1160. *Peptides. Part XVII.*[1] *Synthesis of Peptides and Polymers of Some Sterically Hindered Amino-Acids* via *Oxazolone Intermediates*

By D. S. Jones, G. W. Kenner, J. Preston, and R. C. Sheppard

Further development of the oxazolone method for the synthesis of peptides of αα-disubstituted amino-acids is described. Peptide-oxazolones containing free terminal amino-groups have been prepared by hydrogenolysis of their benzyloxycarbonyl derivatives, and these undergo ready thermal polymerisation. The preparation of isotactic and syndiotactic polymers of α-ethylalanine is reported.

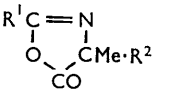
Part XI [2] surveyed the synthesis of peptides derived from the severely hindered amino-acid, α-methylalanine, and further experiments have been reported by Faust and Lange,[3] Faust and Kleppel,[4] and Diehl and Young.[5] In Part XI we concluded that most of the usual coupling methods were of limited value, generally because the peptide-forming reactions were so slow that either the starting materials were largely recovered or alternative, less hindered reactions supervened. However two methods, involving respectively the mixed anhydride of benzyloxycarbonyl-α-methylalanine and pivalic acid (Ia) and oxazolones (II) as activated intermediates, gave excellent yields of peptide derivatives. The latter method was especially applicable in this series, since problems of racemisation and acylation at the activated α-carbon atom which have prevented its use in conventional peptide synthesis, are absent with derivatives of αα-disubstituted amino-acids. This paper describes further examination of these methods and their application to the synthesis of oligo- and poly-peptides of α-methylalanine and DL-, D-, and L-α-ethylalanine (isovaline).

[1] Part XVI, D. S. Jones, G. W. Kenner, and R. C. Sheppard, *J.*, 1965, 4393.
[2] M. T. Leplawy, D. S. Jones, G. W. Kenner, and R. C. Sheppard, *Tetrahedron*, 1960, **11**, 39.
[3] G. Faust and H. Lange, *J. prakt. Chem.*, 1960, [4], **11**, 153.
[4] G. Faust and M. Kleppel, *J. prakt. Chem.*, 1960, [4], **11**, 133.
[5] J. Diehl and E. A. Young, *J. Medicin. Chem.*, 1964, **7**, 820.

Special interest is attached to polymers of the last amino-acid, since the methods to be described allow the preparation of polymers containing alternating D- and L-amino-acid residues, and in principle other defined repeating sequences, as well as stereochemically homogeneous polypeptides. Some observations on the synthesis of peptide derivatives of α-phenylalanine are also described, although the difficulties encountered in the early stages discouraged us from attempting to prepare polymers of this amino-acid.

$Z{\cdot}NH{\cdot}CRMe{\cdot}CO{\cdot}O{\cdot}CO{\cdot}CMe_3$

(Ia, R = Me)
(Ib, R = Et)
(Ic, R = Ph)
($Z = C_6H_5{\cdot}CH_2{\cdot}O{\cdot}CO$)



(IIa, $R^1 = CF_3$, R^2 = Et)
(IIb, $R^1 = CF_3$, R^2 = Ph)
(IIc, R^1 = H, R^2 = Me)
[IId, $R^1 = Z{\cdot}(NH{\cdot}CMe_2{\cdot}CO)_n{\cdot}NHCMe_2$, R^2 = Me]
[IIe, $R^1 = H{\cdot}(NH{\cdot}CMe_2{\cdot}CO)_n{\cdot}NHCMe_2$, R^2 = Me]
(IIf, $R^1 = NH_2{\cdot}CMe{\cdot}Et$, R^2 = Et)

As described in Part XI, benzyloxycarbonyl-α-methylalanine readily formed the crystalline mixed anhydride (Ia) when treated with pivaloyl chloride and triethylamine.[2] The trial of this mixed anhydride for peptide synthesis was originally prompted by the necessity for a stable activated derivative in which the adverse steric effects of the α-methylalanyl residue would be counterbalanced, *i.e.*, alternative reaction paths with amines would be at least as strongly sterically retarded as the desired peptide-forming reaction. The anhydride (Ia) reacted cleanly with α-methylalanine methyl ester in non-polar media yielding the dipeptide derivative almost exclusively. In more polar solvents, however, evidence was obtained for concurrent formation of some pivaloyl-α-methylalanine methyl ester. This must have arisen by attack of the amino-component at the alternative carbonyl group in the mixed anhydride, for the latter showed no tendency to disproportionate, even when heated at 100°. In the α-ethylalanine and α-phenylalanine series, however, the situation was less clear. Treatment of the triethylammonium salt of benzyloxycarbonyl-DL-α-ethylalanine with pivaloyl chloride yielded an oily product with typical anhydride bands in the infrared spectrum; the nuclear magnetic resonance spectrum was complex, and it is likely that the mixed anhydride (Ib) was contaminated with the symmetrical anhydride, as well as with pivalic anhydride. Reaction of the crude anhydride with α-methylalanine methyl ester afforded the crystalline dipeptide ester in 80% yield, but DL-α-ethylalanine methyl ester gave only an oil. The non-crystallinity of this product may be ascribed to its diastereoisomeric composition, but in view of the impure nature of the mixed anhydride, this approach was discontinued in favour of the oxazolone method described below.

The pivalic anhydride method was no more successful in the α-phenylalanine series. Both the benzyloxycarbonyl and *p*-phenylazobenzyloxycarbonyl derivatives of DL-α-phenylalanine were treated with pivaloyl chloride and triethylamine, but only in the latter case could a solid product be obtained. Furthermore, treatment of the crude anhydride from benzyloxycarbonyl-DL-α-phenylalanine with cyclohexylamine in benzene solution yielded 67% of the cyclohexylammonium salt of the acylamino-acid. Although disproportionation of the anhydride may have contributed to this result the high yield of salt shows that even in non-polar solvents, at least two thirds of the mixed anhydride was attacked by cyclohexylamine at the pivaloyl carbonyl group. Evidently the increased steric hindrance in the α-phenylalanine series was sufficient to overcome electronic factors favouring reaction at the acylamino-acid carbonyl. Reaction of the anhydride derived from *p*-phenylazobenzyloxycarbonyl-DL-α-phenylalanine with DL-α-phenylalanine t-butyl ester was very slow, and nuclear magnetic resonance spectroscopy provided clear evidence that disproportionation of the anhydride took place under the vigorous conditions necessary for reaction with the amino-component. Clearly further application of the mixed anhydride

method beyond the α-methylalanine series was unpromising, and attention was therefore turned to the use of oxazolone intermediates.

The use of oxazolones for the stepwise lengthening of benzyloxycarbonyl peptides is limited to the preparation of tri- and higher peptides, since benzyloxycarbonylamino-acids themselves do not form stable oxazolones. One way of avoiding this difficulty which was previously explored in the α-methylalanine series is to commence the synthesis with trifluoroacetylamino-acids, and to exchange the protecting group for benzyloxycarbonyl at the dipeptide or tripeptide stage.

In the past,[2,6] trifluoromethyloxazolones have usually been prepared *via* the acid chlorides of the corresponding trifluoroacetylamino-acids, which cyclised on heating, sometimes in the presence of tertiary base. This procedure worked well with trifluoroacetyl-α-phenylalanine, but yielded impure products in the α-ethylalanine series. A superior method for the preparation of 4-ethyl-4-methyl-2-trifluoromethyloxazolone (IIa) was found in the action of dicyclohexylcarbodi-imide on trifluoroacetyl-α-ethylalanine. Isolation of the oxazolone was unnecessary when prepared in this way, and addition of the appropriate amino-acid ester to the filtered solution yielded the dipeptide derivative directly. The methyl esters of the trifluoroacetyl derivatives of DL-α-ethylalanyl-α-methylalanine, DL-α-ethylalanyl-DL-α-ethylalanine, and the L–L and L–D isomers were prepared in this manner. Both protecting groups were cleaved simultaneously by alkaline hydrolysis, and then the free peptides were converted into their benzyloxycarbonyl derivatives. The α-phenylalanine series proceeded similarly, except that the trifluoroacetyl group proved to be stable to alkaline hydrolysis under the usual conditions. However, it was readily cleaved by methanolic hydrogen chloride. The resulting dipeptide ester showed little tendency to cyclise to the corresponding dioxopiperazine (in the α-methylalanine series the dipeptide ester cyclised too rapidly for isolation), but it could be hydrolysed with aqueous sodium hydroxide and the free dipeptide converted into its benzyloxycarbonyl derivative. The dipeptide ester was also extended to the fully protected tripeptide derivative by further reaction with 4-methyl-4-phenyl-2-trifluoromethyloxazolone (IIb).

