776. *Amino-acids and Peptides. Part XV.* Racemisation during Peptide Synthesis.*

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Although α-benzyloxycarbonylamino-acids normally do not racemise when they are condensed with amino-esters to form peptides, benzyloxycarbonyldipeptides may do so when they are coupled by some of the procedures in current use, which therefore have limited applicability in peptide synthesis. The racemisation occurring when acetyl-L-leucine is condensed with glycine ethyl ester by various methods has now been studied. Coupling through the acid azide gave a product of high optical activity, from which no racemate could be isolated; some racemisation was observed when dicyclohexylcarbodi-imide was the condensing agent (with dichloromethane or tetrahydrofuran as solvent). The racemisation found with the use of diethyl phosphorochloridite or tetraethyl pyrophosphite varied considerably with the conditions. The carbonic mixed anhydride and the "phosphorazo" method caused extensive racemisation. Methods which have been postulated to involve activation of the amino-component may therefore result in racemisation in the carboxylic component. The use of amino-ester hydrochloride with an equivalent of tertiary amine, in place of the free amino-ester, led to increased racemisation in several cases.

Few synthetic steps have received more attention in recent years than that in which the peptide bond is formed. Many of the newer methods afford high yields under mild conditions, but the preservation of optical activity has not always received sufficient study. Even slight racemisation at each step may, in lengthy syntheses, result in the formation of diastereoisomers in amounts sufficient to hinder crystallisation and to make purification troublesome. The problem has been obscured to some extent by the fact that benzyloxycarbonylamino-acids remain active under conditions which rapidly racemise other acylamino-acids; Vaughan [1] showed in 1952 that coupling through the carbonic mixed anhydride of a benzyloxycarbonyldipeptide can result in partial or complete racemisation, in procedures which give fully active material from benzyloxycarbonylamino-acids. This can be correlated with the inability of the latter derivatives (under normal conditions) to form azlactones, which are well known to racemise readily [2] (it is not implied that racemisation proceeds only by this route); when benzyloxycarbonylamino-acid chlorides cyclise, it is to form *N*-carboxyamino-acid anhydrides, not azlactones.† Shortly after Vaughan's publication, similar examinations of the methods using phosphorochloridous esters [4] and tetraethyl pyrophosphite,[5] and of the sulphuric mixed anhydride procedure,[6] were reported; in each case it was found that racemisation could occur under certain conditions when benzyloxycarbonyldipeptides were coupled further, although procedures were described by which, in the reactions studied, racemisation was avoided. It is of course possible to plan syntheses so that benzyloxycarbonylamino-acids are used at each coupling, lengthening the chain from the amino-end at every stage, as in Bodanszky and du Vigneaud's synthesis

* Part XIV, *J.*, 1959, 3868.

† The validity of the statement by Schwarz and Bumpus [3] that "no racemisation is observed when an acylamino-acid is employed" (as the carboxyl component in coupling) is limited, as the present paper shows.

[1] Vaughan, *J. Amer. Chem. Soc.*, 1952, **74**, 6137; Vaughan and Eichler, *ibid.*, 1953, **75**, 5556.
[2] Cornforth, in "The Chemistry of Penicillin," Princeton Univ. Press, 1949, p. 742.
[3] Schwarz and Bumpus, *J. Amer. Chem. Soc.*, 1959, **81**, 890.
[4] (*a*) Anderson, Blodinger, R. W. Young, and Welcher, *J. Amer. Chem. Soc.*, 1952, **74**, 5304; Anderson and R. W. Young, *ibid.*, 1952, **74**, 5307; (*b*) R. W. Young, Wood, Joyce, and Anderson, *ibid.*, 1956, **78**, 2126.
[5] Anderson, Blodinger, and Welcher, *J. Amer. Chem. Soc.*, 1952, **74**, 5309.
[6] (*a*) Kenner and Stedman, *J.*, 1952, 2069; (*b*) Clayton, Farrington, Kenner, and Turner, *J.*, 1957, 1398.

of oxytocin.[7] But greater operational flexibility is obviously desirable, and a study of the general applicability of methods of coupling was begun by North and Young in 1954, and continued by the present authors. The early conclusions have been briefly reported,[8] and others have been summarised recently;[9] detailed results are now presented on the condensation of acetyl-L-leucine with glycine ethyl ester. The Table summarises our results. In many cases, racemic or largely racemic material crystallised from the reaction product, and its identity was confirmed by melting point (120—120·5°; L-isomer, 100—101°) and mixed melting point, and by the infrared absorption spectrum (of a suspension in liquid paraffin), which differs from that of the L-isomer in having a peak at 1681 cm.$^{-1}$. Our main purpose was then to establish that racemisation had occurred. Estimation of the proportion of L-peptide in the total product is complicated by the fact that later fractions were usually syrups, but in the Table a very rough distinction is made between experiments according to the amount of racemate formed. These conclusions are based on the

	Method	Conditions *	Solvent	Product
1	Acid azide	*a*	Ether	α
2	(i) Carbodi-imide	*a*	Dichloromethane	α †
	(ii) ,,	*a*	Tetrahydrofuran	α †
	(iii) ,,	*b*	,,	ρ
	(iv) ,,	*a*	Dimethylformamide	ρ
	(v) ,,	*a*	Aq. tetrahydrofuran	ρ
3	(i) Et_2 phosphorochloridite	Anhydride procedure; *a*	Toluene	α †
	(ii) ,, ,,	,, ,, *b*	,,	ρ
	(iii) ,, ,,	,, ,, *c*	,,	β
	(iv) ,, ,,	Amide procedure; *a*	Ether, toluene	ρ
4	(i) Et_4 pyrophosphite	Anhydride procedure; *a*	Et_2 phosphite	ρ
	(ii) ,,	,, ,, *b*	,,	ρ
	(iii) ,,	Amide procedure; *a*	,,	ρ
	(iv) ,,	,, ,, *b*	,, ,,	β
	(v) ,,	,, ,, *d*	,, ,,	α
	(vi) ,,	*e*	,, ,,	γ
	(vii) ,,	*e*	Pyridine	ρ
5	(i) Carbonic mixed anhydride	*b*	Tetrahydrofuran	γ
	(ii) ,, ,,	*b*	Chloroform	γ
	(iii) ,, ,,	*a*	Tetrahydrofuran	β
	(iv) ,, ,,	*f*	,,	β
6	(i) Phosphorazo ‡	100°; *b*	Pyridine	γ
	(ii) ,,	Room temp.; *b*	,,	γ

* Conditions: *a*, Distilled glycine ethyl ester used. *b*, Ester hydrochloride with triethylamine. *c*, Distilled ester with triethylamine. *d*, An equivalent each of distilled ester and of ester hydrochloride. *e*, Ester hydrochloride only. *f*, Ester previously liberated from the hydrochloride by triethylamine in chloroform. In the anhydride procedure the phosphite reagent reacts with the tertiary amine salt of the carboxylic acid before addition of the amino-component; in the amide procedure the phosphite reagent reacts with the amino-compound before addition of the carboxylic acid.

Product: α, Less than 30% of racemate. β, 30—70% of racemate. γ, At least 70% of racemate. ρ, Contains racemate, but the high proportion of syrup prevents assignment to categories α, β, or γ.

† Some crystalline racemic (or largely racemic) peptide was isolated.

‡ For a discussion see Goldschmidt and Krauss, *Annalen*, 1955, **595**, 193.

composition of solid fractions for which proof of chemical identity is available, from melting point, nitrogen analysis, and infrared absorption spectrum. From the specific rotation, the proportion of optical isomers in these fractions was calculated, and it was then confirmed by the optical rotation of the solution obtained on acid-hydrolysis. Where the high proportion of syrupy product prevented assignment to these categories but there was clear evidence (from the solid fractions) of racemisation, the symbol ρ is used; in such cases, a useful conclusion concerning the minimum degree of racemisation can be

[7] Bodanszky and du Vigneaud, *Nature*, 1959, **183**, 1324; *J. Amer. Chem. Soc.*, 1959, **81**, 5688.

