1260. *Amino-acids and Peptides. Part XXIV.*[1] *The Use of Esters of 1-Hydroxypiperidine and of Other NN-Dialkylhydroxylamines in Peptide Synthesis and as Selective Acylating Agents*

By B. O. Handford, J. H. Jones, G. T. Young, and (in part) T. F. N. Johnson

Esters of acylamino-acids with 1-hydroxypiperidine, dimethylhydroxylamine, diethylhydroxylamine, and dibenzylhydroxylamine, have been prepared in good yield (A) by direct condensation using dicyclohexylcarbodiimide, (B) through the acid chloride, or (C) through the carbonic mixed anhydride. Such esters (1) are themselves slow to react with α-amino-esters but, in the presence of acetic acid, condensation occurs readily at room temperature, giving high yields of pure protected peptides. The amino-ester hydrochlorides with sodium acetate, or with triethylamine and acetic acid, may conveniently be used instead of the amino-ester and acetic acid. The acylamino-esters will also couple, rather more slowly, with the sodium salts of amino-acids, again in high yield. They have remarkable optical stability under basic conditions in which analogous *p*-nitrophenyl esters racemise partially or completely. In stringent racemisation tests, the condensation of benzoyl-L-leucine 1-piperidyl ester with glycine ethyl ester or with the sodium salt of glycine, no racemisation was detected. These esters are therefore stable intermediates which can be activated at will to provide reagents which couple without racemisation, giving products of high purity in excellent yield.

Although acid catalysis is desirable for the reaction of these esters with α-amino-esters, with stronger nucleophiles such as aliphatic amines and ammonia the uncatalysed reaction is also fast. Highly selective acylation can be effected in this way; *e.g.*, 1-benzoyloxypiperidine reacts completely with n-butylamine under conditions in which t-butylamine and aniline are unattacked, and cyclohexylamine and isopropylamine scarcely attacked. Other uses include the benzyloxycarbonylation and *p*-nitrobenzyloxycarbonylation of α-amino-esters by means of benzyl or *p*-nitrobenzyl 1-piperidyl carbonate.

Investigations of the racemisation which may occur during the condensation of acylamino-acids with amino-esters have shown [2-6] that the acid azide route is unique among those examined, in preserving full optical activity in a wide range of cases and conditions. Furthermore, this route alone allows activation of the carboxyl group of an acylpeptide (*via* the alkyl ester and hydrazide) without danger of racemisation. Although, for example, the widely-used *p*-nitrophenyl esters will normally couple without racemisation, an acylpeptide cannot yet be converted into its *p*-nitrophenyl ester without this risk.*

* We have shown [7] that the procedure using diphenylketen, which was reported to give optically pure aryl esters,[8] does in fact give some racemate when used to convert benzyloxycarbonylglycyl-L-phenylalanine into its *p*-nitrophenyl ester.

[1] Part XXIII, preceding Paper.
[2] M. B. North and G. T. Young, *Chem. and Ind.*, 1955, 1597.
[3] N. A. Smart, G. T. Young, and M. W. Williams, *J.*, 1960, 3902.
[4] M. W. Williams and G. T. Young, *J.*, 1963, 881.
[5] G. W. Anderson and R. W. Young, *J. Amer. Chem. Soc.*, 1952, **74**, 5307; G. W. Anderson, J. Blodinger, and A. D. Welcher, *J. Amer. Chem. Soc.*, 1952, **74**, 5309; G. W. Anderson and F. M. Callahan, *ibid.*, 1958, **80**, 2902.
[6] F. Weygand, A. Prox, L. Schmidhammer, and W. König, *Angew. Chem. Internat. Edn.*, 1963, **2**, 183; A. Prox, F. Weygand, W. König, and L. Schmidhammer, Proc. 6th European Peptide Symp., Athens, 1963, Pergamon Press, in the press.
[7] I. Antonovics, A. L. Heard, J. Hugo, M. W. Williams, and G. T. Young, Proc. 6th European Peptide Symp., Athens, 1963, Pergamon Press, in the press.
[8] D. T. Elmore and J. Smyth, *Proc. Chem. Soc.*, 1963, 18.

Unfortunately, acid azides have other disadvantages; the Curtius rearrangement and other side-reactions can form troublesome impurities,[9] and yields are often lower than with alternative methods. These facts have imposed restrictions on the scheme of synthesis of large peptides, which commonly are built up stepwise from the amino-end by condensation with alkoxycarbonylamino-acids (which normally do not racemise whatever method of coupling is used); the fragments so formed are then joined either through an amino-acid (such as glycine or proline) for which racemisation is not a problem, or through the acid azide. It is to remove this limitation of synthetic approach that improved methods of condensation are still being sought. We report now a new method of coupling which, even in unfavourable cases, has been found so far to proceed without racemisation and in high yield.[10]

The " active esters " which are at present used in peptide synthesis gain their reactivity from an inductive effect which inevitably increases the acidity of the α-hydrogen, and in basic conditions even benzyloxycarbonylamino-esters of this kind racemise slowly.[11,12] Our studies on racemisation drew our attention to esters of *NN*-dialkylhydroxylamines (1) which we expected to be relatively inactive but to be activated strongly by protonation (2).

$$\underset{(1)}{R{\cdot}CO{\cdot}ONR'R''} \xrightarrow{H^+} \underset{(2)}{R{\cdot}CO{\cdot}\overset{+}{O}NHR'R''}$$

For our initial examination we chose esters of 1-hydroxypiperidine, which can be obtained by direct oxidation of piperidine with hydrogen peroxide,[13] though the yields are low (*ca.* 25%). It has long been known that the action of heat on 1-ethylpiperidine 1-oxide gives 1-hydroxypiperidine,[14] but a satisfactory preparation of the latter by this route has not been described, and we give details of our procedure for the oxidation of 1-ethylpiperidine to the 1-oxide and for the subsequent Cope elimination reaction; we find this to be the most convenient laboratory route.

Acylamino-acids were condensed with 1-hydroxypiperidine by means of dicyclohexylcarbodi-imide (method A), through the acid chloride (method B), or through the carbonic mixed anhydride (method C). Method A appears to be more generally useful, giving yields of 65—89% in the cases examined, although when the acid chloride is readily available (as for benzyloxycarbonyl- and phthaloyl-glycine) high yields of esters can conveniently be obtained by method B, which is by far the best method for the preparation of esters of simple carboxylic acids. The yields from method C have been rather lower on average. By one or more of these methods have been prepared the 1-piperidyl esters of benzyloxycarbonyl-L-alanine, -β-alanine, -*S*-benzyl-L-cysteine, -glycine, -L-leucine, -L-phenylalanine, -sarcosine, -L-valine, and -glycylglycine, and of phthaloyl-glycine and -L-phenylalanine. All these acylamino-esters (excepting that of benzyloxycarbonylsarcosine) are crystalline compounds, and when pure are stable at room temperature for many weeks; their infrared ester-carbonyl absorption is usually near 1760 cm.$^{-1}$.