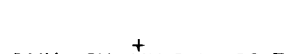
The realisation that dicyclohexylcarbodi-imide rapidly converts certain acylamino-acids into oxazolones* prompted a reinvestigation of our previous attempts to use this reagent for direct peptide synthesis in the α-methylalanine series. In Part XI it was considered that dicyclohexylcarbodi-imide was of negligible value for the joining of α-methylalanyl residues, since no dipeptide derivative was obtained from the condensation of formyl-α-methylalanine and α-methylalanine methyl ester.[2] However it is also known that the corresponding oxazolone (IIc) reacts with sterically hindered amines abnormally at C-2, yielding water soluble amidine derivatives (III) rather than formylamino-acid amides.[2] Reinvestigation of this condensation has shown that dicyclohexylcarbodi-imide does indeed rapidly convert formyl-α-methylalanine into the oxazolone (IIc), and therefore this reaction cannot be considered a fair test of the carbodi-imide method. In cases where oxazolone formation is impossible, *e.g.*, with the tosyl [2] and benzyloxycarbonyl derivatives of α-methylalanine, dicyclohexylcarbodi-imide is moderately efficient for peptide bond formation, and a yield of 64% of benzyloxycarbonyl-α-methylalanyl-α-methylalanine methyl ester has now been obtained with its aid. However the method is not general, for in the more sterically hindered condensation between benzyloxycarbonyl-α-phenylalanine and α-phenylalanine methyl ester, the major product (71%) was the corresponding acyl urea.

In the α-methylalanyl series, the benzyloxycarbonyl dipeptide was extended by successive oxazolone formation and reaction with amino-acid ester up to the hexapeptide level. Methyl esters were unsuitable for this process, since their hydrolysis was much less satisfactory than in the tosyl series.[2] Thus attempted saponification of benzyloxycarbonyl-α-methylalanyl-α-methylalanine methyl ester led to a mixture of the desired acid and the

* It is probable that the recently described [5] condensation reactions of benzyloxycarbonylglycyl-α-methylalanine with alanine and valine benzyl esters passed through the intermediate oxazolone.

[6] F. Weygand and U. Glöckle, *Chem. Ber.*, 1956, **89**, 653.

hydantion (IV). The corresponding tripeptide derivative required much more vigorous conditions for hydrolysis, and then only 10% of the acid and 43% of recovered ester were obtained. Acidic hydrolysis was successful for cleavage of the benzyloxycarbonyl dipeptide ester,[2] but under the same conditions the tripeptide derivative was largely degraded to benzyloxycarbonyl-α-methylalanyl-α-methylalanine. These results showed the



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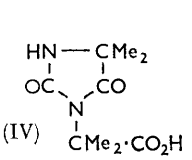

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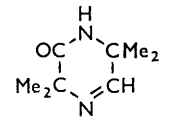

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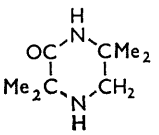

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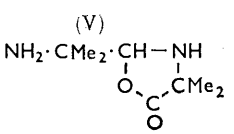

(VII)

need for a C-terminal protecting group removable under non-hydrolytic conditions, and for this purpose the t-butyl esters were selected, especially since their cleavage by anhydrous acid was not expected to depend on steric factors.

Although α-methylalanine t-butyl ester could not be obtained by direct acid-catalysed reaction of the amino-acid with isobutene,[7] it was readily prepared by Anderson and Callahan's method *via* the benzyloxycarbonyl derivative.[8] (In contrast, α-phenylalanine t-butyl ester was obtained in good yield by the former method.) Removal of the t-butyl ester groups from benzyloxycarbonyl-α-methylalanine and homologous peptide derivatives was easily effected by dissolution in trifluoroacetic acid at room temperature. Under more vigorous conditions (trifluoroacetic acid in refluxing benzene) the benzyloxycarbonyl tripeptide t-butyl ester was degraded like the methyl ester, and both the benzyloxycarbonyl dipeptide and free α-methylalanine were isolated.

Reaction of the benzyloxycarbonyl peptide oxazolones (IId, $n = 0$, 1, 2, and 3) with α-methylalanine t-butyl ester proceeded very cleanly, although with increasing chain length some lowering of oxazolone reactivity was evident. By a repetitive process of cleaving t-butyl esters with trifluoroacetic acid and forming oxazolones by brief warming with acetic anhydride, the benzyloxycarbonyl dipeptide was extended through twelve crystalline intermediates to the hexapeptide in better than 50% overall yield. The very high efficiency of this process and its freedom from side reactions suggested the use of oxazolones as intermediates in the polymerisation of α-methylalanine, and possibly also in the synthesis of cyclic peptides. Accordingly the removal of the benzyloxycarbonyl protecting groups from the oxazolones (IId, $n = 0$, 1, 2, 3, and 4) was investigated.

It was found that hydrogenolysis of the amino-protecting groups could be achieved with a palladium–carbon catalyst without serious reduction of the oxazolone double bond. Evaporation of solvent then yielded the rather unstable peptide oxazolones (IIe, $n = 0$, 1, 2, 3, and 4), the first three of which were obtained crystalline. The self-condensation of these peptide oxazolones was examined in the solid state and in concentrated solution, and also in dilute solution in the hope of obtaining cyclic peptides from the higher members (IIe, $n = 3$ and 4). In fact, only in the case of the tripeptide oxazolone (IIe, $n = 1$) was a monomeric product isolated,[9] and structural investigation of this will be described in detail elsewhere. The other peptide oxazolones yielded poly-α-methylalanine under all the conditions investigated, and the readily accessible dipeptide oxazolone (IIe, $n = 0$) was selected for a detailed study of this reaction.

This oxazolone is unusual amongst activated derivatives of dipeptides in not forming the corresponding dioxopiperazine readily. This result was expected since examination

[7] R. Roeske, *J. Org. Chem.*, 1963, **28**, 1251.
[8] G. W. Anderson and F. M. Callahan, *J. Amer. Chem. Soc.*, 1960, **82**, 3359.
[9] D. S. Jones, G. W. Kenner, and R. C. Sheppard, *Experientia*, 1963, **19**, 126; D. S. Jones, G. W. Kenner, J. Preston, and R. C. Sheppard, 6th European Peptide Symposium, Athens, 1963.

of molecular models had shown that the terminal amino-group could not reach the oxazolone carbonyl without serious deformation of bond angles. Traces of dioxopiperazine were formed in some polymerisations, but these only became serious when the solvent or other added reagent participated in the reaction. Thus, attempted polymerisation of the dipeptide oxazolone by warming in acetic acid solution resulted in near quantitative formation of dioxopiperazine, almost certainly *via* the intermediate mixed anhydride with acetic acid. It is probable that traces of dioxopiperazine observed in other polymerisation experiments arose through incomplete removal of acetic acid in the oxazolone-forming reaction. Dioxopiperazine was also formed substantially in an experiment in acetonitrile solution containing added triethylamine hydrochloride, possibly *via* an intermediate acyl triethylammonium ion. Two minor byproducts obtained in admixture from some polymerisations were recognised as the unsaturated oxopiperazine (V), and its saturated analogue (VI). There can be no doubt that these arise by initial reduction of the oxazolone double bond during hydrogenolysis because deliberately prolonged hydrogenation of (IId, $n = 0$) increased the yield of the saturated oxopiperazine (VI) to 66%. Saturation of the oxazolone ring would lead to the flexible oxazolidone (VII), in which the side-chain amino-group could easily attack the lactone carbonyl. The subsequent steps to (V) and (VI) are unexceptional.[10]

In order to polymerise the dipeptide oxazolone (IIe, $n = 0$), it was heated either in the solid state or in anhydrous solvents of varying dielectric constant, usually at 82° In the solutions the concentration of peptide oxazolone was adjusted to 1M for uniformity. In all cases the polymer precipitated during the reaction. Some typical results are collected in the Table. The viscosity measurements in dichloroacetic acid solution were kindly performed by Dr. H. Block, who also computed the approximate mean molecular weights and degrees of polymerisation, using a modified Doty, Bradbury, and Holtzer [11] calibration.