[8] North and G. T. Young, *Chem. and Ind.*, 1955, 1597; North, Smart, and G. T. Young, Abs. Proc. 19th Internat. Congr. Pure Appl. Chem., Paris, 1957, II, p. 238.

[9] G. T. Young, Proc. Symposium on Methods of Peptide Synthesis, Prague, 1958 (*Coll. Czech. Chem. Comm.*, 1959, **24**, Special issue, p. 39).

drawn from the weight of racemate in the solid fractions. No assumptions have been made regarding the composition of syrupy material. For reference, acetyl-L-leucylglycine ethyl ester was synthesised by a route which should not permit racemisation: benzyloxycarbonyl-L-leucine methyl ester was converted into the hydrazide, and the azide obtained therefrom was condensed with glycine ethyl ester; hydrogenation followed by acetylation yielded acetyl-L-leucylglycine ethyl ester. It may be noted here that the acetyl-L-leucine used by Karrer, Escher, and Widmer [10] for the preparation of the methyl ester was low in optical rotation, and we give constants for authentic acetyl-L-leucine methyl ester. The same fully active ester was obtained by Brenner and Huber's method,[11] in which the acid is added to the product of the reaction of thionyl chloride with methanol at $-10°$.

No racemate was isolated from the peptide formed through the acid azide,[12] and the solid fraction alone (of one experiment) contained L-isomer corresponding to 86% of the total product. We are not aware of any report of racemisation by this route, which has lately been used with advantage in, for example, the synthesis of a decapeptide related to gramicidin-S where each acylpeptide was coupled in this way, and all but one of the fifteen intermediates were crystalline.[13] In spite of occasional difficulties (*e.g.*, formation of the amide and the urea as by-products [14]) this old route remains important for the junction of large peptides.

The use of dicyclohexylcarbodi-imide [15] in dichloromethane or anhydrous tetrahydrofuran, with free glycine ethyl ester, gave chiefly L-peptide, but presence of racemate was confirmed by recrystallisation of the first fractions, and there was clear evidence of racemisation when dimethylformamide or aqueous tetrahydrofuran was the solvent. The possibility that racemisation occurs when this method is used to couple benzyloxycarbonylpeptides has since been reported by Anderson and Callaghan [16] (using dichloromethane or tetrahydrofuran as solvent), and by Schwarz and Bumpus [3] (using dimethylformamide or tetrahydrofuran as solvent) who, by fractional crystallisation, isolated the diastereoisomer formed by racemisation at the acylating residue. Some *N*-acetyl-L-leucyl-*NN'*-dicyclohexylurea (identified by synthesis) was isolated in our experiments with dimethylformamide as the solvent. It is particularly remarkable that the synthesis of the acylurea, from acetyl-L-leucine and dicyclohexylcarbodi-imide in boiling pyridine, gave fully active material, since under these conditions any acid anhydride formed would surely be racemised.

In discussing the phosphite reagents, it is convenient to consider first those experiments in which free glycine ethyl ester was used. Diethyl phosphorochloridite [4a] in the " anhydride " procedure [**3** (i)] gave some racemate, but the optical activity of the fractions was high. No racemate was isolated from the product of the " amide procedure " [**3** (iv)] although the properties of the solid fractions indicated its presence. Tetraethyl pyrophosphite, both in the " anhydride " [**4** (i)] and the " amide " [**4** (iii)] procedure, gave products containing racemate. It is a common and convenient practice in peptide synthesis to use the hydrochloride of the amino-ester, or dipeptide ester, with the equivalent of tertiary amine, in place of the free amino-compound, and in several methods of coupling this is the normal procedure. Anderson and his co-workers found, however, that with phosphite reagents this technique resulted in some racemisation, and they concluded [4b] that " no racemisation is observed in using dipeptide acids in the absence of hydrogen chloride, as in pyrophosphite reactions or with chlorophosphites when triethylamine in

[10] Karrer, Escher, and Widmer, *Helv. Chim. Acta*, 1926, **9**, 301.
[11] Brenner and Huber, *Helv. Chim. Acta*, 1953, **36**, 1109.
[12] Curtius, *Ber.*, 1902, **35**, 3226.
[13] Erlanger, Curran, and Kokowsky, *J. Amer. Chem. Soc.*, 1959, **81**, 3051.
[14] Cf., *e.g.*, Prelog and Wieland, *Helv. Chim. Acta*, 1946, **29**, 1128; Hinman, Caron, and Christensen, *J. Amer. Chem. Soc.*, 1950, **72**, 1620.
[15] Sheehan and Hess, *J. Amer. Chem. Soc.*, 1955, **77**, 1067.
[16] Anderson and Callahan, *ibid.*, 1958, **80**, 2902.

inert solvents like benzene and toluene is used. In these solvents, the acid is effectively removed from the solution." The racemisation which they found [5] when using tetraethyl pyrophosphite in the " anhydride procedure," in the absence of chloride, is an exception to this generalisation, but we also have noted lower optical activity in the products of such condensations, when glycine ethyl ester hydrochloride with triethylamine replaced glycine ethyl ester [compare **3** (i) with **3** (ii), in which at least 50% of racemate was found in the products of the third experiment and nearly complete racemisation resulted in one case, when glycine ester hydrochloride was used alone, without tertiary base [**4** (vi)]. A similar effect when ethoxyacetylene was the condensing agent has recently been reported.[17] On the other hand, the presence of ester hydrochloride in addition to free ester, in the tetraethyl pyrophosphite procedure, gave a high proportion of L-peptide [**4** (v)]. Further investigation is clearly required, but we draw attention here to another aspect. Glycine ethyl ester hydrochloride and triethylamine hydrochloride are only slightly soluble in diethyl phosphite, and insoluble in toluene; the liberation of free ester by triethylamine may therefore be incomplete, and the excess of tertiary amine will be expected to favour racemisation [compare **3** (i) and **3** (iii)]. A similar advantage in the use of free ester was observed with the carbodi-imide and mixed carbonic anhydride methods [compare **2** (ii) with **2** (iii), and **5** (iii) with **5** (i)]. The practical difficulty is that solvents sufficiently polar to dissolve hydrochlorides of amino-esters or peptide esters are usually undesirable solvents for the coupling reaction; this can be overcome by liberating the free ester in a more powerful solvent such as chloroform, which is then removed and replaced by the chosen reaction medium [cf. **5** (iv)].

Recently it was recommended [18] that pyridine should replace diethyl phosphite as the solvent for condensations using tetraethyl pyrophosphite. The modification [**4** (vii)] caused considerable racemisation in this reaction.

The use of the carbonic mixed anhydride [19] with ester hydrochloride led to extensive racemisation when tetrahydrofuran or chloroform was the solvent; the use of the free ester in tetrahydrofuran [**5** (iii)] gave products of higher activity. A recent example of racemisation by the use of this method has been given by Schwarz and Bumpus,[3] who coupled benzyloxycarbonyl-L-valyl-L-tyrosine with distilled L-isoleucine methyl ester, using tetrahydrofuran as solvent, and separated benzyloxycarbonyl-L-valyl-D-tyrosyl-L isoleucine methyl ester from the product in 11% yield.

The " phosphorazo " method [20] also resulted in extensive racemisation, showing clearly that methods which have been postulated to involve activation of the amino-component may still cause racemisation in the carboxyl component. More recently, Grassmann, Wünsch, and Riedel [21] coupled benzyloxycarbonylglycyl-L-phenylalanine with L-alanine methyl ester by this means; the crude product was saponified, and fractionation yielded some benzyloxycarbonylglycyl-D-phenylalanyl-L-alanine. Analogous syntheses of tri- and penta-peptides gave products of unsharp melting point, but in these cases the diastereoisomers assumed to be present could not be separated. As pointed out by these authors, such methods are likely to involve an intermediate acid anhydride, which in the solvent pyridine may be expected to racemise.