As expected, these rate of reaction of the esters with α-amino-esters is slow at room temperature (see Exp. 20 in the Table) but the addition of acetic acid equivalent in amount to the amino-component causes rapid and smooth condensation; the results of such coupling reactions are shown in the Table. The progress of these condensations is conveniently followed by means of thin-layer chromatography using ether as solvent, which readily separates unchanged 1-piperidyl ester; it should be noted that the reaction time

[9] See, *e.g.*, J. Honzl and J. Rudinger, *Coll. Czech. Chem. Comm.*, 1961, **26**, 2333.
[10] Preliminary communications: S. M. Beaumont, B. O. Handford, and G. T. Young, Proc. 7th European Peptide Symp., Budapest, 1964, to be published; S. M. Beaumont, B. O. Handford, J. H. Jones, and G. T. Young, *Chem. Comm.*, 1965, 53; U.K. Pat. Appl., 48,881/1964.
[11] M. Bodanszky and C. A. Birkhimer, *Chimia* (*Switz.*), 1960, **14**, 368.
[12] B. Liberek, *Tetrahedron Letters*, 1963, 925.
[13] C. Schöpf, A. Komjak, F. Braun, and E. Jacobi, *Annalen*, 1948, **559**, 1.
[14] W. Wernick and R. Wolffenstein, *Ber.*, 1898, **31**, 1553; M. Auerbach and R. Wolffenstein, *ibid.*, 1899, **32**, 2507.

The synthesis of peptides using esters of *NN*-dialkylhydroxylamines

No.	Active ester [1]	Amino-component	Method [2]	Scale [3] (mmoles)	Solvent	Time [4] (hr.)	Peptide [5] Yield (%)	Peptide [5] M. p.
1	Z.Ala.OPip	Gly.OEt	(I)	1·0	Dioxan	1·25	98	97—98° [6]
2	Z.βAla.OPip	,,	(I)	1·0	,,	3	95	101—102 [7]
3	Z.Cys(Bzl).OPip	,,	(I)	1·0	EtOAc	48	84	95—98 [8]
4	Z.Gly.OPip	,,	(I)	4·7	,,	1	91	78—80 [9]
5	Z.Gly.ONEt$_2$	,,	(I)	1·0	,,	2·5	81	78—80
6	Z.Gly.OPip	Leu.OMe	(II)	5·0	Dioxan	48	72	— [10]
7	,,	Phe.OMe	(II)	5·0	,,	72	80	— [11]
8	,,	Phe.ONa	(III)	5·0	Dioxan–H_2O	15	71	126—128 [12]
9	,,	Tyr.OEt	(II)	2·5	Dioxan	48	76	126 [13]
10	,,	Gly.Gly.OEt	(II)	5·0	,,	14	51	164 [14]
11	Pht.Gly.OPip	Gly.OEt	(II)	4·7	,,	16	91	192—194 [15]
12	Pht.Gly.ONMe$_2$	,,	(I)	1·0	EtOAc	2·5	81	195—197 [16]
13	Pht.Gly.ON(CH$_2$Ph)$_2$	,,	(I)	1·0	,,	3	87	193—194 [16]
14	Pht.Gly.OPip	Leu.OMe	(II)	4·7	$CHCl_3$	60	92	140—143 [17]
15	,,	Phe.OMe	(II)	4·7	,,	60	94	176—179 [18]
16	,,	Gly.Gly.OEt	(II)	4·7	Dioxan	14	61	230—231 [19]
17	Z.Leu.OPip	Gly.OEt	(I)	5·0	Dioxan	36	84	103—104·5 [20]
18	,,	,,	(II)	5·0	,,	2	85	103—104·5 [20]
19	,,	,,	[21]	5·0	$CHCl_3$	5	94	101—104
20	,,	,,	[22]	5·0	Dioxan	5 days	81	103—104·5 [20]
21	Z.Leu.ONMe$_2$	,,	(I)	1·0	EtOAc	2·5 hr.	88	101—102
22	Z.Leu.OPip	Gly.ONa	(III)	5·0	Dioxan–H_2O	24	85	117—119 [23]
23	,,	Leu.OMe	(II)	5·0	Dioxan	84	79	96—98 [24]
24	Bz.Leu.OPip	Gly.OEt	(I)	5·0	,,	24	94	153—156 [25]
25	,,	,,	(II)	5·0	,,	1·75	95	153—156 [25]
26	,,	Gly.ONa	(III)	5·0	Dioxan–H_2O	24	92	— [26]
27	Z.Phe.OPip	Gly.OEt	(II)	2·0	Dioxan	2	87	110—112 [27]
28	,,	Leu.OMe	(II)	2·0	Dioxan (2 ml.)	18	83	108·5—110 [28]
29	Z.Sar.OPip	Gly.OEt	(II)	5·0	Dioxan	24	91	— [29]
30	Z.Val.OPip	,,	(I)	1·0	,,	6	92	165—166 [30]
31	Z.Gly.Gly.OPip	Gly.Gly.OEt	(II)	0·74	,,	24	79	198—207 [31]

[1] The abbreviations for the amino-acid residues are those in the " Revised Tentative Rules for Abbreviations and Symbols for Chemical Names of Special Interest in Biological Chemistry " (Information Bulletin No. 20, I.U.P.A.C.) and their mode of use is that recommended in " Peptides," Proc. Fifth European Peptides Symp., Oxford, 1962," ed. G. T. Young, Pergamon Press, Oxford, 1963, p. 261. Z = CO·OCH$_2$Ph; Pip = 1-piperidyl; Bzl = CH$_2$Ph; Pht = phthaloyl; Sar = sarcosine. Optically active amino-acids were of the L-form. [2] Method (I); using amino-ester with acetic acid; (II), amino-ester hydrochloride with sodium acetate; (III), amino-acid with 1 equivalent of sodium hydroxide. [3] Mmole of active ester. [4] The reaction time is not necessarily the minimum for complete reaction. [5] The constants refer to product obtained in the stated yield; literature constants cited refer to the pure compound. [6] $[\alpha]_D^{22}$ −20·8° (*c* 1·0 in EtOH); H. J. Panneman, A. F. Marx, and J. F. Arens (*Rec. Trav. chim.*, 1958, **78**, 487) give m. p. 99—99·5°, $[\alpha]_D^{20}$ −21·2°. [7] H. J. Panneman, A. F. Marx, and J. F. Arens (*loc. cit.*) give m. p. 100—101°. [8] Recrystallisation from Pr$^i_2$O gave product of m. p. 98—99°, $[\alpha]_D^{22}$ −30·9° (*c* 6·0 in AcOH); S. Goldschmidt and C. Jutz (*Chem. Ber.*, 1953, **86**, 1116) give m. p. 98—99°, $[\alpha]_D^{20}$ −26·8°. [9] Recrystallisation from ethanol–ether gave product of m. p. 82—83°; C. Süss and H. Hoffmann (*Annalen*, 1951, **572**, 96) give m. p. 82·5—83°; G. W. Anderson and R. W. Young (*J. Amer. Chem. Soc.*, 1952, **74**, 5307) give m. p. 86—87°. [10] Chromatographically pure oil. E. L. Smith and N. B. Slonim (*J. Biol. Chem.*, 1948, **176**, 835) give m. p. 64—66°; H. J. Panneman, A. F. Marx, and J. F. Arens (*Rec. Trav. chim.*, 1958, **78**, 487) obtained an oil. [11] Chromatographically pure oil. H. J. Panneman, A. F. Marx, and J. F. Arens (*loc. cit.*) obtained an oil. [12] After crystallisation from ethyl acetate–di-isopropyl ether the product had m. p. 127—128·5°, $[\alpha]_D^{24·5}$ +37·1° (*c* 1·0 in EtOH). G. W. Anderson and F. M. Callahan (*J. Amer. Chem. Soc.*, 1958, **80**, 2902) give m. p. 127·5°, $[\alpha]_D^{24}$ +38·8° (*c* 5 in EtOH). [13] $[\alpha]_D^{22}$ +16·6° (*c* 5·0 in EtOH). G. W. Anderson, J. Blodinger, and A. D. Welcher (*J. Amer. Chem. Soc.*, 1952, **74**, 5309) give m. p. 126—127°, $[\alpha]_D^{23-25}$ +19·2°. [14] G. W. Anderson, A. D. Welcher, and R. W. Young (*J. Amer. Chem. Soc.*, 1951, **73**, 501) give m. p. 166—167°. [15] Recrystallisation from ethyl acetate gave needles of m. p. 194°. R. A. Boissonnas (*Helv. Chim. Acta*, 1951, **34**, 874) gives m. p. 194—195°. [16] The peptide separated during the reaction, and was collected, washed with ethyl acetate, ether, and dried. [17] Recrystallisation from MeOH–H_2O gave needles of m. p. 145—147°, $[\alpha]_D^{24}$ −26·6° (*c* 2·0 in EtOH). H. J. Panneman, A. F. Marx, and J. F. Arens (*Rec. Trav. chim.*, 1958, **78**, 487) give m. p. 145—146°, $[\alpha]_D^{21}$ −27·0°. [18] Recrystallisation from methanol gave product of m. p. 177·5—179°, $[\alpha]_D^{24}$ +92·7° (*c* 1·0 in EtOAc). H. J. Panneman, A. F. Marx, and J. F. Arens (*loc. cit.*) give m. p. 174·5—175·5°, $[\alpha]_D^{21}$ +91·6°. [19] F. E. King, J. W. Clark-Lewis, R. Wade, and W. A. Swindin (*J.*, 1957, 873) give m. p. 229—230°. [20] Yield and m. p. are after one crystallisation from