Evidently addition to oxazolones is not good enough for production of very high polymers under these conditions, despite being impressively efficient in stepwise synthesis of small peptides. There seems to be a terminal molecular weight which may be determined by insolubility; this would explain the slightly favourable effect of hexamethylphosphoramide as solvent. The nature of the side reactions and termination processes is unknown. A similar polymer can be prepared more easily *via* the carboxyanhydride.[12]

Solvent	Additive	Temp.	Time (hr.)	Yield of ppt.	Mean mol. wt.†	Mean D.P.†
Acetonitrile	—	82°	22	64	3710	44
Acetonitrile	LiCl	82	22	67	3730	44
Acetonitrile	NEt_3,HCl	82	15	72 *	4430	52
Pyridine	—	82	17	34	4550	54
Pyridine	—	82	70	20	5240	62
Dimethylformamide	—	82	22	57	4750	56
Dimethylformamide	LiCl	82	22	40	3780	45
Hexamethylphosphoramide	—	82	22	54	5550	65
Hexamethylphosphoramide	—	137	22	42	6380	75
None	—	82	22	—	5020	59

* Including *ca.* 30% dioxopiperazine. † Approximating to weight average.

The optically active dipeptide oxazolones (IIf) of α-ethylalanine were starting materials for preparation of both isotactic and syndiotactic polymers. The yield and chain length of the all-L polymer (61%, mean mol. wt. 8070) were markedly greater than those of the alternating L–D polymer (38%, 3890). These results parallel the coupling of L-4-ethyl-4-methyl-2-trifluoromethyloxazolone with L-α-ethylalanine methyl ester in 98% yield and with the D-ester in only 80% yield. Similarly, in carboxyanhydride polymerisations it has been reported that pure L-anhydrides polymerise more rapidly than DL-anhydrides.[13]

[10] Cf. L. T. Plante, W. G. Lloyd, C. E. Schilling, and L. B. Clapp, *J. Org. Chem.*, 1956, **21**, 82.
[11] P. Doty, J. H. Bradbury, and A. M. Holtzer, *J. Amer. Chem. Soc.*, 1956, **78**, 947.
[12] H. Weingarten, *J. Amer. Chem. Soc.*, 1958, **80**, 352; D. Coleman, *J.*, 1950, 3222.
[13] R. D. Lundberg and P. Doty, *J. Amer. Chem. Soc.*, 1957, **79**, 3961; M. Idelson and E. R. Blout, *ibid.*, 1958, **80**, 2387.

Our intention had been to examine the configurations of the isotactic and syndiotactic polymers by optical rotatory dispersion measurements, but they proved to be much too insoluble, even in *m*-cresol.

Experimental

All evaporations were under reduced pressure.

Derivatives of α-Methylalanine.—Benzyloxycarbonyl-α-methylalanine t-butyl ester. A solution of benzyloxycarbonyl-α-methylalanine (47 g.) in methylene chloride (400 ml.) containing concentrated sulphuric acid (2 ml.) was saturated with isobutene and set aside at room temp. for 67 hr. The solution was then washed with 5% aqueous sodium carbonate and water, dried (Na_2SO_4), and evaporated. Crystallisation of the oily residue from ether–light petroleum yielded the *t-butyl ester* (50·5 g., 88%), m. p. 60—61°, unchanged on further recrystallisation (Found: C, 65·5; H, 7·8; N, 5·0. $C_{16}H_{23}NO_4$ requires C, 65·5; H, 7·9; N, 4·8%).

α-Methylalanine t-butyl ester. Hydrogen was bubbled through a stirred solution of the foregoing benzyloxycarbonyl derivative (45 g.) in methanol (1·5 l.) containing 5% palladium–charcoal (2 g.) until carbon dioxide evolution ceased (12 hr.). The filtered solution was distilled, yielding the *ester* (19·8 g., 81%), b. p. 160—163° (Found: C. 60·6; H, 11·2; N, 9·15. $C_8N_{17}NO_2$ requires C, 60·3; H, 10·8; N, 8·8%).

2-(1′-*Benzyloxycarbonyl-α-methylalanylamino*-1′-*methyl*)*ethyl*-4,4-*dimethyloxazolone* (IId, n = 1). The general method for the preparation of oxazolones [2] yielded directly the pure *oxazolone* (100%), m. p. 124—125°, unchanged by recrystallisation from benzene (Found: C, 61·9; H, 7·1; N, 10·6. $C_{20}H_{27}N_3O_5$ requires C, 61·7; H, 7·0; N, 10·6%).

2-(1′-*Benzyloxycarbonyl*-α-*methylalanyl*-α-*methylalanylamino*-1′-*methyl*)*ethyl*-4,4-*dimethyl-oxazolone* (IId, n = 2). This *oxazolone* (100%) had m. p. 157—158°, raised to 158—159° by recrystallisation from benzene–light petroleum (Found: C, 61·0; H, 6·95; N, 11·9. $C_{24}H_{34}N_4O_6$ requires, C, 60·7; H, 7·2; N, 11·8%).

2-(1′-*Benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-α-methylalanylamino*-1′-*methyl*)-*ethyl*-4,4-*dimethyloxazolone* (IId, n = 3). This *oxazolone* (100%) had m. p. 200—201°, unchanged by recrystallisation from benzene–light petroleum (Found: C, 60·2; H, 7·3; N, 12·4. $C_{28}H_{41}N_5O_7$ requires C, 60·1; H, 7·4; N, 12·5%).

2-(1′-*Benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-α-methylalanyl*-α-*methylalanylamino*-1′-*methyl*)*ethyl*-4,4-*dimethyloxazolone* (IId, n = 4). This *oxazolone* (95%) had m. p. 233—234° after recrystallisation from ethyl acetate–light petroleum (Found: C, 59·5; H, 7·45; N, 12·9. $C_{32}H_{48}N_6O_8$ requires C, 59·6; H, 7·5; N, 13·0%).

Benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-α-methylalanine t-butyl ester. A solution of 2-(1′-benzyloxycarbonylamino-1′-methyl)ethyl-4,4-dimethyloxazolone (0·152 g., 0·5 mmole) and α-methylalanine t-butyl ester (0·12 g., 0·75 mmole) in dry acetonitrile (5 ml.) was heated under reflux during 8 hr. After evaporation of the acetonitrile the residue ws dissolved in ethyl acetate and the neutral fraction (0·205 g., 89%) isolated in the usual manner. The *ester* had m. p. 165—167°, raised by recrystallisation from ethyl acetate–light petroleum to 166·5—167·5° (Found: C, 62·0; H, 8·3; N, 9·1. $C_{24}H_{37}N_3O_6$ requires C, 62·2; H, 8·05; N, 9·1%).

Benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-α-methylalanyl-α-methylalanine t-butyl ester. A solution of 2-(1′-benzyloxycarbonyl-α-methylalanylamino-1′-methyl)ethyl-4,4-dimethyloxazolone (15·7 g., 40 mmoles) and α-methylalanine t-butyl ester (8·0 g., 50 mmoles) in dry acetonitrile (100 ml.) was heated under reflux during 10 hr. Isolation of the neutral product in the usual manner and recrystallisation from ethyl acetate–light petroleum yielded the *tetrapeptide ester* (21·61 g., 98·5%) m. p. 176—178° (Found: C, 61·5; H, 8·1; N, 10·0. $C_{28}H_{44}N_4O_7$ requires C, 61·3; H, 8·1; N, 10·2%).

Benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-α-methylalanyl-α-methylalanyl-α-methyl-alanine t-butyl ester. A solution of 2-(1′-benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-amino-1′-methyl)ethyl-4,4-dimethyloxazolone (4·74 g., 10 mmoles) and α-methylalanine t-butyl ester (2·2 g., 14 mmoles) in dry acetonitrile (40 ml.) was heated under reflux during 20 hr. (During this period the pentapeptide derivative partially crystallised.) Isolation of the neutral fraction in the usual manner and recrystallisation from ethyl acetate–light petroleum yielded the *pentapeptide ester* (5·42 g., 86%) m. p. 234—236° (decomp.) (Found: C, 60·5; H, 8·0; N, 11·3. $C_{32}H_{51}N_5O_8$ requires C, 60·6; H, 8·1; N, 11·05%).

Benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-α-methylalanyl-α-methylalanyl-α-methyl-alanyl-α-methylalanine t-butyl ester. A solution of 2-(1′-benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-α-methylalanylamino-1′-methyl)ethyl-4,4-dimethyloxazolone (3·9 g., 6·9 mmole)

and α-methylalanine t-butyl ester (1·75 g., 11 mmoles) in dry acetonitrile (50 ml.) was heated under reflux during 21 hr. (During this period the hexapeptide derivative partially crystallised.) Isolation of the neutral fraction in the usual manner and recrystallisation from ethyl acetate–light petroleum yielded the *hexapeptide ester* (4·15 g., 84%) m. p. 215—218° (Found: C, 60·3; H, 8·0; N, 11·9. $C_{36}H_{58}N_6O_9$ requires C, 60·1; H, 8·1; N, 11·7%).

Benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-α-methylalanine. (a) *From trifluoroacetyl-α-methylalanyl-α-methylalanyl-α-methylalanine methyl ester.* A mixture of trifluoroacetyl-α-methylalanyl-α-methylalanyl-α-methylalanine methyl ester [2] (15·3 g., 40 mmoles) and N-sodium hydroxide (120 ml.) was kept at room temp. overnight. The solution was then acidified to pH 6 with trifluoroacetic acid and evaporated. The crystalline residue was dissolved in a mixture of water (120 ml.) and acetone (100 ml.), and the solution brought to pH 10·8 by addition of N-sodium hydroxide. Benzyl chloroformate (14·4 g.) was added slowly to the cooled (0°) and stirred solution, the pH being maintained at 10·8 by concurrent addition of N-sodium hydroxide. Stirring was continued for 1 hr. at room temp. and the solution was then concentrated. Isolation of the acidic fraction in the usual manner and recrystallisation from aqueous methanol yielded the *benzyloxycarbonyl derivative* (15·6 g., 96%), m. p. 205—206° (Found: C, 59·15; H, 7·3; N, 10·1. $C_{20}H_{29}N_3O_6$ requires C, 58·95; H, 7·2; N, 10·3%).

(b) *From benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-α-methylalanine t-butyl ester.* A solution of the t-butyl ester (0·116 g.) in trifluoroacetic acid (2·5 ml.) was kept at room temp. for 1 hr. and then evaporated. Isolation of the acidic fraction in the usual manner yielded the benzyloxycarbonyl-tripeptide (0·096 g., 94%), m. p. 196—200°.

Benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-α-methylalanyl-α-methylalanine. (a) *From trifluoroacetyl-α-methylalanyl-α-methylalanyl-α-methylalanyl-α-methylalanine methyl ester.* A mixture of trifluoroacetyl-α-methylalanyl-α-methylalanyl-α-methylalanyl-α-methylalanine methyl ester (0·936 g., 2 mmole) and N-sodium hydroxide (6 ml.) was kept at room temp. overnight. Trifluoroacetic acid was added to pH 6 and the solution evaporated. The residue was dissolved in water (44 ml.) and acetone (36 ml.), and brought to pH 10·6 by addition of N-sodium hydroxide. Benzyl chloroformate (0·42 g.) in acetone (8 ml.) was added slowly to the cooled and stirred solution, the pH being maintained at 10·6 by further addition of alkali. Stirring was continued at room temp. for 1 hr. and the acidic product isolated in the usual manner. The *benzyloxycarbonyl-tetrapeptide* (0·975 g., 99%) had m. p. 239—243° (decomp.); recrystallisation from aqueous methanol yielded 0·901 g. (92%), m. p. 241—244° (decomp.). For analysis, a sample was recrystallised once further, m. p. 243—244° (Found: C, 58·5; H, 7·1; N, 11·3. $C_{24}H_{36}N_4O_7$ requires C, 58·5; H, 7·4; N, 11·4%).

(b) *From benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-α-methylalanyl-α-methylalanine t-butyl ester.* The t-butyl ester (19·2 g., 35 mmoles) dissolved in trifluoroacetic acid (50 ml.) was kept at room temp. for 1 hr. and the solution was then evaporated. Isolation of the acidic product yielded the benzyloxycarbonyl-tetrapeptide (16·6 g., 96·5%) m. p. 236—241° (decomp.), raised to 243—245° by one recrystallisation from aqueous methanol.

Benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-α-methylalanyl-α-methylalanyl-α-methylalanine. A solution of benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-α-methylalanyl-α-methylalanyl-α-methylalanine t-butyl ester (4·00 g., 0·3 mmole) in trifluoroacetic acid (10 ml.) was kept at room temp. during 1 hr. and then evaporated. Isolation of the acidic product yielded the *benzyloxycarbonyl-pentapeptide* (3·54 g., 97·5%) m. p. 245—248° (decomp.). Recrystallisation from aqueous methanol yielded 3·36 g. (92·5%), m. p. 254—256° (decomp.) (Found: C, 58·2; H, 7·5; N, 12·1. $C_{28}H_{43}N_5O_8$ requires C, 58·2; H, 7·5; N, 12·1%).

Benzyloxycarbonyl-α-methylalanyl-α-methylalanyl-α-methylalanyl-α-methylalanyl-α-methylalanyl-α-methylalanine. A solution of the t-butyl ester (3·81 g., 5·3 mmoles) in trifluoroacetic acid (10 ml.) was kept at room temp. during 1 hr. and then evaporated. The residue was shaken with 5% aqueous sodium carbonate and ethyl acetate. Acidification of the sodium carbonate layer precipitated the *benzyloxycarbonyl-hexapeptide*, which was recrystallised from aqueous methanol. Yield 3·35 g. (96%), m. p. 243—245° (decomp.) (Found: C, 57·8; H, 7·9; N, 12·9. $C_{32}H_{50}N_6O_9$ requires C, 58·0; H, 7·6; N, 12·7%).

Trifluoroacetyl-α-methylalanyl-α-methylalanyl-α-methylalanine methyl ester. The following procedure gives an improved yield over that previously reported.[2] Dry hydrogen chloride was bubbled through a boiling solution of trifluoroacetyl-α-methylalanyl-α-methylalanine methyl ester (3·0 g., 10 mmoles) in dry methanol (200 ml.) during 2½ hr. The solution was evaporated and the hygroscopic residue dissolved in dry acetonitrile (40 ml.). Triethylamine (1·09 g.,

10·7 mmole) was added, the solution filtered and 4,4-dimethyl-2-trifluoromethyloxazolone (1·95 g., 10·7 mmole) added to the ice-cooled filtrate. The mixture was kept for 10 min. at 0° before being boiled for 10 min. and evaporated. Isolation of the neutral product in the usual manner and crystallisation from ethyl acetate yielded the tripeptide derivative (2·98 g., 78%), m. p. 197—198°. Leplawy *et al.*[2] report 60% yield and m. p. 197—199°.

Trifluoroacetyl-α-methylalanyl-α-methylalanyl-α-methylalanyl-α-methylalanine methyl ester. The above procedure applied to trifluoroacetyl-α-methylalanyl-α-methylalanyl-α-methylalanine methyl ester furnished the *tetrapeptide-derivative* (54%) m. p. 238—240° (Found: C, 49·0; H, 6·7; N, 12·25. $C_{19}H_{31}F_3N_4O_6$ requires C, 48·7; H, 6·7; N, 12·0%).

2-(1′-*Amino*-1′-*methyl*)*ethyl*-4,4-*dimethyloxazolone* (IIe, $n = 0$). A solution of 2-(1′-benzyloxycarbonylamino-1′-methyl)ethyl-4,4-dimethyloxazolone (6 mmoles) in dry ethyl acetate (90 ml.) containing 5% palladium–charcoal catalyst (0·5 g.) was stirred and hydrogen was passed over the surface until evolution of carbon dioxide ceased (3½ hr.). After filtration, the solution was evaporated at room temperature yielding the crystalline *oxazolone* (97%), m. p. 68° (Found: C, 56·7; H, 8·6; N, 16·3. $C_8H_{14}N_2O_2$ requires C, 56·45; H, 8·3; N, 16·5%).

Similarly, hydrogenolysis of the benzyloxycarbonyl derivatives (IId, $n = 1$) and (IId, $n = 2$) yielded the crystalline amino-oxazolones (IIe, $n = 1$) (100%, m. p. 82—87°, and (IIe, $n = 2$) (97%, m. p. 130—132°), both of which polymerised rapidly on standing at room temp. Hydrogenolysis of the benzyloxycarbonyl derivatives (IId, $n = 3$) and (IId, $n = 4$) yielded unstable oily oxazolones.