Several of the methods of coupling not so far used in our study have been examined by other workers, who have looked for racemic material in the product of reactions having benzyloxycarbonyldipeptides as the acyl component. Farrington, Hextall, Kenner, and Turner [22] investigated the use of *p*-nitrophenyl thiol esters in this way; Sheehan and

[17] Panneman, Marx, and Arens, *Rec. Trav. chim.*, 1959, **78**, 487.
[18] Maclaren, *Austral. J. Chem.*, 1958, **11**, 360.
[19] Boissonnas, *Helv. Chim. Acta*, 1951, **34**, 874; Vaughan, *J. Amer. Chem. Soc.*, 1951, **73**, 3547; Wieland and Bernhard, *Annalen*, 1951, **572**, 190.
[20] Goldschmidt, *Angew. Chem.*, 1950, **62**, 538; Goldschmidt and Lautenschlager, *Annalen*, 1953, **580**, 68.
[21] Grassmann, Wünsch, and Riedel, *Chem. Ber.*, 1958, **91**, 455.
[22] Farrington, Hextall, Kenner, and Turner, *J.*, 1957, 1407.

Hlavka [23] and (more fully) Panneman, Marx, and Arens [17] examined the use of ethoxyacetylene, and Anderson and Paul [24] reported one such experiment in the use of *NN'*-carbonyldi-imidazole. In these cases again, it was found that racemisation can occur during the coupling of benzyloxycarbonyldipeptides, but attention to conditions may almost eliminate this. It must however be emphasised that, as shown by Clayton *et al.*,[6b] the extent of racemisation will depend on the peptide components as well as on the condensing agent, and it is most desirable that a variety of model reactions should be studied. Early in our work, we had supposed that racemisation would be more likely where the acyl group concerned is acetyl rather than acylaminoacetyl, but the lack of evidence of racemisation by the azide route, and the confirmation of our findings in several cases by other workers using benzyloxycarbonyldipeptides, indicate that the model reaction we have used may not be unduly sensitive. We do not suggest that those methods of coupling which we have found to cause racemisation will do so in all reactions. There are many examples of syntheses in which racemisation would be expected but optically pure material has been isolated by careful purification. But clearly those methods are generally to be preferred which have been shown, in tests such as those we have described, to cause little or no racemisation.

Experimental

M. p.s were taken on a Kofler block. Infrared spectra were recorded on a Perkin–Elmer model 21 spectrophotometer.

Acetyl-L-leucylglycine Ethyl Ester.—Benzyloxycarbonyl-L-leucylglycine ethyl ester [25] {3·5 g.; m. p. 103·5°, $[\alpha]_D^{17}$ −26·2° (*c* 2·8 in EtOH)} in ethanol (30 ml.) containing acetic acid (1·2 ml.) was hydrogenated in the presence of palladium black (0·35 g.). 1·8 g. of the syrupy acetate so obtained were dissolved in a mixture of chloroform (2 ml.) and benzene (8 ml.), and acetic anhydride (0·7 ml.) was added. After 12 hr. at room temperature, alcohol (1 ml.) was added (to decompose the excess of anhydride) and then ethyl acetate (30 ml.); the solution was washed with small volumes of dilute hydrochloric acid, 2N-potassium hydrogen carbonate, and water, and dried ($MgSO_4$). Evaporation gave a residue which solidified under light petroleum (b. p. 60—80°) at 5°, giving a white solid (0·78 g., 60%), m. p. 98·5—99·5°. Recrystallisation from isopropyl ether–light petroleum (b. p. 80—100°) gave material of m. p. 99—100°, $[\alpha]_D^{24}$ −55·0° (*c* 1·0 in EtOH). Distillation in a molecular still at 10^{-4} mm. gave *ester* of m. p. 100—101°, $[\alpha]_D^{16}$ −56·0° (*c* 1·2 in EtOH), $\nu_{max.}$ 1751, 1637 cm.$^{-1}$ (in liquid paraffin), at 1742, 1667 cm.$^{-1}$ (in $CHCl_3$) (Found: C, 55·8; H, 8·5; N, 10·8. $C_{12}H_{22}O_4N_2$ requires C, 55·8; H, 8·6; N, 10·8%).

Acetylation of L-leucylglycine ethyl ester by means of acetic acid with dicyclohexylcarbodiimide in tetrahydrofuran proceeded in 60% yield, but the crude product contained *N*-acetyl-*NN'*-dicyclohexylurea, m. p. 126—127°.

N-*Acetyl*-NN'-*dicyclohexylurea* (cf. ref. 26).—To dicyclohexylcarbodi-imide (2·0 g.) in boiling pyridine (50 ml.; dried and redistilled) was added dropwise glacial acetic acid (0·6 ml.). The mixture was heated under reflux for a further 30 min., the solvent was removed under reduced pressure, and the residue was crystallised and recrystallised from acetone–isopropyl ether, giving the *acylurea*, m. p. 127—128° (Found: C, 67·7; H, 9·7; N, 10·3. $C_{15}H_{26}O_2N_2$ requires C, 67·7; H, 9·8; N, 10·5%), $\nu_{max.}$ 1718, 1672 cm.$^{-1}$ (in liquid paraffin), at 1712, 1661 cm.$^{-1}$ (in $CHCl_3$).

Acetyl-L-leucylglycine.—Acetyl-L-leucylglycine ethyl ester (0·50 g.) was hydrolysed at room temperature by N-sodium hydroxide (2·15 ml.) in water (2 ml.) for 1 hr. The solution was made acid to Congo Red, and ether (15 ml.) was added; the *product* (0·35 g., 78%) crystallised. Recrystallisation from water gave needles, m. p. 193—194°, $[\alpha]_D^{17}$ −50° (*c* 2·0 in EtOH) (Found: C, 52·4; H, 7·8; N, 12·2. $C_{10}H_{18}O_4N_2$ requires C, 52·2; H, 7·9; N, 12·2%).

[23] Sheehan and Hlavka, *J. Org. Chem.*, 1958, **23**, 635.
[24] Anderson and Paul, *J. Amer. Chem. Soc.*, 1958, **80**, 4423.
[25] Vaughan and Osato, *ibid.*, 1952, **74**, 676.
[26] Zetzsche and Fredrich, *Chem. Ber.*, 1939, **72**, 1735.

By the action of diazoethane in ether, the acid was converted quantitatively into the ethyl ester, m. p. 97—98·5°, $[\alpha]_D^{15}$ −55° (*c* 1·2 in EtOH). Distillation of this product at 10^{-4} mm. gave material of m. p. 101°, $[\alpha]_D^{16}$ −55·4° (*c* 1·5 in EtOH).

*Acetyl-*DL*-leucylglycine Ethyl Ester.*—Acetyl-DL-leucine [27] (m. p. 161°; 4·7 g.) with triethylamine (3·8 ml.) was dissolved in anhydrous tetrahydrofuran (50 ml.), then cooled to −5°, and isobutyl chloroformate (3·6 ml.) was added slowly with stirring. After 5 min., glycine ethyl ester hydrochloride (3·8 g.) and triethylamine (3·8 ml.) in tetrahydrofuran (50 ml.) were added, and the temperature was allowed to rise. Next morning, the *ester* was isolated in the usual fashion, and recrystallised from ethyl acetate–cyclohexane, from benzene, and finally from ethyl acetate–light petroleum (b. p. 60—80°), giving needles, m. p. 120—120·5° (Found: C, 56·1; H, 8·7; N, 10·7%), $\nu_{max.}$ 1742, 1681 m, 1645 cm.$^{-1}$ (in liquid paraffin), at 1742, 1667 cm.$^{-1}$ (in $CHCl_3$).