given in the Table is not always the minimum required for completion. Instead of using the free amino-ester with acetic acid (method I) it is possible to use the amino-ester hydrochloride with an equivalent of sodium acetate (method II); with dioxan as solvent this heterogeneous reaction proceeds smoothly. Although we used dioxan in our early experiments with method (I), other solvents, such as ethyl acetate or chloroform, have been found to be equally satisfactory (and more convenient) when the free amino-ester is used with acetic acid. A convenient variant is to use the amino-ester hydrochloride with equivalents of triethylamine and acetic acid, *e.g.*, in chloroform solution (Exp. 19). As with other active esters, the time required for condensation is sensitive to steric factors but even with extended reaction times the yield and purity of the product are still high. We have noted particularly the absence of by-products; the co-product, 1-hydroxypiperidine, being soluble in all common solvents, is readily removed by washing. In most cases the crude product left after evaporation of the solvent (after the usual washing procedure) was chromatographically pure, and the melting point was often close to the best literature figure

The *N*-protecting groups (benzyloxycarbonyl and phthaloyl) used in most of these condensations do not allow racemisation under normal conditions, whatever method of coupling is used, but Expts. 24 and 25 constitute our racemisation test,[4] the condensation of benzoyl-L-leucine with glycine ethyl ester. The preparation of the optically pure benzoyl-L-leucine 1-piperidyl ester required for these tests presented difficulties. Attempts to obtain L-leucine 1-piperidyl ester by the action of hydrogen bromide in acetic acid, or hydrogen in the presence of palladium black, on benzyloxycarbonyl-L-leucine 1-piperidyl ester failed because both reagents removed the ester as well as the benzyloxycarbonyl group. Fortunately we were able to separate pure benzoyl-L-leucine 1-piperidyl ester by fractional crystallisation of the partly racemic mixture obtained (not unexpectedly, since dicyclohexylcarbodi-imide causes racemisation during the coupling of benzoyl-L-leucine) [4] using method A with benzoyl-L-leucine.* When this L-isomer was condensed with glycine ethyl ester in the presence of an equivalent of acetic acid, or with the ester hydrochloride and an equivalent of sodium acetate trihydrate, with dioxan as solvent, analytically pure benzoyl-L-leucylglycine ethyl ester of high rotation was obtained in 94—95% yield; saponification of the whole product and fractional crystallisation of the acid gave no

di-isopropyl ether. After a further crystallisation the product had m. p. 104—105°, $[\alpha]_D^{23}$ −25·7° ±0·1° (*c* 1·0 in EtOH). G. W. Anderson and R. W. Young (*J. Amer. Chem. Soc.*, 1952, **74**, 5307) give m. p. 104—105°, $[\alpha]_D^{25}$ −26·4° (*c* 5·0 in EtOH). [21] Using method (II) but replacing the sodium acetate by triethylamine (7·0 mmoles) and acetic acid (7·0 mmoles). After recrystallisation from di-isopropyl ether the product had m. p. 103·5—104·5°, $[\alpha]_D^{24}$ −24·8° (*c* 1·0 in EtOH). [22] Free glycine ester used without acid catalysis. [23] Yield and m. p. are after one crystallisation from ethyl acetate–di-isopropyl ether. After a further crystallisation the product had m. p. 118·5—120°, $[\alpha]_D^{24}$ −23·8° (*c* 1·0 in EtOH). W. Grassmann and E. Wünsch (*Chem. Ber.*, 1958, **91**, 449) give m. p. 117°; F. Weygand and W. Steglich (*ibid.*, 1960, **93**, 2983) give m. p. 112—114°, $[\alpha]_D^{25}$ −23·8° (*c* 2·43 in EtOH). [24] $[\alpha]_D^{22}$ −37·8° (*c* 5 in EtOH), after recrystallisation from di-isopropyl ether. G. W. Anderson, J. Blodinger, R. W. Young, and A. D. Welcher (*J. Amer. Chem. Soc.*, 1952, **74**, 5304) give m. p. 93—95°, $[\alpha]_D^{21}$ −36·2°; M. A. Nyman and R. M. Herbst (*J. Org. Chem.*, 1950, **15**, 108) give m. p. 97—98°. [25] $[\alpha]_D^{25}$ −31·4° ±0·2° (*c* 1·0 in EtOH). M. W. Williams and G. T. Young (*J.*, 1963, 881) give m. p. 156·5—157°, $[\alpha]_D^{20}$ −34·0° for pure product. [26] $[\alpha]_D^{25}$ −24·9° (*c* 1·0 in EtOH), glass; analysed satisfactorily. M. W. Williams and G. T. Young (*loc. cit.*) give m. p. 134—135°, $[\alpha]_D^{20}$ −26·4° (*c* 4·1 in EtOH) for pure product. [27] M. p. 110·5—112·5°, $[\alpha]_D^{25}$ −15·3° (*c* 1·0 in EtOH) after crystallisation from ethyl acetate–di-isopropyl ether. R. W. Young, K. H. Wood, R. J. Joyce, and G. W. Anderson (*J. Amer. Chem. Soc.*, 1956, **78**, 2126) give m. p. 110—111°, $[\alpha]_D^{25}$ −16·9° (*c* 5·0 in EtOH). [28] Yield and m. p. are after crystallisation from di-isopropyl ether. Recrystallisation gave product of m. p. 109·5—110·5°, $[\alpha]_D^{25\cdot5}$ −24·2° (*c* 1·0 in MeOH); R. B. Woodward, R. A. Olofson, and H. Mayer (*J. Amer. Chem. Soc.*, 1961, **83**, 1010) give m. p. 109—109·5°. [29] New compound: see Experimental section. [30] $[\alpha]_D^{21}$ −25·3° (*c* 1·0 in EtOH). W. Grassmann and E. Wünsch (*Chem. Ber.*, 1958, **91**, 449) give m. p. 166°. [31] M. p. 210—212° after recrystallisation from water. S. Goldschmidt and M. Wick (*Annalen*, 1952, **575**, 217) give m. p. 185°, and S. Goldschmidt and W. Lautenschlager (*ibid.*, 1953, **580**, 68) give m. p. 205°, and suggest dimorphism.