Polymerisation of 2-(1′-*amino*-1′-*methyl*)*ethyl*-4,4-*dimethyl-oxazolone* (IIe, $n = 0$). (a) *General procedure.* Freshly prepared oxazolone (2—6 mmoles) was dissolved in anhydrous solvent (2—6 ml.) to give a M-solution, which was heated for 15—70 hr. usually at 82°. The progress of the polymerisation could be followed by the diminution of the oxazolone carbonyl absorption at *ca.* 1820 cm.$^{-1}$. The precipitated poly-α-methylalanine was collected after cooling and washed well. Specific viscosities were determined for solutions in dichloroacetic acid (*ca.* 0·12 g. of polymer in 25 ml. solvent) using a U-tube viscometer at 25°.

(b) *In acetic acid solution.* The oxazolone (IIe, $n = 0$) (0·96 g., 6 mmoles) was dissolved exothermically in anhydrous acetic acid (6 ml.), and the solution was then heated at 82°. After 1 hr., a sample (0·5 ml.) of the solution was withdrawn and evaporated. The infrared spectrum of the residue showed no oxazolone absorption at 1820 cm.$^{-1}$. After 17 hr. at 82°, the crystalline precipitate (0·058 g.) was collected and identified as pure 2,2,5,5-tetramethyl-3,6-dioxopiperazine by its infrared spectrum. Evaporation of the filtrate yielded a crystalline residue (0·882 g.) with identical infrared spectrum.

(c) *In dimethylformamide solution.* The oxazolone (IIe, $n = 0$) (0·96 g., 6 mmoles) was dissolved in dry dimethylformamide and the solution heated at 82° for 22 hr. The precipitated polymer (0·59 g.) was collected and the filtrate evaporated yielding a solid residue (0·285 g.). This product (0·188 g.) was heated at 150—155°/0·1 mm. yielding a crystalline sublimate and residual polymer. The sublimate was resublimed at 100—110°/0·1 mm. yielding involatile 2,2,5,5-tetramethyl-3,6-dioxopiperazine (0·01 g., 1%) and volatile 3,4-dehydro-2,2,5,5-tetramethyl-6-oxopiperazine (0·07 g., 7%) (Found: C, 62·0; H, 9·3; N, 18·0. Calc. for $C_8H_{14}N_2O$: C, 62·3; H, 9·15; N, 18·2%), identified by its infrared and nuclear magnetic resonance spectra. Electrometric titration in 78·5% aqueous methylcellosolve solution indicated the presence of the saturated analogue (12%) with pK^*_{MCS} 5·1.

Prolonged catalytic hydrogenation of 2-(1′-*benzyloxycarbonylamino*-1′-*methyl*)*ethyl*-4,4-*dimethyl-oxazolone.* A solution of 2-(1′-benzyloxycarbonylamino-1′-methyl)ethyl-4,4-dimethyloxazolone (0·619 g., 2 mmoles) in dry ethyl acetate (100 ml.) containing 5% palladium–charcoal catalyst (1·0 g.) was stirred and hydrogen passed over the surface until evolution of carbon dioxide ceased (3 hr.) and for 42 hr. further. The filtered solution was evaporated and the residue (0·245 g., 78%) dissolved in ethyl acetate (3 ml.), filtered and re-evaporated. The residue (0·218 g.) was heated at 100—110°/0·5 mm. in a sublimation tube for 21 hr. The crystalline sublimate (0·184 g.) was identified by its infrared and nuclear magnetic resonance spectra as 2,2,5,5-tetramethyl-3-oxopiperazine (Found: C, 61·7; H, 10·3; N, 17·8. Calc. for $C_8H_{16}N_2O$: C, 61·5; H, 10·3; N, 17·9%). Electrometric titration gave pK^*_{MCS} 5·1, Equiv., 170 (calc. 156); the impurity was shown to be the 3,4-dehydro-derivative by n.m.r. and infrared spectroscopy.

Reaction of formyl-α-methylalanine with NN′-dicyclohexylcarbodi-imide. Dicyclohexylcarbodi-imide (1·03 g., 5 mmoles) was added to a suspension of formyl-α-methylalanine (0·66 g.,

5 mmole) in dry methylene dichloride (23 ml.), and the mixture shaken overnight. After removal of precipitated dicyclohexylurea (1·06 g., 95%), the solution was shown to contain dimethyloxazolone (infrared spectrum [2]). α-Methylalanine methyl ester (0·59 g., 5 mmole) was added to the methylene dichloride solution, and the mixture set aside overnight. Dilution with ethyl acetate precipitated *NN'*-bis-(1-carboxy-1-methyl-ethyl)formamidine monomethyl ester [2] (0·82 g., 75%), m. p. 145—146° (decomp.), with infrared spectrum identical to that already described.[2]

Benzyloxycarbonyl-α-methylalanyl-α-methylalanine methyl ester. To a solution of benzyloxycarbonyl-α-methylalanine (0·239 g., 1 mmole) and α-methylalanine methyl ester (0·117 g.) in tetrahydrofuran (10 ml.) was added dicyclohexylcarbodi-imide (0·206 g., 1 mmole) dissolved in tetrahydrofuran (10 ml.). After 2 days at room temp., the dicyclohexylurea was collected, and the filtrate evaporated. Isolation of the neutral fraction and recrystallisation from ethyl acetate–light petroleum yielded benzyloxycarbonyl-α-methylalanine methyl ester [2] (64%), identified by m. p. and mixed m. p. (107—109°), and infrared spectrum.

*Derivatives of α-Ethylalanine.—Benzyloxycarbonyl-*DL*-α-ethyl-alanine.* To an ice-cold solution of DL-α-ethylalanine (11·7 g., 0·1 mole) in 2N-sodium hydroxide (50 ml.) and acetone (50 ml.), adjusted to pH 10·9, was added benzyl chloroformate (37 g.) in acetone (50 ml.) during 1 hr. with stirring. The pH was maintained at 10·8—10·9 by simultaneous addition of 2N-sodium hydroxide. The mixture was stirred for 2 hr. at room temp. before being concentrated and extracted with ether. The aqueous solution was acidified yielding an oil which was extracted into ethyl acetate. Evaporation of the dried ($MgSO_4$) ethyl acetate solution yielded *benzyloxycarbonyl-*DL*-α-ethylalanine* (25·2 g., 100%), m. p. 85—89°, raised to 88—89° by recrystallisation from benzene–light petroleum (Found: C, 62·1; H, 6·7; N, 5·7. $C_{13}H_{17}NO_4$ requires C, 62·1; H, 6·8; N, 5·6%).

*Reaction of benzyloxycarbonyl-*DL*-α-ethylalanine with pivaloyl chloride.* Pivaloyl chloride (1·20 g., 10 mmoles) was added to a stirred and cooled (−5°) solution of benzyloxycarbonyl-DL-α-ethylalanine (2·514 g., 10 mmoles) and triethylamine (1·01 g., 10 mmoles) in dry toluene (20 ml.). After 1½ hr. at −5° and 1½ hr. at room temp. the solution was filtered and the filtrate evaporated to yield a viscous oil (3·40 g., 101%) which could not be crystallised ($\nu_{max.}$ 1005, 1245, 1500, 1730, 1820, and 3400 cm.$^{-1}$).

*Benzyloxycarbonyl-*DL*-α-ethylalanyl-α-methylalanine methyl ester.* A solution of the foregoing crude anhyride (2·546 g.) and α-methylalanine methyl ester (2·0 g.) in dry toluene (50 ml.) was heated under reflux for 3½ hr. Isolation of the neutral fraction in the usual manner and recrystallisation from ethyl acetate–light petroleum yielded the *dipeptide-derivative* (2·129 g., 80%), m. p. 76—77° (Found: C, 61·9; H, 7·55; N, 8·1. $C_{18}H_{26}N_2O_5$ requires C, 61·7; H, 7·5; N, 8·0%).

When a solution of the foregoing anhydride (1·675 g.) and DL-α-ethylalanine methyl ester (0·818 g., b. p. 140—152°, prepared by esterification of the amino-acid with methanolic hydrogen chloride and liberation of the free base with potassium carbonate) in dry toluene (25 ml.) was heated under reflux during 4 hr., the neutral product (1·67 g.) could not be crystallised.

