1. [b] Some melting and recrystallisation occurred at 114°. Recrystallised from benzene–ether. [c] *c* 1·0 in MeOH. [d] Recrystallised from dichloromethane–ether. [e] Recrystallised from benzene–ether. [f] Some melting and recrystallisation occurred at 150—154°. Recrystallised from MeOH–$H_2O$ (9 : 1). [g] *c* 0·85 in MeOH. [h] Recrystallised from dichloromethane–di-isopropyl ether. [i] *c* 0·5 in MeOH. [j] Prepared as described by Dr. K. K. Gupta (to be published). [k] Recrystallised from MeOH–$H_2O$. [l] Additional dioxan (1 ml.) was added during the reaction to assist stirring, but a thick suspension of the product, which is sparingly soluble in dioxan, remained. [m] The crude product was triturated with di-isopropyl ether, because it was partially soluble in diethyl ether. [n] Additional dioxan (0·5 ml.) was added during the reaction to assist stirring. Recrystallisation was from methanol–ether. [o] *c* 1·0 in $HCONMe_2$. [p] The crude product dissolved readily in dichloromethane, from which it crystallised rapidly, the purified product being insoluble. [q] *c* 0·8 in $HCONMe_2$.

*The Use of* 1,2,4-*Triazole as Catalyst*.[5]—To L-valine methyl ester (0·10 g.) and benzyloxycarbonyl-L-valine 1-piperidyl ester [2] (0·16 g.) in dimethylformamide (1 ml.) was added 1,2,4-triazole (0·05 g.). After 11 days at room temperature the reaction was still incomplete and some unchanged 1-piperidyl ester was recovered.

*Comparisons of Couplings of* 1-*Piperidyl Esters with* L-*Leucine Methyl Ester and with* L-*Leucyl-*L-*leucylglycine Methyl Ester* (*Table* 2).—In Experiments 3—6 the procedure was essentially as described above for the synthesis of protected dipeptides. In Experiments 1 and 2, 0·5 mmole each of the 1-piperidyl ester,[2] of amino-ester hydrochloride, and of sodium acetate trihydrate, were used in 1 ml. of dioxan. The reactions were stopped at the stated time in order to compare the yields; the reactions with the tripeptide ester were in each case incomplete, and the crude product was therefore triturated with anhydrous ether and the solid product so obtained was washed well with ether to remove unchanged 1-piperidyl ester. The results are shown in Table 2; the constants for the purified protected tetrapeptides are reported in Table 3.

*Synthesis of Protected Tetra- and Penta-peptides* (*Table* 3).—The procedure was essentially as described for the preparation of the protected dipeptides (Table 1), except that 1·25 mmole of the 1-piperidyl ester [2] was used with 0·5 mmole each of the tripeptide ester hydrochloride (described below) and of sodium acetate trihydrate, in 1 ml. of dioxan; the time in each case was 72 hr., at room temperature. The crude product was triturated with ether, filtered off, and dried; the yield and m. p. are recorded in Table 3, which gives also the solvents for recrystallisation and the constants of the recrystallised product.

*Benzyloxycarbonylglycyl-*L-*leucylglycine Methyl Ester*.—Benzyloxycarbonyl-L-leucylglycine methyl ester [8] (0·673 g.) in methanol (10 ml.) containing acetic acid (0·137 ml.) was hydrogenated in the presence of palladium on charcoal (10%; 0·040 g.) for 5 hr. The catalyst was filtered off, and the filtrate was evaporated to dryness. To the oily residue of L-leucylglycine methyl ester acetate was added benzyloxycarbonylglycine 1-piperidyl ester [2] (0·585 g.) and chloroform (3 ml.). After 4 days, ethyl acetate (30 ml.) was added and the solution was washed and dried. After evaporation of the solvent the residue immediately crystallised; it was triturated with water, filtered, and washed with ether, giving crude protected tripeptide (0·56 g., 71%), m. p. 128—132° (lit.,[9] m. p. 131°).

*Benzyloxycarbonylglycyl-*L-*leucyl-*L-*leucine Methyl Ester*.—This was prepared as described above for benzyloxycarbonylglycyl-L-leucylglycine methyl ester by hydrogenation of benzyloxycarbonyl-L-leucyl-L-leucine methyl ester [10] and reaction of the unstable solid acetate so formed with benzyloxycarbonylglycine 1-piperidyl ester. The crude product (86% yield) had m. p. 126—131° (lit., m. p. 131—132°; [11] m. p. 133—134° [8]).

*Benzyloxycarbonyl-*L-*phenylalanyl-*L-*leucyglycine Methyl Ester*.—Benzyloxycarbonyl-L-leucylglycine methyl ester [8] (1·346 g.) in methanol (20 ml.) containing acetic acid (0·275 ml.) was hydrogenated in the presence of palladium on charcoal (10%; 0·08 g.) for 5 hr. The syrupy L-leucylglycine methyl ester acetate so obtained was dissolved in chloroform (8 ml.) and benzyloxycarbonyl-L-phenylalanine p-nitrophenyl ester [12] (1·68 g.) was added. After 72 hr., ethyl acetate (50 ml.) was added and the solution was washed with N-potassium hydroxide, 2N-hydrochloric acid, saturated sodium hydrogen carbonate, and water and was then dried. Evaporation of the solvent left a crystalline residue which was triturated with ether and collected, giving protected tripeptide (1·46 g., 76%), m. p. 173—174° (unchanged from benzene) (lit.,[3] m. p. 166—167°)$[\alpha]_{546}^{20}$ −40° (*c* 0·8 in MeOH) (Found: C, 64·6; H, 6·6; N, 8·55. Calc. for $C_{26}H_{33}N_3O_6$: C, 64·6; H, 6·9; N, 8·7%). We are

[8] R. W. Young, K. H. Wood, R. J. Joyce, and G. W. Anderson, *J. Amer. Chem. Soc.*, 1956, **78**, 2126.