* We have since prepared this ester by the reaction of benzoyl-L-leucyl azide with 1-hydroxypiperidine.

racemate. This procedure will detect 2% or more of racemate, and is a stringent test, since oxazolone formation is exceptionally easy in this case. It must be emphasised that the validity of the deductions made from the optical rotation of the crude product depends on the establishment of its chemical purity by elemental analysis; confirmation of the presence or absence of racemate is then obtained by fractional crystallisation of the ester (if much racemate is present) or of the acid (if small proportions are being sought).[4] We are grateful to Professor F. Weygand for allowing us to add here that in racemisation tests carried out in his laboratory 1-piperidyl esters of acylpeptides have been found to couple without detectable racemisation.

The absence of racemisation in these tests is paralleled by the remarkable optical stability of these esters in the presence of base. A solution of benzyloxycarbonyl-L-leucine 1-piperidyl ester in dimethylformamide containing triethylamine had an unchanged optical rotation after 28 hr. at room temperature; after this period the rotation of a similar solution of the analogous *p*-nitrophenyl ester had fallen by 11%. A solution of benzyloxycarbonyl-β-cyano-L-alanine 1-piperidyl ester in pyridine had an unchanged rotation after 25 hr. at room temperature, whereas the analogous *p*-nitrophenyl ester loses 65% of its rotation under these conditions.[15] More striking are the effects of triethylamine on the esters of benzyloxycarbonyl-β-cyano-L-alanine, phthaloyl-L-phenylalanine, and *S*-benzyl-*N*-benzyloxycarbonyl-L-cysteine: under conditions which result in complete racemisation of the *p*-nitrophenyl esters,[12,16,17] the rotations of the 1-piperidyl analogues were unchanged. Further, a solution of benzoyl-L-leucine *p*-nitrophenyl ester in chloroform containing a tertiary aliphatic amine rapidly racemises and forms 4-isobutyl-2-phenyloxazolone, detected by the infrared carbonyl absorption at 1832 $cm.^{-1}$ (it has been shown that racemisation in this case proceeds chiefly, if not exclusively, through the oxazolone);[18] the optical rotation of the corresponding 1-piperidyl ester in ethyl acetate solution containing an equivalent of triethylamine was unchanged after 14 days at room temperature, and no oxazolone absorption developed in a similar solution in chloroform during 7 days. The exceptional optical stability of 1-piperidyl esters is presumably due to the low reactivity of the ester in the basic conditions which otherwise favour oxazolone formation or ionisation of the α-hydrogen atom. Activation occurs only in the presence of acid which represses these side-reactions; thus, a solution of benzoyl-DL-leucine 1-piperidyl ester in chloroform containing one equivalent of acetic acid again developed no oxazolone absorption during 7 days at room temperature. We have therefore a new method of coupling which is reasonably rapid, proceeds nearly quantitatively, and (as far as our experience goes) is free from any danger of racemisation during coupling. It should, however, be noted that the methods used so far for the preparation of these esters (excepting that through the acid azide: see the footnote on p. 6817) must be expected to involve a risk of racemisation when they are applied to acyldipeptides.

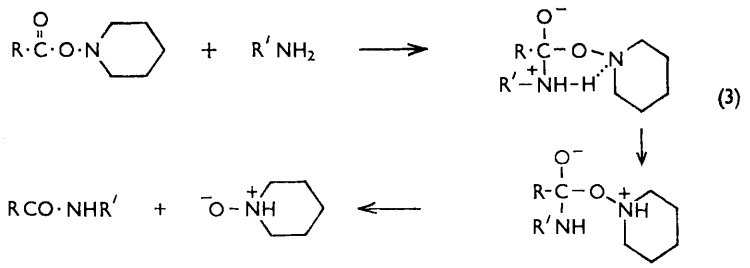
We have prepared also the esters of benzyloxycarbonylglycine with *NN*-diethylhydroxylamine (by method B), of benzyloxycarbonyl-L-leucine with *NN*-dimethylhydroxylamine (by method C), and of phthaloylglycine with *NN*-dimethyl- and -dibenzylhydroxylamines using minor modifications of method (B). After our original communication concerning 1-piperidyl esters, Bittner, Knobler, and Frankel[19] described the preparation of benzyloxycarbonylamino-esters of *NN*-diethylhydroxylamine (by method A) and their condensation with amino-esters to form peptides, but the condensations were carried out without acid catalysis and were therefore undesirably slow; "some days" were required at room temperature for the condensation *e.g.*, of *O*-(benzyloxycarbonylglycyl)-*NN*-diethylhydroxylamine with glycine ethyl ester, which with acid catalysis under our

[15] B. Liberek and A. Nowicka, *Zeszyty Nauk. Wyzszej Szkoly Pedagogicznej w Gdansku*, 1963, **3**, 105.
[16] B. Liberek, *Bull. Acad. polon. Sci., Ser. Sci. chim.*, 1963, **11**, 677.
[17] I. Antonovics, unpublished work.
[18] M. W. Williams and G. T. Young, *J.*, 1964, 3701.
[19] S. Bittner, Y. Knobler, and M. Frankel, *Tetrahedron Letters*, 1965, 95.

conditions is complete within 2·5 hr. (Expt. 5 in the Table). Condensation reactions of these analogues also are reported in the Table. We find that they behave similarly to the 1-piperidyl esters, which we chose to examine first because it seemed likely that they would crystallise more readily than the acyclic derivatives. In view of the lability of esters of this kind under the conditions required for the removal of common amino-protecting groups, the preparation of the amino-ester hydrochlorides by the reaction of *N*-carboxyamino-acid anhydrides with the dialkylhydroxylamine hydrochloride is likely to be particularly useful.[19]

Although, in the absence of acid, the reaction of 1-piperidyl esters with α-amino-esters is slow, with the stronger nucleophile benzylamine the acylation is rapid; benzyloxycarbonylglycine 1-piperidyl ester gave a nearly quantitative yield of the *N*-benzylamide in 30 min. at room temperature, and other examples are given in the Experimental section. It seemed possible, therefore, that the amino-group of glycine anion, which is of comparable basicity, might react similarly, and although the reaction is slower, nearly quantitative yields have been obtained by the condensation of acylamino-1-piperidyl esters with amino-acids in dioxan containing one equivalent of sodium hydroxide. Examples are shown in the Table (method III). Bittner, Knobler, and Frankel [19] have also used this procedure. We have applied a modification of our racemisation test under these conditions, condensing benzoyl-L-leucine 1-piperidyl ester with glycine sodium salt (Expt. 26). Benzoyl-L-leucylglycine is normally a glass, whilst the racemate crystallises readily (it is this difference which allows the detection of DL-isomer in our racemisation test).[18] No racemate could be found in this case.

The ready reaction of these esters with aliphatic amines was unexpected. We suggest that the transition complex for the formation of the adduct (3), and the adduct itself, may be stabilised by hydrogen-bonding; the subsequent proton transfer would greatly accelerate the final stage, as shown. There is a marked selectivity in this reaction. For example, although n-butylamine is completely benzoylated by 1-benzoyloxypiperidine in 48 hr.



at 24°, t-butylamine is unattacked, and cyclohexylamine and isopropylamine are scarcely attacked in that time; possibly steric hindrance prevents the amines from approaching sufficiently closely for hydrogen bonding to be effective. Under similar conditions aniline was unattacked after 10 days, and selective benzoylation of benzylamine admixed with aniline was effected in this way; benzanilide could not be detected in the reaction products. Ammonia reacts readily and hydrazine more slowly; benzyloxycarbonyl-L-leucine 1-piperidyl ester with ammonia in methanol gave a nearly quantitative yield of amide in 30 min. at room temperature. These esters are, however, quite unreactive towards alcohols; *e.g.*, benzyloxycarbonyl-L-leucine 1-piperidyl ester was recovered substantially unchanged after being heated in boiling ethanol for 1·75 hr.; chromatography revealed no ethyl ester.