*Trifluoroacetyl-*DL*-α-ethylalanine.* Trifluoroacetic anhydride (22·0 g.) was added slowly to an ice-cooled solution of DL-α-ethylalanine (11·7 g., 0·1 mole) in trifluoroacetic acid (42·5 g.). After standing overnight at room temp. the solution was evaporated and the crystalline residue extracted with ether. Concentration of the ethereal solution and dilution with light petroleum afforded *trifluoroacetyl-*DL*-α-ethylalanine* (20·0 g., 94%), m. p. 120—122°. The analytical sample was sublimed at 140°/0·1 mm. (Found: C, 39·6; H, 4·9; N, 6·1. $C_7H_{10}F_3NO_3$ requires C, 39·4; H, 4·7; N, 6·6%).

Trifluoroacetyl-L-α-ethylalanine was prepared similarly and purified by sublimation at 120—140°/0·1 mm., m. p. 121—123°, $[\alpha]_D^{20}$ +9·95° (*c* 2·1 in ethanol).

*Trifluoroacetyl-*DL*-α-ethylalanyl-α-methylalanine methyl ester.* Trifluoroacetyl-DL-α-ethylalanine (2·131 g., 10 mmoles) was dissolved in methylene dichloride (60 ml.) and a solution of dicyclohexylcarbodi-imide (2·062 g., 10 mmoles) in methylene chloride (20 ml.) added. After 2 hr. at room temp. the mixture was filtered and a solution of α-methylalanine methyl ester hydrochloride (1·689 g., 11 mmoles) and triethylamine (1·121 g., 11 mmoles) in methylene chloride (40 ml.) added to the filtrate. The solution was left at room temp. for 21 hr., concentrated to 40 ml., and then heated under reflux for 45 min. Isolation of the neutral fraction in the usual manner yielded the *dipeptide-derivative* (2·643 g., 85%) m. p. 105—107° (Found: C, 46·45; H, 6·3; N, 9·3. $C_{12}H_{19}F_3N_2O_4$ requires C, 46·1; H, 6·1; N, 9·0%).

Trifluoroacetyl-DL-α-*ethylalanyl*-DL-α-*ethylalanine methyl ester*. Dicyclohexylcarbodi-imide (5·12 g., 25 mmoles) dissolved in methylene dichloride (20 ml.) was added to a solution of trifluoroacetyl-DL-α-ethylalanine (5·33 g., 25 mmoles) in methylene dichloride (120 ml.). After 3½ hr. at room temp. the precipitated dicyclohexylurea (5·47 g., 98%) was collected. (The filtrate had $\nu_{max.}$ 994, 1105, 1130, 1175, 1215, 1360, 1675, and 1835 cm.$^{-1}$, indicating formation of 2-trifluoromethyl-4-ethyl-4-methyloxazolone.) To the filtrate was added a solution of DL-α-ethylalanine methyl ester hydrochloride (6·14 g., 37 mmoles) and triethylamine (4·60 g., 45 mmole) in methylene dichloride (40 ml.) and the mixture set aside overnight. Next morning the solution was concentrated to *ca.* 50 ml., heated under reflux for 1 hr. and then evaporated. Isolation of the neutral fraction in the usual manner and recrystallisation from ethyl acetate–light petroleum yielded the *dipeptide derivative* (6·17 g., 76%), m. p. 68—70° (Found: C, 48·1; H, 6·5; N, 8·5. $C_{13}H_{21}F_3N_2O_4$ requires C, 47·8; H, 6·5; N, 8·6%).

The same procedure applied to trifluoroacetyl-L-α-ethylalanine and L-α-ethylalanine methyl ester hydrochloride ($[\alpha]_D^{20}$ +6·0°, *c* 5·2 in ethanol) and the D-isomer ($[\alpha]_D^{20}$ −6·2°, *c* 2·1 in ethanol) yielded *trifluoroacetyl*-L-α-*ethylalanyl*-L-α-*ethylalanine methyl ester* (98%) m. p. 77—78°, $[\alpha]_D^{20}$ +9·2° (*c* 0·25 in ethanol) (Found: C, 47·9; H, 6·6; N, 8·4%), and the L–D isomer (80%) $[\alpha]_D^{20}$ +7·8° (*c* 0·16 in ethanol), both of which had infrared spectra identical with that of the racemic compound.

Benzyloxycarbonyl-DL-α-*ethylalanyl*-DL-α-*ethylalanine*. A mixture of trifluoroacetyl-DL-α-ethylalanyl-DL-α-ethylalanine methyl ester (2·614 g., 8 mmoles) and N-sodium hydroxide (25 ml.) was kept at room temp. for 62 hr. The solution was extracted with ethyl acetate, acidified to pH 1 and again extracted with ethyl acetate. The aqueous solution was diluted with acetone (30 ml.) and brought to pH 10·9 using 4N-sodium hydroxide. Benzyl chloroformate (10 g.) in acetone (20 ml.) was added to the cooled and stirred solution during 30 min., the pH being kept at 10·9 by concurrent addition of N-sodium hydroxide. Stirring was continued for 2½ hr. at room temp., the solution concentrated and the acidic fraction (1·518 g., 54%) isolated. Recrystallisation from aqueous methanol yielded the *benzyloxycarbonyl derivative* (0·563 g., 20%) m. p. 126—128° (Found: C, 62·0; H, 7·6; N, 7·7. $C_{18}H_{26}N_2O_5$ requires C, 61·7; H, 7·5; N, 8·0%).

The same procedure applied to trifluoroacetyl-L-α-ethylalanyl-L-α-ethylalanine methyl ester and the L–D isomer yielded *benzyloxycarbonyl*-L-α-*ethylalanyl*-L-α-*ethylalanine* (42%), m. p. 136—138°, $[\alpha]_D^{20}$ +9·5° (*c* 0·3 in ethanol) (Found: C, 61·75; H, 7·4; N, 7·75%), and *benzyloxycarbonyl*-L-α-*ethylalanyl*-D-α-*ethylalanine* (58%) $[\alpha]_D^{20}$ −13·2° (*c* 0·13 in ethanol) (Found: C, 61·9; H, 7·5; N, 7·7%).

Poly-α-*ethylalanine*. Benzyloxycarbonyl-DL-α-ethylalanyl-DL-α-ethylalanine (1·056 g., 3 mmoles) in acetic anhydride (14 ml.) was heated at 110—120° for 15 min., and the solution then evaporated. Last traces of acetic anhydride were removed by addition of toluene (15 ml). and evaporation, leaving the oily benzyloxycarbonyl dipeptide oxazolone (1·001 g., 100%), $\nu_{max.}$ (film) 900, 1025, 1255, 1455, 1510, 1670, 1725, and 1820 cm.$^{-1}$. This oxazolone was dissolved in anhydrous ethyl acetate (100 ml.) containing 5% palladium–charcoal catalyst (0·5 g.) and hydrogen passed over the surface of the stirred solution until carbon dioxide evolution ceased (5½ hr.). After filtration, evaporation of the solvent left residual 2-(1′-amino-1′-ethyl)ethyl-4-ethyl-4-methyloxazolone (0·592 g., 100%) as an oil, $\nu_{max.}$ (film) 895, 1020, 1075, 1195, 1380, 1470, 1670, and 1820 cm.$^{-1}$. A solution of this amino-oxazolone (0·590 g.) in anhydrous acetonitrile (15 ml.) was heated under reflux for 70 hr. The precipitated solid was collected, washed with acetonitrile and dried. The solid was heated at 140—160°/0·2 mm. when 2,5-diethyl-2,5-dimethyl-3,6-dioxopiperazine (0·031 g., 5%) sublimed. The involatile residue of *poly*-DL-α-*ethylalanine* (0·288 g., 49%) had $\nu_{max.}$ 1170, 1210, 1270, 1380, 1470, 1545, and 1670 cm.$^{-1}$.

The same procedure applied to benzyloxycarbonyl-L-α-ethylalanyl-L-α-ethylalanine and the L–D isomer yielded *poly*-L-α-*ethylalanine* (61%, DP_w 82) and *poly*-(L-α-*ethylalanyl*-D-α-*ethylalanine*) (38%, DP_w 39), respectively.