[9] M. Bergmann, L. Zervas, and J. S. Fruton, *J. Biol. Chem.*, 1935, **111**, 225.

[10] G. W. Anderson, J. Blodinger, R. W. Young, and A. D. Welcher, *J. Amer. Chem. Soc.*, 1952, **74**, 5304.

[11] S. Simmonds, J. I. Harris, and J. S. Fruton, *J. Biol. Chem.*, 1951, **188**, 251.

[12] M. Goodman and K. C. Stueben, *J. Amer. Chem. Soc.*, 1959, **81**, 3980.

grateful to Professor F. Weygand for informing us that the specific rotation was erroneously reported in ref. 3, the correct value being $[\alpha]_{546}^{20}$ −39° (*c* 0·8 in absolute MeOH).

In analogous fashion was prepared benzyloxycarbonyl-L-leucyl-L-leucyglycine methyl ester of (70% yield), m. p. 110° (from benzene–ether) (lit.,[13] m. p. 108°), $[\alpha]_D^{20}$ −51° (*c* 1·0 in MeOH).

*Benzyloxycarbonyl-L-valyl-L-leucylglycine Methyl Ester.*—This was prepared as described for benzyloxycarbonyl-L-phenylalanyl-L-leucylglycine methyl ester except that the initial hydrogenation was carried out in the presence of hydrogen chloride (1 mol.) instead of acetic acid; the solvent for the coupling reaction was dimethylformamide (5 ml.) and triethylamine (1 mol.) was added to neutralise the hydrogen chloride. The crude product crystallised and had m. p. 174—175° (yield, 81%). Recrystallisation from dichloromethane–di-isopropyl ether gave the *ester* of unchanged m. p. and $[\alpha]_D^{20}$ −53° (*c* 1·0 in MeOH) (Found: C, 60·9; H, 7·5; N, 9·8. $C_{22}H_{33}N_3O_6$ requires C, 60·7; H, 7·6; N, 9·65%).

*L-Phenylalanyl-L-leucylglycine Methyl Ester Hydrochloride.*—The benzyloxycarbonyl derivative (0·967 g., 2 mmoles) was hydrogenated in anhydrous methanol (30 ml.) containing hydrogen chloride (2 mmoles) in the presence of palladium on charcoal (10%; 0·080 g.) for 4 hr. The catalyst was filtered off and the filtrate was evaporated to dryness. Anhydrous ether was added, and next day the crystalline product was collected and washed with ether; yield, nearly quantitative, m. p. 194—197°. For analysis the product was reprecipitated from methanol by ether, giving the *ester hydrochloride*, m. p. 196—199° (Found: C, 55·9; H, 7·3; Cl, 9·4; N, 10·25. $C_{18}H_{28}ClN_3O_4$ requires C, 56·0; H, 7·3; Cl, 9·2; N, 10·9%).

*L-Leucyl-L-leucylglycine Methyl Ester Hydrochloride.*—This was prepared as described for the L-phenylalanyl analogue above, giving a nearly quantitative yield of the *ester hydrochloride*, m. p. 170—171°, unchanged after reprecipitation from methanol by ether (Found: C, 50·6; H, 8·3; Cl, 10·3; N, 12·3. $C_{15}H_{30}ClN_3O_4$ requires C, 51·2; H, 8·5; Cl, 10·1; N, 11·95%).

*L-Valyl-L-leucylglycine Methyl Ester Hydrochloride.*—This was prepared as described for the L-phenylalanyl analogue above, giving the *ester hydrochloride* of m. p. 188—189° (Found: C, 49·95; H, 8·5; N, 11·9; Cl, 10·15. $C_{14}H_{28}ClN_3O_4$ requires C, 49·7; H, 8·4; Cl, 10·5; N, 12·4%).

*Methyl α-Aminoisobutyrate Hydrochloride.*—Thionyl chloride (2·0 ml.) was added dropwise to methanol (18 ml.) at −6°, and this temperature was maintained while α-aminoisobutyric acid (2·06 g.) was added in portions. The temperature was then raised to boiling point during 2·5 hr., and after 1 hr. further at boiling point the solvent was distilled off. The residue was then recrystallised from methanol–ether, giving amino-ester hydrochloride (2·34 g., 76%), m. p. 185—190° (lit.,[14] m. p. 180—181°).

We thank the S.R.C. for a Grant for a Special Research, the British Council for a Bursary (held by B. L.), the Salters' Company for a Scholarship (held by J. H. J.), and Imperial Chemical Industries Ltd., Pharmaceuticals Division, for financial support.

[7/778 *Received, June 26th,* 1967]

[13] M. Bergmann, L. Zervas, and J. S. Fruton, *J. Biol. Chem.*, 1936, **115**, 593.

[14] A. L. Barker and G. S. Skinner, *J. Amer. Chem. Soc.*, 1924, **46**, 403.