Investigation of Racemisation during Coupling: General.—The acetyl-L-leucine had m. p. 186°, $[\alpha]_D^{18}$ −24·2° (*c* 4·0 in MeOH) (lit.,[28] m. p. 185—185·5°, $[\alpha]_D^{25}$ −23·0°). Glycine ethyl ester was distilled immediately before use. The normal procedure after each coupling reaction was to wash the products, dissolved in an organic solvent (usually ethyl acetate), with small volumes of 2N-hydrochloric acid, 2N-sodium carbonate or 2N-potassium hydrogen carbonate, and water; the solution was dried ($MgSO_4$) and evaporated to dryness *in vacuo*. The crude product was triturated with light petroleum (b. p. 60—80°), and the solid was collected. The filtrate was concentrated to give further solid or syrupy fractions. The specific rotations of the fractions were measured with ethanol as solvent (*c* 1); the percentage of L-peptide (excluding that present in any racemate), calculated from these values and by using $[\alpha]_D$ −56·0° for pure L-peptide, is placed in parentheses immediately after the specific rotation.* Hence the weights of L-peptide and of racemate in the solid fraction may be calculated, and from these the *minimum* percentage of each in the total product. The composition of the solid fractions was confirmed by hydrolysis in 6N-hydrochloric acid at 100° for 3 hr.; the solution was made up to a known volume, and the optical rotation was measured (at 20—23°). From the weight of peptide taken, the weight of leucine obtainable was calculated, and the results are expressed as the specific rotation of the leucine (*c* 2—3·5 in 3N-HCl). Under these conditions, authentic acetyl-L-leucylglycine ethyl ester gave a solution whose rotation corresponded to that of leucine of $[\alpha]_D$ +14·7°; by using this figure for the hydrolysis product of the pure L-peptide, the percentage of L-peptide in excess of the D-isomer was calculated for each fraction, and is given in parentheses after the specific rotation of the leucine so obtained. The identity of the solid fractions was confirmed further by infrared absorption spectra (of a suspension in liquid paraffin, unless otherwise stated; the peaks in the carbonyl region only are given). The m. p. of partly racemised peptide is depressed by addition of impurities other than racemate, and mixed m. p. of this kind have been useful in confirming identity.

(1) *Acid Azide Method.*[12]—*Acetyl-*L*-leucine methyl ester.* Acetyl-L-leucine (6·1 g.) was esterified by diazomethane in ether; the resulting syrup distilled at 80°/0·1 mm., and the distillate crystallised (5·4 g., 82%). Recrystallisation from cyclohexane–light petroleum (b. p. 40—60°) gave *ester* of m. p. 41·5—42·5°, $[\alpha]_D^{17}$ −42·0° (*c* 3·3 in MeOH), $[\alpha]_D^{16}$ −57·8° (*c* 4·0 in H_2O) (Found: C, 57·9; H, 9·2; N, 7·5. $C_9H_{17}O_3N$ requires C, 57·7; H, 9·2; N, 7·5%). The literature [10] records m. p. 74—75°, $[\alpha]_D^{16}$ −17·22°, for this compound prepared from acetyl-L-leucine of m. p. 167° (sintering at 155°), $[\alpha]_D^{16}$ −12·09° in EtOH. For comparison, the racemate was prepared as described below.

The fully active ester was obtained in 70% yield by treating acetyl-L-leucine with thionyl chloride and methanol under the conditions of Brenner and Huber.[11]

*Acetyl-*DL*-leucine methyl ester.* Acetyl-DL-leucine (3·5 g.) was esterified with diazomethane in ether; the *ester* (90%) had m. p. 77° after recrystallisation from cyclohexane (Found: C, 57·9; H, 9·2; N, 7·6%).

*Acetyl-*L*-leucylhydrazide.* Hydrazine hydrate (1·85 g.) was added to acetyl-L-leucine methyl ester (4·6 g.) in ethanol (40 ml.); after 36 hr. the solvent was removed, giving white crystals (4·6 g., 100%). Recrystallisation from ethyl acetate–light petroleum (b. p. 60—80°) gave

* In the subsequent descriptions, "L-peptide" is to be understood as excluding any L-peptide present as racemate.

[27] Fischer, *Ber.*, 1901, **34**, 433.
[28] DeWitt and Ingersoll, *J. Amer. Chem. Soc.*, 1951, **73**, 3359.

hydrazide of m. p. 143·5°, $[\alpha]_D^{13}$ −40·5° (*c* 3·8 in MeOH), $[\alpha]_D^{13}$ −39·8° (*c* 3·8 in 0·2N-HCl) (Found: C, 51·3; H, 9·1; N, 22·3. $C_8H_{17}O_2N_3$ requires C, 51·3; H, 9·2; N, 22·5%).

Coupling through the acid azide. To a solution of acetyl-L-leucylhydrazide (1·87 g.) in water (10 ml.), glacial acetic acid (2 ml.), and concentrated hydrochloric acid (2 ml.) at 0°, was added a solution of sodium nitrite (1·4 g.) in the minimum amount of water, with stirring. The resulting oil was extracted into ether (6 × 15 ml.), and the combined extracts were concentrated to 50 ml. in the presence of anhydrous magnesium sulphate. To the filtered solution at 0° was added distilled glycine ethyl ester (2·00 ml.). After 18 hr., ethyl acetate (30 ml.) was added to dissolve the oil which had separated, and the coupling product was isolated in the usual fashion. One experiment gave 1·25 g. of solid {m. p. 98—99°, $[\alpha]_D^{15}$ −53·0° (95%); N, 10·8%; $\nu_{max.}$ 1751, 1637 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +14·4° (calc. as leucine) (98%)} and 0·34 g. of syrup, $[\alpha]_D^{15}$ −43·6°. In another experiment 1·31 g. of acetyl-L-leucylhydrazide were used, with corresponding reductions in the quantities of the other reagents, and there were obtained 0·85 g. of solid {m. p. 97·5—99°; $[\alpha]_D^{18}$ −53·1° (95%); $\nu_{max.}$ 1751, 1639 cm.$^{-1}$}, and 0·14 g. of syrup, $[\alpha]_D^{18}$ −41·9°. Recrystallisation of the solid fractions failed to reveal any DL-material. The weight of L-peptide in the solid fraction of the former experiment is 1·19 g., 86% of the total yield (including syrup).

(2) *The Carbodi-imide Method.*[15]—(i) *In dichloromethane.* Redistilled *NN'*-dicyclohexylcarbodi-imide (2·27 g.) was dissolved in dried and distilled dichloromethane (30 ml.), and acetyl-L-leucine (1·73 g.) and glycine ethyl ester (1·00 ml.) were added. The mixture was stirred for 8 hr., a few drops of acetic acid were added, and after a further 30 min. the dicyclohexylurea was filtered off and washed with ethyl acetate. The combined washings and filtrate were evaporated to dryness, and the residue was taken up in ethyl acetate. One experiment gave 2·00 g. of solid {m. p. 90—93°; $[\alpha]_D^{17}$ −44·1° (79%); C, 55·3; H, 8·2; N, 10·9%; $\nu_{max.}$ 1757, 1645 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +12·0° (calc. as leucine) (82%)} and 0·23 g. of syrup, $[\alpha]_D^{19}$ −6·7°. A second experiment gave 1·73 g. of solid {m. p. 92—94°; $[\alpha]_D^{15}$ −44·6° (80%); C, 56·3; H, 8·4; N, 10·6%; $\nu_{max.}$ 1751, 1639 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +11·7° (calc. as leucine) (80%)} and 0·32 g. of syrup, $[\alpha]_D^{15}$ −5·2°. Recrystallisation of the solid fractions from the first experiment gave material of m. p. 117—119°, $[\alpha]_D^{19}$ −5·7° and from the second experiment solid of m. p. 117—118°, $[\alpha]_D^{20}$ −6·4°, neither m. p. being depressed on admixture with DL-peptide. The weight of L-peptide (1·58 g.) in the solid fraction of the first experiment is 71% of the total product.