Derivatives of α-Phenylalanine.—Benzyloxycarbonyl-DL-α-*phenylalanine*. Benzyl chloroformate (16 g.) dissolved in acetone (25 ml.) was added during 70 min. to a stirred solution of DL-α-phenylalanine (8·25 g., 50 mmoles) in 1·67 N-aqueous sodium hydroxide (30 ml.) and acetone (25 ml.), adjusted to pH 10·9. This pH was maintained by concurrent addition of N-aqueous sodium hydroxide. The mixture was stirred for a further 2 hr. at room temp. before being concentrated and acidified. Isolation in the usual manner and precipitation from sodium carbonate solution by acidification yielded the *benzyloxycarbonyl derivative* (13·1 g., 87%), m. p. 93—97°.

Two recrystallisations from benzene–light petroleum raised the m. p. to 104—106° (Found: 68·5; H, 5·7; N, 4·75. $C_{17}H_{17}NO_4$ requires C, 68·2; H, 5·7; N, 4·7%).

DL-α-*Phenylalanine methyl ester*. A solution of DL-α-phenylalanine (4·13 g., 25 mmoles) in dry methanol (30 ml.) was saturated with dry hydrogen chloride. The solution was set aside overnight at room temp., heated under reflux for 8 hr., and then evaporated. The ester hydrochloride was ground very finely under chloroform (20 ml.) and the suspension added slowly to chloroform (70 ml.) containing a large excess of liquid ammonia. The mixture was well stirred, set aside overnight at room temp., and then filtered. DL-α-*Phenylalanine methyl ester* (2·41 g., 54%) distilled at 138—142°/15 mm. (Found: C, 66·7; H, 7·0; N, 7·8. $C_{10}H_{13}NO_2$ requires C, 67·0; H, 7·3; N, 7·8%).

*Reaction of benzyloxycarbonyl-*DL-α-*phenylalanine with pivaloyl chloride*. Pivaloyl chloride (1·20 g., 10 mmoles) was added to a stirred and cooled (−5°) solution of benzyloxycarbonyl-DL-α-phenylalanine (3·00 g., 10 mmoles) and triethylamine (1·01 g., 10 mmole) in dry toluene (50 ml.). After being stirred for 2 hr. at −5° and 1 hr. at room temp. the mixture was set aside overnight. Precipitated triethylamine hydrochloride (1·36 g., 96%) was removed by filtration and the filtrate evaporated at room temp. to yield an oil (3·60 g.), $\nu_{max.}$ (film) 1010, 1040, 1250, 1355, 1430, 1490, 1710, 1730, 1800, and 3300 $cm.^{-1}$.

A solution of the foregoing product (0·9 g.) in dry benzene (25 ml.) was cooled as cyclohexylamine (0·25 g., 2·5 mmoles) was added. The mixture was shaken at room temp. for 2 hr. and the crystalline precipitate collected. *Benzyloxycarbonyl-*DL-α-*phenylalanine cyclohexylammonium salt* (0·67 g., 67%) had m. p. 168—172°, raised by recrystallisation from aqueous methanol to 175—176° (Found: C, 69·15; H, 7·3; N, 6·7. $C_{23}H_{30}N_2O_4$ requires C, 69·3; H, 7·6; N, 7·0%).

p-*Phenylazobenzyloxycarbonyl-*DL-α-*phenylalanine*. A mixture of DL-α-phenylalanine (2·49 g., 15 mmole) and magnesium oxide (3·0 g.) suspended in water (15 ml.) and dioxan (100 ml.) was stirred as *p*-phenylazobenzyloxycarbonyl chloride (4·14 g., 15 mmoles) was added. The mixture was heated under reflux for 3 hr. and then stirred at room temp. overnight. The acidic *product* (3·51 g., 58%) was isolated in the usual manner, and was recrystallised from ethyl acetate–light petroleum, m. p. 145—146° (Found: C, 68·7; H, 5·6; N, 10·1. $C_{23}H_{21}N_3O_4$ requires C, 68·5; H, 5·25; N, 10·4%).

p-*Phenylazobenzyloxycarbonyl-*DL-α-*phenylalanine pivalic acid mixed anhydride*. Pivaloyl chloride (0·21 ml., 1·5 mmoles) was added to a stirred and cooled (0°) solution of *p*-phenylazobenzyloxycarbonyl-DL-α-phenylalanine (0·60 g., 1·5 mmoles) and triethylamine (0·21 ml., 1·5 mmoles) in dry benzene (25 ml.). The solution was stirred for a further 2 hr. at 5° and 2 hr. at room temp. After standing overnight, precipitated triethylamine hydrochloride was collected (0·20 g., 97%), and the filtrate evaporated to yield the *mixed anhydride* (0·62 g., 85%), m. p. 62—64° after recrystallisation from ether (Found: C, 68·5; H, 6·3; N, 8·4. $C_{28}H_{29}N_3O_5$ requires C, 69·0; H, 6·0; N, 8·6%).

*Benzyloxycarbonyl-*DL-α-*phenylalanine-t-butyl ester*. A solution of benzyloxycarbonyl-DL-α-phenylalanine (2·68 g.) and concentrated sulphuric acid (0·1 ml.) in methylene dichloride (20 ml.) was saturated with isobutene. After 3 days at room temp. the solution was added to 5% sodium carbonate solution (5 ml.), the methylene dichloride layer separated and washed with water, dried (Na_2SO_4), and evaporated. The *ester* (2·97 g., 93·5%), m. p. 54—55°, was recrystallised for analysis from ether–light petroleum (Found: C, 70·9; H, 6·95; N, 4·1. $C_{21}H_{25}NO_4$ requires C, 71·0; H, 7·1; N, 3·9%).

DL-α-*Phenylalanine t-butyl ester*. Concentrated sulphuric acid (6 ml.) was added to a suspension of DL-α-phenylalanine (5·0 g.) in dioxan (50 ml.). The solution was placed in an autoclave (capacity 270 ml.) and cooled to −70° and liquid isobutene (170 ml.) was added. After 16 hr. at room temp. the pressure was released and the solution basified with aqueous sodium hydroxide. The solution was extracted with ether and the extract washed with water and dried (Na_2SO_4). Evaporation of the ether yielded an oil (5·4 g., 80·5%) which was distilled at 86°/0·6 mm. The distillate crystallised as large needles. In later preparations the *ester* was recrystallised from light petroleum and had m. p. 46—47° (Found: C, 70·5; H, 8·8; N, 6·4. $C_{13}H_{19}NO_2$ requires C, 70·55; H, 8·65; N, 6·3%). The *picrate* had m. p. 162—163°, after recrystallisation from benzene–light petroleum (Found: C, 50·75; H, 5·1; N, 12·3. $C_{19}H_{22}N_4O_9$ requires C, 50·7; H, 4·9; N, 12·4%).

*Trifluoroacetyl-*DL-α-*phenylalanine*. Trifluoroacetic anhydride (4·41 g.), was added gradually to an ice-cooled solution of DL-α-phenylalanine (3·30 g., 20 mmoles, dried at 80°) in trifluoroacetic

acid (10 g., distilled from a little phosphoric oxide). After standing overnight at room temp. the solution was evaporated and the white crystalline residue extracted into ether and filtered. Dilution of the ether with light petroleum and concentration afforded crystalline *trifluoroacetyl*-DL-α-*phenylalanine* (5·04 g., 96·5%), m. p. 126—128° (Found: C, 50·4; H, 3·8; N, 5·8. $C_{11}H_{10}F_3NO_3$ requires C, 50·6; H, 3·9; N, 5·4%).

DL-2-*Trifluoromethyl*-4-*methyl*-4-*phenyloxazolone*. Throughout the preparation, atmospheric moisture was excluded by drying tubes containing phosphoric oxide. Trifluoroacetyl-DL-α-phenylalanine (2·61 g., 10 mmoles, dried at 80°) was dissolved in thionyl chloride (6 ml.) and the solution was maintained at 50—70° for 1 hr. Excess of thionyl chloride was evaporated at room temp. and the residue distilled yielding the trifluoromethyloxazolone (1·98 g., 82%), b. p. 53—57°/0·6 mm. The infrared spectrum of the product had $\nu_{max.}$ (film) 3000, 1850, 1670, 1475, 1365, 1220, 1160, 1000, 885, 845, 757, 712, and 695 cm.$^{-1}$.