We distinguish, therefore, the acid-catalysed reaction of 1-piperidyl esters, which we use with amino-compounds such as α-amino-esters (pK_a *ca.* 7·75), and the uncatalysed reaction which we use with more basic amines such as benzylamine, and with ammonia and hydrazine. It is clear that the effectiveness of acid catalysis will depend on the strength of both the acid and the amine, since there will be competition for protonation

between the piperidyl group and the amine; acetic acid is most satisfactory for use with α-amino-esters. We intend to investigate this factor in more detail.

The usefulness of esters of *NN*-dialkylhydroxylamines in peptide synthesis is not confined to coupling reactions. Benzyl and *p*-nitrobenzyl 1-piperidyl carbonates can, for example, be used to benzyloxycarbonylate amino-esters; the former and other analogous carbonates were prepared by Zinner [20] from the appropriate chloroformate in a Schotten–Baumann reaction, but we find that the direct reaction of the benzyl chloroformate with 1-hydroxypiperidine in ether (method B) is preferable. We are examining similar applications. We are investigating also the reactions of the simple carboxylic esters of this type, which seem likely to be of interest in general organic chemistry.

Experimental

Melting points were determined on a Kofler hot-stage apparatus, and optical rotations on an Ericsson automatic polarimeter with solutions in a 0·2-dm. tube. Thin-layer chromatography was on Kieselgel G (unbaked), using ether as solvent and iodine vapour for detection, unless otherwise stated; R_F values are not precisely reproducible. Evaporation was usually by rotary evaporator and solutions in organic solvents were dried over magnesium sulphate. Light petroleum was of b. p. 40—60°. Infrared spectra were measured on a Perkin-Elmer 237 spectrophotometer; usually, only the carbonyl absorption of the active ester is quoted.

Preparation of 1-*Hydroxypiperidine*.—Wernick and Wolffenstein [14] showed that 1-ethylpiperidine was oxidised by hydrogen peroxide to the 1-oxide, which on heating gave 1-hydroxypiperidine, but satisfactory preparations have not been described.

1-*Ethylpiperidine* 1-*oxide*. The method is based on that used by Cope and LeBel [21] for the oxidation of 1-methylpiperidine. Hydrogen peroxide (30%; 680 ml.) was added slowly to a stirred solution of 1-ethylpiperidine (274 ml.) and ethanol (200 ml.) maintained at 0—5°. When the addition was complete, the temperature was maintained at 0—5° for a further 4 hr. and then allowed to rise to room temperature. After 5 days, a test with phenolphthalein showed that amine was absent; excess of peroxide was then destroyed by cautious addition of a slurry of platinum black (0·25 g.) in water, in portions over 8 hr., with stirring and cooling in ice; the solution then gave no immediate coloration with acidified starch–iodide paper (the amine oxide gives a weak starch–iodide test), and the platinum black was then filtered off and the filtrate concentrated in the rotary evaporator at room temperature, at first at 12 mm. and finally at 1 mm. pressure. When crystals of hydrated amine oxide appeared, the product was transferred to a 3-necked 500-ml. flask by means of aqueous ethanol, which was removed at 12 mm., and the residue was finally heated at 1 mm., until no more liquid distilled at 90°; an off-white, crystalline residue remained on cooling. This product, which was hygroscopic, was used directly for the next stage.

1-*Hydroxypiperidine*. The procedure is based on that used in other cases by Cope, Foster, and Towle.[22] The 3-necked flask containing the amine oxide was fitted with a thermometer (to the bottom of the flask), a nitrogen inlet, and a wide-bore condenser (to facilitate the removal of the large volume of ethylene produced). The bath was raised to, and maintained at, 115° while the amine oxide melted; when this was complete, the bath temperature was adjusted to provide gentle reflux in the first 15 min., moderate reflux for the next 30 min., and more vigorous reflux during a further 30 min., by which time no ethylene was detected in the effluent gas (tested by bromine water). After cooling, the reaction mixture was distilled under nitrogen through a short (15 cm.) column, and fractions boiling between *ca.* 70 and 90° at 17 mm. were collected in receivers cooled to −30°; if any pale yellow oil begin to distil the process was stopped. The distillate was recrystallised from light petroleum at 0°, giving pure white 1-hydroxypiperidine (*ca.* 55% yield overall) of m. p. 38—40° (lit.,[23] 39°). It should be stored in a stoppered vessel at 0° or below; it should be recrystallised or redistilled before use if the product becomes discoloured. Improvements in this preparation have been effected by Mr. W. Sabel [24] (of the Oxford College of Technology) to whom we are grateful for additional supplies of 1-hydroxypiperidine.

[20] G. Zinner, *Chem. Ber.*, 1958, **91**, 302.
[21] A. C. Cope and N. A. LeBel, *J. Amer. Chem. Soc.*, 1960, **82**, 4656.
[22] A. C. Cope, T. T. Foster, and P. H. Towle, *J. Amer. Chem. Soc.*, 1949, **71**, 3929.
[23] R. Wolffenstein, *Ber.*, 1892, **25**, 2777.
[24] W. Sabel, to be published; U.K. Pat. Appl., 14,350/1965.

Hydroxylamines.—*NN*-Diethylhydroxylamine was prepared by the oxidation of diethylamine with 30% hydrogen peroxide;[25] the excess of peroxide was decomposed by the addition of a slurry of platinum black in water.

NN-Dimethylhydroxylamine hydrochloride was prepared in 75% yield by the oxidation of *NN*-dimethylcyclohexylamine to the 1-oxide and then thermal elimination of cyclohexene, as described[26] for the analogous preparation from *NN*-dimethylcyclohexylmethylamine.

NN-Dibenzylhydroxylamine was prepared by a modification of the method of de la Mare and Copinger,[27] from the reaction of benzyl chloride with hydroxylamine hydrochloride and triethylamine in boiling chloroform for 2 hr.; after recrystallisation from ethanol the product (57%) had m. p. 123·5—124·5°, (lit.,[27] 123—124°).

Preparation of Active Esters of NN-*Dialkylhydroxylamines.*—Typical cases are described first for each general method. *Method* (*A*). *Benzyloxycarbonyl-*L-*leucine* 1-*piperidyl ester.* Benzyloxycarbonyl-L-leucine (2·65 g., 0·01 mole) and 1-hydroxypiperidine (1·11 g., 0·011 mole) were dissolved in AnalaR ethyl acetate (50 ml.) and dicyclohexylcarbodi-imide (2·06 g., 0·01 mole) was added at room temperature with stirring. Dicyclohexylurea began to separate, and after 6 hr. it was filtered off and the filtrate was washed with N-hydrochloric acid (2 × 30 ml.), brine (1 × 30 ml.), saturated sodium hydrogen carbonate (2 × 30 ml.), and brine (2 × 30 ml.), and dried. The solvent was evaporated at room temperature, and the residue was taken up in a small volume of ether and filtered to remove some dicyclohexylurea. Light petroleum was added to the filtrate until turbidity, when a few drops of ether were added to restore a clear solution. On standing, monoclinic crystals were deposited, and cooling to 0° gave further yield, totalling 2·90 g. (83%), m. p. 65—67°. Recrystallisation from the same solvents gave *ester* of m. p. 66—67°, $[\alpha]_D^{24}$ −10·8° (*c* 1·0 in EtOAc), $\nu_{max.}$ 1760 cm.$^{-1}$ (in paraffin paste) R_F 0·54 (Found: C, 65·3; H, 8·0; N, 8·0. $C_{19}H_{28}N_2O_4$ requires C, 65·5; H, 8·1; N, 8·0%). This ester has also been made by method C, using dichloromethane as solvent, giving a 60% yield of crude product of m. p. 65—66·5°; recrystallisation from di-isopropyl ether gave ester of m. p. 67—68°, $[\alpha]_D^{21·5}$ −11·3° (*c* 1·0 in EtOAc), $[\alpha]_D^{20·5}$ −20·6° [*c* 1·0 in dimethylformamide (DMF)].