(ii) *In tetrahydrofuran.* Dried, redistilled tetrahydrofuran (40 ml.) replaced the dichloromethane in procedure 2(i). One experiment gave 1·74 g. of solid {m. p. 95·5—98·5°; $[\alpha]_D^{13}$ −49·2° (88%); C, 56·4; H, 8·4; N, 11·1%; $\nu_{max.}$ 1754, 1642 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +13·0° (calc. as leucine) (88%)} and 0·27 g. of syrup, $[\alpha]_D^{15}$ +7·1°. A second experiment gave 2·00 g. of solid {m. p. 94·5—95·5°; $[\alpha]_D^{13}$ −47·6° (85%); C, 55·7; H, 8·6; N, 11·0%; $\nu_{max.}$ 1751, 1637 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +12·5° (calc. as leucine) (85%)} and 0·31 g. of syrup, $[\alpha]_D^{13}$ +11·5°. Recrystallisation of the solid fraction gave material of m. p. 116—118·5° (undepressed by admixture with DL-peptide), $[\alpha]_D^{18}$ −13°; $\nu_{max.}$ 1751, 1686, 1653 cm.$^{-1}$. The weight of L-peptide (1·70 g.) in the solid fraction of the second experiment is 74% of the total product.

(iii) *In tetrahydrofuran, with glycine ester hydrochloride and triethylamine.* Glycine ethyl ester hydrochloride (1·40 g.) and triethylamine (1·36 ml.) replaced the glycine ethyl ester used in procedure 2 (i). One experiment gave 1·28 g. of solid {m. p. 114—116°; $[\alpha]_D^{16}$ −13·1° (23%); C, 56·3; H, 8·2; N, 10·8%; $\nu_{max.}$ 1745, 1681m, 1639 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +3·4° (calc. as leucine) (23%)}, 0·30 g. of solid {m. p. 90—93°; $[\alpha]_D^{17}$ −40° (71·5%); C, 56·4; H, 8·7; N, 11·1%; $\nu_{max.}$ 1745, 1639 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +11·6° (calc. as leucine) (79%)}, and 0·66 g. of syrup, $[\alpha]_D$ 0°. A second experiment gave 0·87 g. of solid {m. p. 108—112°; $[\alpha]_D^{16}$ −14·8° (26·5%) C, 56·1; H, 8·5; N, 10·9%; $\nu_{max.}$ 1742, 1692w, 1656 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +4·2° (calc. as leucine) (29%)} and 0·94 g. of syrup, $[\alpha]_D^{17}$ −10·3°. The weight of racemate (1·08 g.) in the solid fractions of the first experiment is 48% of the total product.

(iv) *In dimethylformamide.* Dimethylformamide (40 ml.) replaced dichloromethane in procedure 2(i). One experiment gave 1·04 g. of solid {m. p. 97·5—99°; $[\alpha]_D^{18}$ −37·5° (67%); C, 56·1; H, 8·9; N, 10·95%; $\nu_{max.}$ 1751, 1637 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +9·2° (calc. as leucine) (63%)} and 1·17 g. of syrup, $[\alpha]_D^{19}$ +34·0°. Recrystallisation of the solid fraction gave some material of m. p. 119—120° (unchanged by admixture with DL-peptide), $[\alpha]_D^{18}$ −6·4°. After several months the syrup deposited crystals of *N*-acetyl-L-leucyl-*NN'*-dicyclohexylurea, which after recrystallisation from light petroleum (b. p. 60—80°) had m. p. 145—146°, $[\alpha]_D^{19}$ +58·5°;

the m. p. was unchanged by admixture with the authentic acylurea described below. A second experiment gave 1·20 g. of solid {m. p. 96—98°; $[\alpha]_D^{18}$ −36·5° (65%); C, 55·6; H, 8·5; N, 10·9%; $\nu_{max.}$ 1754, 1639 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +9·0° (calc. as leucine) (61%)} and 1·16 g. of syrup, $[\alpha]_D^{18}$ +27·5°. Recrystallisation of the solid material gave a first fraction of m. p. 110—113°, $[\alpha]_D^{19}$ +20°, $\nu_{max.}$ 1745, 1681w, 1647 cm.$^{-1}$. The weight of DL-peptide (0·42 g.) in the solid fraction of the second experiment is 18% of the total product.

(v) *In aqueous tetrahydrofuran.* A mixture of tetrahydrofuran (30 ml.) and water (10 ml.) replaced the dichloromethane in procedure 2(i). One experiment gave 1·73 g. of solid {m. p. 114—116° (undepressed on admixture with DL-peptide); $[\alpha]_D^{18}$ −13·9° (25%); C, 55·6; H, 8·5; N, 10·8%; $\nu_{max.}$ 1736, 1684w, 1647 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +3·5° (calc. as leucine) (24%)} and 0·31 g. of syrup, $[\alpha]_D^{19}$ −16·3°. A second experiment gave 1·60 g. of solid {m. p. 106—115°; $[\alpha]_D^{19}$ −14·6° (26%); C, 55·8; H, 8·5; N, 10·8%; $\nu_{max.}$ 1745, 1686w, 1650 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +3·2° (calc. as leucine) (22%)}, 0·1 g. of m. p. 100—115°, $[\alpha]_D^{18}$ −27°, and 0·21 g. of syrup, $[\alpha]_D^{18}$ −2°. The weight of DL-peptide (1·3 g.) in the first solid fraction of the first experiment is 64% of the total product.

N-*Acetyl*-L-*leucyl*-NN′-*dicyclohexylurea* (cf. ref. 26).—To dicyclohexylcarbodi-imide (2·06 g.) in boiling pyridine (50 ml.) was added acetyl-L-leucine (1·73 g.) portionwise in 45 min., and the temperature was allowed to fall slowly (2 hr.) to room temperature. The pyridine was removed *in vacuo* and the syrupy residue was extracted with light petroleum (b. p. 60—80°). Dicyclohexylurea was filtered off, the solvent was removed, and the residue was taken up in ethyl acetate, washed with hydrochloric acid, aqueous sodium carbonate, and water, and dried ($MgSO_4$). The solvent was removed, and the residue was finally crystallised from light petroleum (b. p. 60—80°) by slow evaporation, giving the *acylurea*, m. p. 146°, $[\alpha]_D^{25}$ +57·5° (*c* 0·8 in EtOH), $\nu_{max.}$ 1718, 1686, 1672, 1661sh, 1642 cm.$^{-1}$ (in liquid paraffin), at 1712, 1661 cm.$^{-1}$ (in $CHCl_3$) (Found: C, 66·4; H, 9·8; N, 11·0. $C_{21}H_{37}O_3N_3$ requires C, 66·4; H, 9·8; N, 11·1%). Hydrolysis by 6N-hydrochloric acid at 100° for 3 hr. gave a solution which (after removal of dicyclohexylurea) was made up to a known volume, then having $[\alpha]_D^{25}$ +14·2° (calc. as leucine; *c* 1·8 in 4·5N-HCl).