Trifluoroacetyl-DL-α-*phenylalanine cyclohexylamide*. A solution of the foregoing oxazolone (0·110 g.) in acetonitrile (5 ml.) was cooled as cyclohexylamine (0·100 g.) was added. The solution was kept at room temp. for 10 min. before being heated (steam-bath) for 10 min. and then set aside overnight. Isolation of the neutral product and recrystallisation from aqueous methanol afforded the *cyclohexylamide* (0·126 g., 81%) as fine white needles, m. p. 140—142° (Found: C, 59·6; H, 6·2; N, 8·4. $C_{17}H_{21}F_3N_2O_2$ requires C, 59·6; H, 6·2; N, 8·2%).

Trifluoroacetyl-DL-α-*phenylalanine anilide*. A solution of the trifluoromethyloxazolone (0·240 g.) in acetonitrile (5 ml.) was cooled and aniline (0·200 g.) added. The solution was heated under reflux for 30 min. and then set aside at room temp. overnight. Isolation of the neutral fraction and recrystallisation from benzene–light petroleum yielded the *anilide* (0·298 g., 90%) as large white needles, m. p. 137—138·5° (Found: C, 60·5; H, 4·5; N, 8·4. $C_{17}H_{15}F_3N_2O_2$ requires C, 60·7; H, 4·5; N, 8·3%).

Trifluoroacetyl-DL-α-*phenylalanyl*-DL-α-*phenylalanine methyl ester*. DL-α-Phenylalanine methyl ester (4·00 g.) was added gradually to an ice-cooled solution of the trifluoromethyloxazolone (4·66 g.) in dry acetonitrile (15 ml.). The solution was warmed on the steam-bath for 15 min. and set aside at room temp. overnight. Large crystals of the *dipeptide derivative* (1·85 g.), m. p. 162° separated. Evaporation of the filtrate and isolation of the neutral product in the usual manner yielded a further 5·96 g., m. p. 160—162°. The total yield was 7·81 g. (96·7%). The analytical sample had m. p. 160—162·5° after recrystallisation from benzene (Found: C, 59·6; H, 5·0; N, 6·9. $C_{21}H_{21}F_3N_2O_4$ requires C, 59·7; H, 5·0; N, 6·6%).

Alkaline hydrolysis of trifluoroacetyl-DL-α-*phenylalanyl*-DL-α-*phenylalanine methyl ester*. A mixture of trifluoroacetyl-DL-α-phenylalanyl-DL-α-phenylalanine methyl ester (1·95 g.) and N-sodium hydroxide solution (15 ml.) was kept overnight at room temp. An attempted benzylcarbonylation of the resulting alkaline solution was unsuccessful, and the acidic product (1·54 g., m. p. 142—145°) isolated in the usual manner proved to be *trifluoroacetyl*-DL-α-*phenylalanyl*-DL-α-*phenylalanine*. Recrystallisation from methanol yielded the methanol solvate (Found: C, 57·35; H, 5·1; N, 6·45. $C_{20}H_{19}F_3N_2O_4$,CH_3OH requires C, 57·25; H, 5·2; N, 6·4%). The *dicyclohexylammonium salt* had m. p. 246—248° (from methanol) (Found: C, 65·3; H, 7·25; N, 7·0. $C_{32}H_{42}F_3N_3O_4$ requires C, 65·25; H, 7·1; N, 7·1%). Treatment of the free acid with ethereal diazomethane in the usual manner regenerated the starting ester, m. p. and mixed m. p. 160—162°.

Trifluoroacetyl-DL-α-*phenylalanyl*-DL-α-*phenylalanyl*-DL-α-*phenylalanine methyl ester*. (a) *From trifluoroacetyl*-DL-α-*phenylalanyl*-DL-α-*phenylalanine*. A solution of trifluoroacetyl-DL-α-phenylalanyl-DL-α-phenylalanine (143 mg.) in acetic anhydride (4 ml.) was heated at 110—120° (oil-bath) for 15 min. and then evaporated. Last traces of acetic anhydride were removed by addition of toluene (5 ml.) and evaporation. The oily oxazolone (127 mg., 100%) had $\nu_{max.}$ 3380, 1834, 1750, 1668, 1480, 1450, 1378, 1275, 1210, 1160, and 885 cm.$^{-1}$. A solution of the oxazolone in acetonitrile (5 ml.) was cooled to 0° and DL-α-phenylalanine methyl ester (318 mg.) added slowly. The mixture was kept for 10 min. before being heated (steam-bath) for 10 min. and then set aside at room temp. overnight. Isolation of the neutral product yielded the crystalline *tripeptide-derivative* (97 mg., 50%), m. p. 187—191°. The analytical sample was recrystallised from aqueous methanol (Found: C, 63·2; H, 5·6; N, 7·2. $C_{30}H_{30}F_3N_3O_5$ requires C, 63·25; H, 5·3; N, 7·4%).

(b) *From trifluoroacetyl*-DL-α-*phenylalanyl*-DL-α-*phenylalanine methyl ester*. Hydrogen chloride was bubbled through a boiling solution of trifluoroacetyl-DL-α-phenylalanyl-DL-α-phenylalanine methyl ester (1·69 g., 4 mmoles) in dry methanol (100 ml.) during 5 hr. After

evaporation, the hygroscopic hydrochloride (1·35 g.) was dissolved in dry acetonitrile (20 ml.), triethylamin e(0·41 g., 4 mmoles) added and the solution filtered. DL-4-Methyl-4-phenyl-2-trifluoromethyloxazolone (0·972 g., 4 mmoles) was added to the ice-cooled filtrate. The solution was kept for 10 min. at room temp. then heated (steam-bath) for 10 min., and set aside for 2 days at room temp. Isolation of the neutral fraction yielded the tripeptide-derivative (1·68 g., 74%) m. p. 189—191°, mixed m. p. 188—190° with the material from the previous preparation.

Benzyloxycarbonyl-DL-α-*phenylalanyl*-DL-α-*phenylalanine dicyclohexylammonium salt.* Dry hydrogen chloride was passed through a boiling solution of trifluoroacetyl-DL-α-phenylalanyl-DL-α-phenylalanine methyl ester (3·38 g., 8 mmoles) in dry methanol (150 ml.) for 5 hr. The solution was evaporated and the residue (3·34 g.) dissolved in 2N-hydrochloric acid, washed with ethyl acetate and evaporated. To the residue (2·69 g.) was added N-sodium hydroxide solution (20 ml.) and the mixture shaken vigorously for 2 hr. when most of the solid had dissolved. Acetone (15 ml.) was added and the solution brought to pH 10·9 by addition of trifluoroacetic acid. Benzyl chloroformate (3·20 g.) in acetone (20 ml.) was added during 30 min. to the cooled (0°) and stirred solution, the pH being maintained at 10·9—11 by further addition of N-sodium hydroxide. Stirring was continued at room temp. for 1½ hr. before the solution was acidified and extracted with ethyl acetate. When the dried ($MgSO_4$) extract was evaporated it yielded the benzyloxycarbonyl dipeptide as an oil (1·85 g., 52%), which could not be crystallised. To a solution of the oil in ethyl acetate (20 ml.) was added dicyclohexylamine (1·0 g.). After standing overnight at room temp. the crystalline *dicyclohexylammonium salt* (2·11 g., 42%) was collected, m. p. 219—221° (Found: C, 72·8; H, 7·9; N, 6·8. $C_{38}H_{49}N_3O_5$ requires C, 72·7; H 7·9; N, 6·7%).

N-(*Benzyloxycarbonyl*-DL-α-*phenylalanyl*)-NN′-*bis*(*cyclohexyl*)*urea.* To a solution of benzyloxycarbonyl-DL-α-phenylalanine (0·663 g.) and DL-α-phenylalanine methyl ester (0·392 g.) in tetrahydrofuran (10 ml.) was added a solution of dicyclohexylcarbodi-imide (0·472 g.) in tetrahydrofuran (10 ml.). After standing for 2 days at room temp. the solution was filtered and the filtrate evaporated. The residue was dissolved in ethyl acetate and the neutral fraction isolated as an oil (0·881 g., 86%). The *acylurea* (0·798 g., 71%), crystallised from ethyl acetate–light petroleum, m. p. 123—123·5° (Found: C, 71·4; H, 8·0; N, 8·15. $C_{30}H_{39}N_3O_4$ requires C, 71·3; H, 7·8; N, 8·3%).

We thank Monsanto Chemicals for a studentship (to D. S. J.).

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