Method (*B*). *Phthaloylglycine* 1-*piperidyl ester.* To 1-hydroxypiperidine (4·0 g., 0·04 mole) in dry ether (200 ml.) at 0° was added, over 2 min. with stirring, a solution of recrystallised phthaloylglycyl chloride[28] (6·2 g., 0·023 mole) in ether (100 ml.). The hydrochloride of the ester precipitated immediately. After stirring for 5 min. more, the suspension was shaken with saturated aqueous sodium carbonate (100 ml.), and the ether layer was separated. The aqueous layer was extracted again with ether (100 ml.), the combined ether extracts were dried and evaporated, leaving a pale yellow syrup. Addition of dry ether (70 ml.) gave white crystalline product (5·7 g., 75%) of m. p. 94—96°; recrystallisation from ether gave the *ester*, m. p. 97—98°, $\nu_{max.}$ 1769, 1729 cm.$^{-1}$, R_F 0·46 (Found: C, 61·8; H, 5·6; N, 9·5. $C_{15}H_{16}N_2O_4$ requires C, 62·2; H, 5·6; N, 9·7%).

Method (*C*). *Benzyloxycarbonyl-*β*-alanine* 1-*piperidyl ester.* To a solution of benzyloxycarbonyl-β-alanine (11·2 g., 0·05 mole) and triethylamine (5·05 g., 0·05 mole) in dry chloroform (100 ml.) at −5° was added dropwise (5 min.) a solution of ethyl chloroformate (5·9 g., 0·05 mole) in chloroform (5 ml.) with stirring. After 15 min., 1-hydroxypiperidine (7·0 g., 0·07 mole) in chloroform (50 ml.) was added. After 2 hr. the solution was washed with water, 2N-hydrochloric acid, aqueous sodium hydrogen carbonate, and water, and dried. Evaporation below 30° left a colourless syrup, (R_F 0·6, with weak spot at origin), which was crystallised by freezing to a glass at −60°, adding a little ether–light petroleum mixture, and slowly thawing with scratching. The white crystals (11·5 g., 70%) had m. p. 50—52°; recrystallisation gave *ester* of m. p. 50—51°, $\nu_{max.}$ 1760 cm.$^{-1}$ (in $CHCl_3$), R_F 0·39 (Found: C, 62·2; H, 7·0; N, 9·4. $C_{16}H_{22}N_2O_4$ requires C, 62·7; H, 7·25; N, 9·15%).

*Benzyloxycarbonyl-*L-*alanine* 1-*Piperidyl Ester.*—Method (C) was used, with dichloromethane as solvent; the reaction mixture was left overnight. Crystallisation was induced with dry ether, giving a 61% yield of product of m. p. 74—77°; several recrystallisations from di-isopropyl ether gave *ester* of m. p. 77—78°, R_F 0·48, $[\alpha]_D^{22}$ −23·1° (*c* 1·0 in DMF), $[\alpha]_D^{22}$ −9·4° (*c* 1·0 in

[25] A. A. R. Sayigh and H. Ulrich, *J.*, 1963, 3144.
[26] *Org. Synth.*, Coll. Vol. IV, 1963, p. 612.
[27] H. E. de la Mare and G. M. Copinger, *J. Org. Chem.*, 1963, **28**, 1068.
[28] S. Gabriel, *Ber.*, 1907, **40**, 2647.

EtOAc), $\nu_{max.}$ 1760 cm.$^{-1}$ (in CCl_4) (Found: C, 62·4; H, 7·5; N, 9·3. $C_{16}H_{22}N_2O_4$ requires C, 62·7; H, 7·25; N, 9·15%).

S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteine* 1-*Piperidyl Ester*.—Method (A) gave a 65% yield of product of m. p. 70—74°, which after three recrystallisations from di-isopropyl ether gave *ester* of m. p. 72—73°, $[\alpha]_D^{23}$ −43·1° (*c* 1·0 in EtOAc) R_F 0·81, $\nu_{max.}$ 1760 cm.$^{-1}$ (in $CHCl_3$) (Found: C, 64·2; H, 6·6; N, 6·2; S, 7·6. $C_{23}H_{29}N_2O_4S$ requires C, 64·3; H, 6·8; N, 6·5; S, 7·5%).

Benzyloxycarbonylglycine 1-*Piperidyl Ester*.—Method (A) gave *product* of m. p. 113—114° after crystallisation from carbon tetrachloride (yield, 83%); the m. p. was unchanged by recrystallisation, R_F 0·42 (Found: C, 61·7; H, 7·2; N, 9·6. $C_{15}H_{20}N_2O_4$ requires C, 61·6; H, 6·9; N, 9·6%). Di-isopropyl ether can be used with advantage for the recrystallisation. Method (B) gave a 68% yield of product of m. p. 110—113°; the solution of benzyloxycarbonylglycyl chloride was prepared by the reaction of benzyloxycarbonylglycine with phosphorus pentachloride, according to Bergmann and Zervas [29] except that the phosphorus oxychloride was removed by washing the cooled ether solution rapidly with ice–water, and then drying for 5 min.

O-(*Benzyloxycarbonylglycyl*)-NN-*diethylhydroxylamine*.—Method (B) using diethylhydroxylamine in ether (see also the preparation of the 1-piperidyl analogue above) gave crude product which after two recrystallisations from di-isopropyl ether gave *ester* of m. p. 84—85° (lit.,[19] 81—82°) (46%) R_F 0·50, $\nu_{max.}$ 1772, 1728 cm.$^{-1}$ (in CCl_4) (Found: C, 59·9; H, 7·25; N, 9·6. $C_{14}H_{20}N_2O_4$ requires C, 60·0; H, 7·1; N, 10·0%).

NN-*Dimethyl*-O-(*phthaloylglycyl*)*hydroxylamine*.—Method (B) was modified as follows. To a solution of dimethylhydroxylamine hydrochloride (6·0 g., 0·062 mole) and triethylamine (16·6 ml., 0·12 mole) in dichloromethane (200 ml.) was added a solution of phthaloylglycyl chloride [28] (13·4 g., 0·06 mole) in dichloromethane (150 ml.). After stirring for 20 min. at 20°, the solution was washed (water, N-hydrochloric acid, saturated aqueous sodium carbonate, and brine) and dried. Evaporation left a white solid which was recrystallised from di-isopropyl ether to give product of m. p. 107—110° (12·5 g., 84%); two more crystallisations gave *ester* of m. p. 110·5—111·5°, $\nu_{max.}$ 1775, 1728 cm.$^{-1}$ (in CCl_4) (Found: C, 58·05; H, 5·2; N, 11·3. $C_{12}H_{12}N_2O_4$ requires C, 58·1; H, 4·8; N, 11·3%).