(3) *With Diethyl Phosphorochloridite.*[4]— (i) *Anhydride procedure.* Acetyl-L-leucine (1·73 g.) was suspended in dried toluene (50 ml.) containing triethylamine (1·36 ml.) and the temperature raised to 20—25°. Diethyl phosphorochloridite (1·46 ml.; b. p. 52—56°/28 mm., n_D^{25} 1·4345) in toluene (10 ml.) was slowly added, with stirring, the temperature being kept below 25°. After 10 min., the solution was filtered and glycine ethyl ester (100 ml.) in toluene (25 ml.) was added. The mixture was heated on the water-bath for 15 min., then cooled, and ethyl acetate (50 ml.) was added. The final product was dried at 70°/0·5 mm. to remove traces of diethyl phosphite. One experiment gave 0·87 g. of solid {m. p. 94—95°; $[\alpha]_D^{16}$ −46·4° (83%); N, 10·55%; $\nu_{max.}$ 1754, 1639 cm.$^{-1}$} and 0·23 g. of syrup, $[\alpha]_D^{15}$ −13·8°. A second experiment gave 0·80 g. of solid {m. p. 95—96°; $[\alpha]_D^{12}$ −48·5° (87%); N, 10·4%; $\nu_{max.}$ 1751, 1639 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +12·7° (calc. as leucine) (86%)} and 0·11 g. of syrup, $[\alpha]_D^{15}$ −10·6°. Recrystallisation of the first fraction gave material of m. p. 111—114° (undepressed on admixture with DL-peptide), $[\alpha]_D^{14}$ −17·8°; $\nu_{max.}$ 1748, 1684w, 1650 cm.$^{-1}$. A second experiment gave 0·66 g. of solid {m. p. 92—94°, $[\alpha]_D^{15}$ −45·1° (80·5%); N, 10·6%; $\nu_{max.}$ 1751, 1642 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +12·0° (calc. as leucine) (82%)} and 0·15 g. of syrup, $[\alpha]_D^{17}$ −15·8°. The weight of L-peptide (0·70 g.) in the solid fraction of the second experiment is 77% of the total product.

(ii) *Anhydride procedure, with glycine ester hydrochloride and triethylamine.* Acetyl-L-leucine (3·5 g., 0·02 mole) was suspended in toluene (100 ml.) containing triethylamine (2·8 ml.). The mixture was heated to 25°, then diethyl phosphorochloridite (2·9 ml.) in toluene (20 ml.) was slowly added (10 min.). After a further 10 min., triethylamine hydrochloride was filtered off, and glycine ethyl ester hydrochloride (2·8 g.), suspended in toluene (50 ml.) containing triethylamine (2·8 ml.), was added. After 30 min. on the boiling-water bath, the mixture was cooled, and ethyl acetate (100 ml.) was added. One experiment gave 0·60 g. of solid {m. p. 104—107° (undepressed by admixture with DL-peptide), $[\alpha]_D^{17}$ −0·6° (1%); N, 10·8%; $\nu_{max.}$ 1742, 1698m, 1681m, 1647 cm.$^{-1}$ (in liquid paraffin), at 1742, 1672 cm.$^{-1}$ (in $CHCl_3$)}, and 1·06 g. of syrup, $[\alpha]_D^{17}$ −17·8°. A second experiment, with 0·01 mole of reactants, gave 0·42 g. of solid {m. p. 102—105° (undepressed by admixture with DL-peptide); $[\alpha]_D^{15}$ −4·5° (8%); N, 10·55%; $\nu_{max.}$ 1745, 1704m, 1684m, 1653 cm.$^{-1}$ (in liquid paraffin), at 1739, 1672 cm.$^{-1}$ (in $CHCl_3$); after hydrolysis, $[\alpha]_D$ +1·8° (calc. as leucine) (12%)} and 0·40 g. of syrup, $[\alpha]_D^{15}$ −18·2°. A third experiment (0·01-molar scale) gave 0·65 g. of solid {m. p. 103—106°; $[\alpha]_D^{16}$ −14·4° (26%);

N, 10·35%; $\nu_{max.}$ 1739, 1667 cm.$^{-1}$ (in $CHCl_3$); after hydrolysis, $[\alpha]_D$ +4·7° (calc. as leucine) (32%)} and 0·31 g. of syrup, $[\alpha]_D^{16}$ −15·1°. The weight of DL-peptide (0·48 g.) in the solid fraction of the third experiment is 50% of the total product.

(iii) *Anhydride procedure, with glycine ester and triethylamine.* The procedure is described under 3 (i) above, but additional triethylamine (1·36 ml.) was added with the glycine ethyl ester. One experiment gave 0·51 g. of solid {m. p. 93—96°; $[\alpha]_D^{18}$ −26·7° (48%); N, 11·2%; $\nu_{max.}$ 1748, 1645 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +8·3° (calc. as leucine) (56·5%)} and 0·14 g. of syrup, $[\alpha]_D^{18}$ −11·3°. A second experiment gave 0·36 g. of solid {m. p. 83—86°; $[\alpha]_D^{17}$ −30·2° (54%); N, 10·4%; $\nu_{max.}$ 1739, 1661 cm.$^{-1}$ (in $CHCl_3$); after hydrolysis, $[\alpha]_D$ +6·9° (calc. as leucine) (47%)} and 0·29 g. of syrup, $[\alpha]_D^{15}$ −10·3°. The weight of L-peptide (0·24 g.) in the solid fraction of the first experiment is 37% of the total yield, and the weight of racemate in this fraction, 0·27 g., is 41% of the total product.

(iv) *Amide procedure.* Glycine ethyl ester (1·00 ml.) and triethylamine (1·36 ml.) were dissolved in dried ether, and diethyl phosphorochloridite (1·46 ml.) in ether was added slowly. After 30 min. at room temperature the triethylamine hydrochloride was filtered off and washed with ether, and the combined ethereal solutions were evaporated *in vacuo*. The residue was taken up in dried toluene (50 ml.), acetyl-L-leucine (1·73 g.) was added, and the reactants were heated on the water-bath for 15 min.; not all the acetyl-L-leucine dissolved. Ethyl acetate (50 ml.) was added. A first experiment gave 0·55 g. of solid {m. p. 88—91°; $[\alpha]_D^{16}$ −42·9° (77%); N, 10·45%} and 0·18 g. of syrup, $[\alpha]_D^{17}$ −8·0°. A second experiment gave 0·63 g. of solid {m. p. 92—94°; $[\alpha]_D^{16}$ −44·0° (79%); N, 10·7%; $\nu_{max.}$ 1739, 1667 cm.$^{-1}$ (in $CHCl_3$); after hydrolysis, $[\alpha]_D$ +11·9° (calc. as leucine) (81%)} and 0·23 g. of syrup, $[\alpha]_D^{17}$ −10·5°. The weight of DL-peptide (0·13 g.) in the solid fraction of the second experiment is 15% of the total product. Recrystallisation of the solid fractions, which were discoloured, failed to yield racemate.

(4) *With Tetraethyl Pyrophosphite.*[5]—(i) *Anhydride procedure.* Acetyl-L-leucine (1·73 g.) and tetraethyl pyrophosphite (b. p. 62—64°/0·2 mm., n_D^{19} 1·4341) in diethyl phosphite (7 ml.) were heated on a boiling-water bath for 2 min. and glycine ethyl ester (1·00 ml.) was then added. The reactants were heated on the water-bath for 30 min., water (30 ml.) was added, and the solution was extracted with light petroleum (b. p. 60—80°). The aqueous layer was then extracted with ethyl acetate, and the coupling product was isolated from the extract in the normal manner. The final product was dried at 70°/0·5 mm. to remove traces of diethyl phosphite. One experiment gave 0·47 g. of solid {m. p. 119—120·5° (undepressed on admixture with DL-peptide); $[\alpha]_D^{21}$ −11·1° (20%); N, 10·55%; $\nu_{max.}$ 1751, 1689, 1654 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +1·7° (calc. as leucine) (12%)} and 1·46 g. of syrup {$[\alpha]_D^{21}$ −45·0° (80%); after hydrolysis, $[\alpha]_D$ +11·7° (calc. as leucine) (80%)}. A second experiment gave 0·38 g. of solid {m. p. 118·5—120° undepressed on admixture with DL-peptide, $[\alpha]_D^{21}$ −8·8° (16%); N, 11·0%; $\nu_{max.}$ 1751, 1692, 1656 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +2·0° (calc. as leucine) (14%)} and 1·09 g. of syrup {$[\alpha]_D^{21}$ −44·4° (79%); N, 10·3%; after hydrolysis, $[\alpha]_D$ +10·0° (calc. as leucine) (68%)}. The weight of racemate (0·32 g.) in the solid fraction of the second experiment is 22% of the total product.