NN-*Dibenzyl*-O-(*phthaloylglycyl*)*hydroxylamine*.—Method (B) was modified as follows. A solution of *NN*-dibenzylhydroxylamine (4·26 g., 0·02 mole) and triethylamine (2·7 ml., 0·02 mole) in dichloromethane was added slowly to a solution of phthaloylglycyl chloride [28] (4·49 g., 0·02 mole) in dichloromethane. After stirring at 20° for 20 min. the solution was washed with saturated aqueous sodium carbonate, N-hydrochloric acid, and brine, and dried. Evaporation left chromatographically pure product of m. p. 135—137° (7·85 g., 98%). Two recrystallisations from propan-2-ol and one from methanol gave *ester* of m. p. 141—142°, $\nu_{max.}$ 1735, 1768 cm.$^{-1}$ (in CCl_4) (Found: C, 72·1; H, 5·1; N, 7·1. $C_{24}H_{20}N_2O_4$ requires C, 72·0; H, 5·0; N, 7·0%).

Benzoyl-L-*leucine* 1-*Piperidyl Ester*.—Method (A) gave an 89% yield of partly-racemised crystalline product, which after three recrystallisations from warm ether gave L-*ester* of m. p. 112—113·5°, $[\alpha]_D^{25}$ +12·4° (*c* 1·0 in EtOAc), $\nu_{max.}$ 1755 cm.$^{-1}$ (in paraffin paste), (Found: C, 67·8; H, 8·0; N, 8·7. $C_{18}H_{26}N_2O_3$ requires C, 67·9; H, 8·2; N, 8·8%). Proof of optical purity is given by the racemisation tests described below.

Benzoyl-DL-*leucine* 1-*Piperidyl Ester*.—This was prepared as for the L-isomer but was crystallised from ethyl acetate–light petroleum at 20°, giving an 82% yield of *ester* of m. p. 97—98° (Found: C, 68·1; H, 7·9; N, 8·8%).

O-(*Benzyloxycarbonyl*-L-*leucyl*)-NN-*dimethylhydroxylamine*.—Method (C), using dichloromethane as solvent, and dimethylhydroxylamine hydrochloride with an equivalent of triethylamine, gave an oil which was crystallised from di-isopropyl ether to give product of m. p. 63·5—64·5° (yield, 65%). Recrystallisation from di-isopropyl ether gave ester of m. p. 65—66°, $[\alpha]_D^{23}$ −11·9° (*c* 1·0 in EtOAc), $[\alpha]_D^{25}$ −22·8° (*c* 1·0 in DMF), $\nu_{max.}$ 1765, 1730 cm.$^{-1}$ (in CCl_4) (Found: C, 62·2; H, 7·6; N, 9·1. $C_{16}H_{24}N_2O_4$ requires C, 62·3; H, 7·8; N, 9·1%).

Benzyloxycarbonyl-L-*phenylalanine* 1-*Piperidyl Ester*.—Method (A) gave ester (yield, 72%) of m. p. 68—70°; recrystallisation from ethyl acetate–light petroleum gave product of m. p. 69·5—71°, $[\alpha]_D^{20·5}$ +18·2° (*c* 1·0 in $CHCl_3$), $[\alpha]_D^{24·5}$ +0·8° (*c* 1·0 in EtOAc) (Found: C, 68·7; H, 6·6; N, 7·2. $C_{22}H_{26}N_2O_4$ requires C, 69·1; H, 6·9; N, 7·3%).

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

Phthaloyl-L-phenylalanine 1-Piperidyl Ester.—Phthaloyl-L-phenylalanine (2·95 g.) was mixed intimately with phosphorus pentachloride (2·25 g.) and warmed at 50° for 1 min. to initiate the reaction; benzene (35 ml.) was then added and the mixture was heated at 50° for 1 hr. Evaporation gave crude phthaloyl-L-phenylalanyl chloride,[30] which was dissolved in dry ether, washed rapidly with ice–water and dried. The preparation was continued as in method (B); recrystallisation of the crude product from di-isopropyl ether gave *ester* (1·82 g., 46% overall) of m. p. 127—129°, raised by a further crystallisation to 128—130°, R_F 0·84, $[\alpha]_D^{24}$ −184° (*c* 1·0 in EtOAc), $\nu_{max.}$ 1780, 1760, 1720 (in $CHCl_3$) (Found: C, 69·6; H, 6·1; N, 7·1. $C_{22}H_{22}N_2O_4$ requires C, 69·8; H, 5·9; N, 7·4%).

Benzyloxycarbonylsarcosine 1-Piperidyl Ester.—Method (C) was used, with dichloromethane as solvent; the reaction mixture was left overnight. The *ester* (79% yield) could not be crystallised but was chromatographically pure, having R_F 0·36, $\nu_{max.}$ 1770 cm.$^{-1}$ (liquid film). The product was characterised by formation of the *N*-benzylamide and by coupling with glycine ethyl ester (see below).

Benzyloxycarbonyl-L-valine 1-Piperidyl Ester.—Method (C), using dichloromethane as solvent, gave (after evaporation of the solvent) a chromatographically pure syrup which was crystallised by cooling to −60° and triturating with ether–light petroleum. The product (72% yield) was recrystallised from di-isopropyl ether–light petroleum to give *ester* of m. p. 46—47°, R_F 0·53, $[\alpha]_D^{21·5}$ −3·4 (*c* 1·0 in EtOAc), $[\alpha]_D^{21·5}$ −13·9° (*c* 1·0 in DMF), $\nu_{max.}$ 1764 cm.$^{-1}$ (in $CHCl_3$) (Found: C, 64·3; H, 7·5; N, 8·6. $C_{18}H_{26}N_2O_4$ requires C, 64·6; H, 7·85; N, 8·4%).

Benzyloxycarbonylglycylglycine 1-Piperidyl Ester.—Method (C) was used, with chloroform as solvent; the reaction mixture was left overnight. Evaporation yielded a syrup which rapidly crystallised and was triturated with light petroleum, giving chromatographically pure product of m. p. 106—108° (47% yield). Recrystallisation from methanol and then from propan-2-ol gave *ester* of m. p. 110—111°, R_F 0·05, $\nu_{max.}$ 1770 cm.$^{-1}$ (in paraffin paste) (Found: C, 58·5; H, 6·75; N, 11·6. $C_{17}H_{23}N_3O_5$ requires C, 58·5; H, 6·65; N, 12·0%).

Coupling Reactions.—General. The time required for complete consumption of the active ester was usually determined by thin-layer chromatography on Kieselgel G, using ether as solvent and iodine vapour for detection. It is advisable, for comparison, to chromatograph a similar solution of the pure ester adjacent to the reaction mixture, as R_F values, and the intensities of the spots given by iodine, can vary. When a water-miscible solvent was used in methods (I) and (II), this was evaporated at room temperature; the residue was taken up in ethyl acetate (and water, for method II), and the solution was washed with 2N-hydrochloric acid, saturated aqueous sodium hydrogen carbonate, and brine, and dried. The ethyl acetate was evaporated, and the product was crystallised as in the literature reference or as indicated in the Table.

Method (I). Acid-catalysed coupling with amino-esters. The active ester (0·005 mole) was dissolved in dioxan (5 ml.) and the amino-ester (0·006 mole) and AnalaR acetic acid (0·35 ml., 0·006 mole) were added at room temperature (20—23°). Other solvents are equally satisfactory (see Table).

Method (II). Acid-catalysed coupling using amino-ester hydrochlorides and sodium acetate. The active ester (0·005 mole) was dissolved in dioxan (10 ml.), and amino-ester hydrochloride (0·006 mole) and sodium acetate trihydrate (0·82 g., 0·006 mole), both finely powdered, were added. The suspension was shaken or stirred at room temperature throughout the reaction.