(ii) *Anhydride procedure, with glycine ester hydrochloride and triethylamine.* The procedure followed that of 4(i), but glycine ethyl ester hydrochloride (1·40 g.) with triethylamine (1·36 ml.) replaced the glycine ethyl ester. One experiment gave 0·57 g. of solid {m. p. 113—116°; $[\alpha]_D^{13}$ −9·0° (16%); N, 11·0%; $\nu_{max.}$ 1748, 1678w, 1650 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +2·9° (calc. as leucine) (20%)} and 0·68 g. of syrup, $[\alpha]_D^{12}$ −32·5°. A second experiment gave 0·45 g. of solid {m. p. 117—118·5° (undepressed by admixture with DL-peptide); $[\alpha]_D^{16}$ −6·5° (12%); N, 11·2%; $\nu_{max.}$ 1745, 1681w, 1647 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +1·4° (calc. as leucine) (10%)} and 0·98 g. of syrup, $[\alpha]_D^{16}$ −32·4°. The weight of racemate (0·48 g.) in the solid fraction of the first experiment is 38% of the total product.

(iii) *Amide procedure.* Glycine ethyl ester (1·00 ml.) and tetraethyl pyrophosphite (2·7 ml.) in diethyl phosphite (7 ml.) were heated on the water-bath for 2 min., acetyl-L-leucine (1·73 g.) was then added, and heating was continued for 30 min. The subsequent procedure is described in 4(i). One experiment gave 0·25 g. of solid {m. p. 115—117° (undepressed on admixture with DL-peptide), $[\alpha]_D^{10}$ −9·9° (18%); N, 10·6%; $\nu_{max.}$ 1745, 1704w, 1684m, 1647 cm.$^{-1}$ (in liquid paraffin), at 1745, 1664 cm.$^{-1}$ (in $CHCl_3$); after hydrolysis, $[\alpha]_D$ +2·4° (calc. as leucine) (16%)} and 1·25 g. of syrup, $[\alpha]_D^{11}$ −39·4° ($\nu_{max.}$ 1751, 1639 cm.$^{-1}$, liquid film). A second experiment gave 0·68 g. of solid {m. p. 98·5—100°; $[\alpha]_D^{17}$ −47·1° (84%); N, 10·9%; $\nu_{max.}$ 1751, 1681w, 1639 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +12·1° (calc. as leucine) (82%)} and 0·67 g. of syrup, $[\alpha]_D^{16}$

$-27{\cdot}5°$. A third experiment gave 1·16 g. of solid {m. p. 96—98°; $[\alpha]_D^{15}$ $-38{\cdot}9°$ (70%); N, 11·2%; $\nu_{max.}$ 1751, 1639 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ $+12{\cdot}1°$ (calc. as leucine) (82·5%)} and 0·17 g. of syrup, $[\alpha]_D^{17}$ $-9{\cdot}8°$. The weight of DL-peptide (0·35 g.) in the solid fraction of the third experiment is 26% of the total yield.

(iv) *Amide procedure, with glycine ester hydrochloride and triethylamine.* The procedure followed that of 4 (iii), except that glycine ethyl ester hydrochloride (1·40 g.) and triethylamine (1·36 ml.) replaced glycine ethyl ester. One experiment gave 0·95 g. of solid {m. p. 105—112°; $[\alpha]_D^{13}$ $-30{\cdot}1°$ (54%); N, 10·9%; $\nu_{max.}$ 1745, 1664 cm.$^{-1}$ (in $CHCl_3$); after hydrolysis, $[\alpha]_D$ $+8{\cdot}9°$ (calc. as leucine) (61%)} and 0·53 g. of syrup, $[\alpha]_D^{13}$ $-27{\cdot}8°$. A second experiment gave 0·50 g. of solid {m. p. 117·5—119° (undepressed by admixture with DL-peptide); $[\alpha]_D^{17}$ $-8{\cdot}2°$ (15%); N, 10·4%; $\nu_{max.}$ 1751, 1689m, 1656 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ $+1{\cdot}7°$ (calc. as leucine) (12%)} and 0·71 g. of syrup, $[\alpha]_D^{17}$ $-36{\cdot}0°$. The weights of L-peptide (0·51 g.) and of racemate (0·44 g.) in the solid fraction of the first experiment are 35% and 30% of the total product, respectively.

(v) *Amide procedure, with glycine ester and glycine ester hydrochloride.* The procedure followed that of 4(iii), but glycine ethyl ester hydrochloride (1·40 g.) was present in addition to the glycine ethyl ester. One experiment gave 1·16 g. of solid {m. p. 94—96°; $[\alpha]_D^{17}$ $-47{\cdot}6°$ (85%); N, 11·2%; $\nu_{max.}$ 1751, 1642 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ $+13{\cdot}4°$ (calc. as leucine) (91%)} and 0·11 g. of syrup, $[\alpha]_D^{18}$ $-20°$. A second experiment gave 1·32 g. of solid {m. p. 95—97°; $[\alpha]_D^{18}$ $-42{\cdot}3°$ (76%); N, 10·95%; $\nu_{max.}$ at 1751, 1639 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ $+11{\cdot}5°$ (calc. as leucine) (78%)} and 0·16 g. of syrup, $[\alpha]_D^{18}$ $-14{\cdot}8°$. The weight of L-peptide in the solid fraction of the first experiment is 78% of the total product.

(vi) *With glycine ester hydrochloride but without tertiary amine.* Acetyl-L-leucine (1·73 g.), glycine ethyl ester hydrochloride (1·40 g.), and tetraethyl pyrophosphite (2·7 ml.) in diethyl phosphite (7 ml.) were heated on the steam-bath for 30 min., dissolution occurring. After cooling, water (30 ml.) was added, and the procedure then followed that described in 4(i). One experiment gave 0·91 g. of solid {m. p. 117—119° (undepressed on admixture with DL-peptide), $[\alpha]_D^{16}$ $-3{\cdot}7°$ (7%); N, 10·5%; $\nu_{max.}$ 1743, 1686w, 1650 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ $+1{\cdot}5°$ (calc. as leucine) (10%)} and 0·19 g. of syrup, $[\alpha]_D^{16}$ $-3{\cdot}8°$. A second experiment gave 0·80 g. of solid {m. p. 117—119°; $[\alpha]_D^{18}$ $-5{\cdot}2°$ (9%); N, 10·8%; $\nu_{max.}$ 1745, 1684m, 1647 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ $+1{\cdot}7°$ (calc. as leucine) (12%)} and 0·23 g. of syrup, $[\alpha]_D^{14}$ $-7{\cdot}5°$. The weight of racemate in the solid fraction of the first experiment is 0·85 g., 77% of the total product.

(vii) *With pyridine as solvent.*[18] Acetyl-L-leucine (1·73 g.) and glycine ethyl ester hydrochloride (1·40 g.) were dissolved in dried pyridine (50 ml.). Tetraethyl pyrophosphite (5 ml.) was added slowly, followed by triethylamine (1·38 ml.), and the mixture was heated for 1 hr. on the water-bath. The solvent was removed *in vacuo* at 50°, and the residue was taken up in ethyl acetate. Traces of diethyl phosphite were removed from the final product at 70°/0·5 mm. One experiment gave 0·78 g. of solid {m. p. 104—108°; $[\alpha]_D^{15}$ $-4{\cdot}9°$ (9%); N, 11·0%; $\nu_{max.}$ 1742, 1669 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ $+1{\cdot}4°$ (calc. as leucine) (10%)} and 1·38 g. of syrup, $[\alpha]_D^{17}$ $-6{\cdot}7°$. A second experiment gave 0·80 g. of solid {m. p. 106—111° (undepressed on admixture with DL-peptide), $[\alpha]_D^{15}$ $-2{\cdot}3°$ (4%); N, 11·0%; $\nu_{max.}$ 1742, 1701w, 1681w, 1650 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ $+0{\cdot}7°$ (calc. as leucine) (5%)} and 1·32 g. of syrup, $[\alpha]_D^{17}$ $-16{\cdot}0°$. The weight of racemate (0·77 g.) in the solid fraction of the second experiment is 36% of the total product.

(5) *Carbonic Mixed Anhydride Method.*[19]—(i) *In tetrahydrofuran.* A solution of acetyl-L-leucine (1·73 g.) and triethylamine (1·36 ml.) in anhydrous tetrahydrofuran (25 ml.) was cooled to $-5°$ and isobutyl chloroformate (1·31 ml.) was slowly added with stirring. After 5 min., a suspension of glycine ethyl ester hydrochloride (1·68 g., 0·012 mole) and triethylamine (1·36 ml., 0·010 mole) in tetrahydrofuran (25 ml.) at $-5°$ was slowly added, and the temperature was allowed to rise. Next day, triethylamine hydrochloride was filtered off and washed with ethyl acetate; the washings and filtrate were evaporated to dryness *in vacuo* and the residue was taken up in ethyl acetate. One experiment gave 1·38 g. of solid {m. p. 118—119° (undepressed on admixture with DL-peptide); $[\alpha]_D^{16}$ 0° (0%); N, 10·6%; $\nu_{max.}$ 1745, 1684m, 1647 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ $+0{\cdot}7°$ (calc. as leucine) (5%)} and 0·34 g. of syrup, $[\alpha]_D^{16}$ $-34{\cdot}5°$. A second experiment gave 1·65 g. of solid {m. p. 118—119°; $[\alpha]_D^{16}$ $-11{\cdot}3°$ (20%); N, 10·7%; $\nu_{max.}$ 1745, 1681m, 1642 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ $+2{\cdot}3°$ (calc. as leucine) (16%)} and 0·30 g. of syrup, $[\alpha]_D^{17}$ $-33{\cdot}6°$. The solid fraction (racemate) of the first experiment is 80% of the total product.

(ii) *In chloroform.* Chloroform replaced the tetrahydrofuran used in 5(i). One experiment gave 1·65 g. of solid {m. p. 118·5—120°; $[\alpha]_D^{18}$ $-0{\cdot}5°$ (1%); N, 10·8%; $\nu_{max.}$ 1745, 1681m, 1647

cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +0·2° (calc. as leucine) (1%)}, and 0·37 g. of syrup, $[\alpha]_D^{18}$ −7·0°. Another experiment gave 1·80 g. of solid {m. p. 118—120° (undepressed on admixture with DL-peptide), $[\alpha]_D^{19}$ −0·5° (1%); N, 10·9%; $\nu_{max.}$ at 1748, 1681m, 1647 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ 0°}, and 0·16 g. of syrup, $[\alpha]_D^{20}$ −15°. The weight of racemate (1·78 g.) in the solid fraction of the second experiment is 91% of the total product.

(iii) *In tetrahydrofuran, with glycine ester.* The procedure described in 5(i) was followed, except that after anhydride formation the triethylamine hydrochloride was filtered off, and distilled glycine ethyl ester (1·00 ml.) was used in place of the ester hydrochloride and triethylamine. One experiment gave 0·37 g. of solid {m. p. 120—120·5°, $[\alpha]_D^{18}$ −5·7° (10%); N, 10·7%; $\nu_{max.}$ 1754, 1694, 1657 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +0·4° (calc. as leucine) (3%)}, 1·04 g. of solid {m. p. 98—99°; $[\alpha]_D^{18}$ −52·3° (93%); N, 10·65%; $\nu_{max.}$ 1751, 1639 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +14·0° (calc. as leucine) (95%)}, and 0·09 g. of syrup, $[\alpha]_D^{17}$ −17°. A second experiment gave 1·72 g. of solid {m. p. 95—110°; $[\alpha]_D^{15}$ −33·0° (59%); N, 10·45%; $\nu_{max.}$ 1745, 1642 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +9·2° (calc. as leucine) (63%)} and 0·07 g. of syrup, $[\alpha]_D^{13}$ −9·8°. The weights of L-peptide (1·01 g.) and of racemate (0·61 g.) in the solid fraction of the second experiment are 57% and 40% of the total product, respectively.

(iv) *In tetrahydrofuran, with glycine ester liberated in chloroform solution.* Glycine ethyl ester hydrochloride (1·40 g.) and triethylamine (1·38 ml.) were dissolved in chloroform (15 ml.); the solvent was removed *in vacuo* at room temperature, and the suspension of the residue in tetrahydrofuran (25 ml.) was used in a coupling reaction similar to that of 5(i), except that 15 min. were allowed for formation of the anhydride. One experiment gave 1·17 g. of solid {m. p. 105—108°; $[\alpha]_D^{15}$ −24·4° (44%); N, 10·8%; $\nu_{max.}$ 1742, 1639 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +7·0° (calc. as leucine) (48%)} and 0·61 g. of syrup, $[\alpha]_D^{15}$ −41·8°. A second experiment gave 1·09 g. of solid {m. p. 96—103°; $[\alpha]_D^{16}$ −19·6° (35%); N, 10·6%; $\nu_{max.}$ 1745, 1639 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +5·4° (calc. as leucine) (37%)} and 0·74 g. of syrup which subsequently solidified {m. p. 90—94°; $[\alpha]_D^{16}$ −46·0° (82%); N, 10·1%; $\nu_{max.}$ 1751, 1637 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +12·1° (calc. as leucine) (82%)}. The product from the second experiment contained 54% of L-peptide and 46% of racemate.

(6) *Phosphorazo Method.*[20]—(i) *Coupling at* 100°. A solution of glycine ethyl ester hydrochloride (1·40 g.) in dried pyridine (10 ml.) was cooled to 0°, and phosphorus trichloride (0·44 ml., redistilled from dimethylaniline) in pyridine (4·7 ml.) was slowly added. The orange solution was set aside at room temperature for 30 min., with occasional shaking, then acetyl-L-leucine (1·73 g.) and pyridine (10 ml.) were added, and the mixture was heated on the boiling-water bath for 3 hr. The pyridine was removed *in vacuo*, water (1 ml.) was added, and the product was extracted into ethyl acetate (50 ml.). One experiment gave 1·07 g. of solid {m. p. 117·5—118·5° (undepressed on admixture with DL-peptide); $[\alpha]_D^{17}$ −3·6° (6%); N, 10·55%; $\nu_{max.}$ 1742, 1681m, 1647 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +0·7° (calc. as leucine) (5%)} and 0·44 g. of syrup, $[\alpha]_D^{17}$ −21·2°. A second experiment gave 1·11 g. of solid {m. p. 114·5—116°; $[\alpha]_D^{17}$ −4·8° (9%); N, 10·75%; $\nu_{max.}$ 1745, 1684m, 1647 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +1·0° (calc. as leucine) (7%)} and 0·24 g. of syrup, $[\alpha]_D^{17}$ 0°. The weight of racemate (1·01 g.) in the solid fraction of the second experiment is 75% of the total product.

(ii) *Coupling at room temperature.* The procedure followed that of 6(i), except that coupling was effected during 3 days at room temperature. One experiment gave 1·33 g. of solid {m. p. 117—118·5°; $[\alpha]_D^{17}$ −11·5° (21%); N, 10·6%; $\nu_{max.}$ 1748, 1689w, 1653 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +2·0° (calc. as leucine) (14%)} and 0·16 g. of syrup, $[\alpha]_D^{17}$ −9·8°. A second experiment gave 1·30 g. of solid {m. p. 118—119° (undepressed on admixture with DL-peptide); $[\alpha]_D^{16}$ −11·1° (20%); N, 10·65%; $\nu_{max.}$ 1745, 1684w, 1650 cm.$^{-1}$; after hydrolysis, $[\alpha]_D$ +2·9° (calc. as leucine) (20%)} and 0·16 g. of syrup, $[\alpha]_D^{18}$ −11·9°. The weight of racemate (1·05 g.) in the solid fraction of the first experiment is 71% of the total product.

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