Method (III). Coupling with the sodium salt of amino-acids. The powdered active ester (0·005 mole) was stirred with a solution of the amino-acid (0·006 mole) in N-sodium hydroxide (6 ml.), water (4 ml.), and dioxan (15 ml.) at room temperature. After the required reaction time, most of the dioxan was evaporated and the solution was diluted with water and washed with ethyl acetate. The aqueous layer was brought to pH 2 with hydrochloric acid, and the product was extracted into ethyl acetate, which was washed with brine and dried. Evaporation left a residue which was treated as in the literature reference or as indicated in the Table.

Benzyloxycarbonylsarcosylglycine Ethyl Ester. The syrup obtained as indicated in Experiment 29 in the Table solidified after 2 months. Trituration with light petroleum gave silky needles (1·01 g., 66%) of m. p. 69—70°. Recrystallisation from di-isopropyl ether gave *ester* of m. p. 70—71°, $\nu_{max.}$ 1740, 1705sh, 1680 cm.$^{-1}$ (in $CHCl_3$) (Found: C, 58·6; H, 6·3; N, 8·6. $C_{15}H_{20}N_2O_5$ requires C, 58·4; H, 6·5; N, 9·0%).

[30] J. C. Sheehan, D. W. Chapman, and R. W. Roth, *J. Amer. Chem. Soc.*, 1952, **74**, 3822.

Tests for Racemisation during Coupling.—The method is that described by Williams and Young.[4]

(i) To a solution of benzoyl-L-leucine 1-piperidyl ester (1·59 g., 0·005 mole) in dioxan (5 ml.) were added glycine ethyl ester (0·62 ml., 0·006 mole) and AnalaR acetic acid (0·35 ml., 0·006 mole). After 24 hr. at 20—23°, the crude product was isolated by the general procedure described above, giving ester (1·51 g., 94%) of m. p. 153—156°, $[\alpha]_D^{25}$ −31·6° (*c* 1·0 in EtOH) (Found: C, 63·5; H, 7·4; N, 8·7. Calc. for $C_{17}H_{24}N_2O_4$: C, 63·7; H, 7·6; N, 8·8%). Authentic benzoyl-L-leucylglycine ethyl ester has m. p. 156·5—157°, $[\alpha]_D^{20}$ −34·0° (*c* 3·1 in EtOH).[4] The ester was saponified as described by Williams and Young, and the acid so obtained was fractionally crystallised; the first fraction had m. p. 137—139°, and was shown (by mixed m. p.) to be benzoyl-DL-leucine (which is formed from authentic L-peptide and does not indicate racemisation of the peptide), and no racemic benzoyl-DL-leucylglycine was detected.

(ii) A similar experiment was carried out by general coupling method (II), using benzoyl-L-leucine 1-piperidyl ester (1·59 g., 0·005 mole), glyine ethyl ester hydrochloride (0·83 g., 0·006 mole), and sodium acetate trihydrate (0·82 g., 0·006 mole), with a reaction time of 1·75 hr. The crude product (1·52 g., 95%) had m. p. 153—156°, $[\alpha]_D^{25\cdot5}$ −31·3° (*c* 1·0 in EtOH) (Found: C, 63·6; H, 7·6; N, 8·6%). Saponification followed by fractional crystallisation of the acid revealed no racemate.

(iii) Benzoyl-L-leucine 1-piperidyl ester (1·59 g., 0·005 mole) was condensed with glycine (0·45 g., 0·006 mole) in the presence of N-sodium hydroxide (6 ml.) by general coupling method (III), with a reaction time of 24 hr. After removal of the dioxan, the solution was diluted with brine, and the general procedure was then followed. The crude product (1·35 g., 92%) was a glass of $[\alpha]_D^{25}$ −24·9° (*c* 1·0 in EtOH) (Found: C, 62·0; H, 7·2; N, 9·2. Calc. for $C_{15}H_{20}N_2O_4$: C, 61·6; H, 6·9; N, 9·6%); a 2% solution in water yielded no racemate, but after 10 weeks deposited crystalline benzoyl-L-leucylglycine, m. p. 135·5—136°, $[\alpha]_D^{23}$ −27·5° (*c* 1·0 in EtOH). Authentic L-peptide has m. p. 135°, $[\alpha]_D^{20}$ −26·4° (*c* 4·1 in EtOH).[4] The reluctance of the L-isomer to crystallise (which allows detection of the racemate by crystallisation) is characteristic, and the fact that the first crystals deposited were L-isomer is clear confirmation of the absence of racemate.

Benzyloxycarbonyl-β-cyano-L-alanine 1-Piperidyl Ester.—Benzyloxycarbonyl-β-cyano-L-alanine was prepared by the method of Ressler and Ratzkin.[31] Evaporation of the pyridine left a white solid instead of a syrup; several recrystallisations from 1,2-dichloroethane gave product of m. p. 131—134°, $[\alpha]_D^{20}$ −43·5° (*c* 1·0 in DMF), $\nu_{max.}$ 2250w, 1745, 1695 cm.$^{-1}$ (in paraffin paste) {lit., m. p. 133—134°; [31] m. p. 133—134°, $[\alpha]_D^{25}$ −44·2° (*c* 0·93 in DMF); [32] m. p. 126—128°, $[\alpha]_D$ −43·9° (*c* 0·95 in DMF) [33]}. This product (2·31 g.) and 1-hydroxypiperidine (1·5 g.) were dissolved in ethyl acetate (50 ml.) and cooled to 0° with stirring; dicyclohexylcarbodi-imide (2·05 g.) in ethyl acetate (20 ml.) was added dropwise during 5 min. After a further 2 hr., the ester (2·0 g., 61%) was isolated as usual but could not be crystallised, remaining as an amorphous white solid, R_F 0·42, $\nu_{max.}$ 2245w, 1760, 1720 cm.$^{-1}$ (in $CHCl_3$). It was characterised by formation of the *N*-benzylamide (see below).

The Effect of Tertiary Amines on 1-Piperidyl Esters of Acylamino-acids.—(1) *Optical stability.* (*a*) *Benzyloxycarbonyl-L-leucine 1-piperidyl ester.* The ester (0·670 g.) and triethylamine (0·30 ml.) were dissolved in purified dimethylformamide and the volume was made up to 50 ml.; $[\alpha]_D^{22\pm2}$ −24·2 ± 0·1° was unchanged after 28 hr. at 22° ± 2°; a similar solution of the benzyloxycarbonyl-L-leucine *p*-nitrophenyl ester had $[\alpha]_D^{22\pm2}$ −28·9° falling to −25·6° after 28 hr.

(*b*) *Benzyloxycarbonyl-β-cyano-L-alanine 1-piperidyl ester.* (i) The ester (0·505 g.) and triethylamine (0·30 ml.) were dissolved in dry acetone to give 25 ml.; $[\alpha]_D^{22\pm2}$ −26·4° ± 0·1° was unchanged after 26 hr. Liberek's data [16] for the analogous *p*-nitrophenyl ester show complete racemisation within 4 hr.

(ii) The 1-piperidyl ester (0·475 g.) in purified pyridine (to give 25 ml.) had $[\alpha]_D^{22\pm2}$ −33·2° ± 0·1°, unchanged after 25 hr. at 22° ± 2°. Liberek and Nowicka's data [15] for the analogous *p*-nitrophenyl ester show a fall of 65% in the optical rotation under these conditions.

(*c*) *Phthaloyl-L-phenylalanine 1-piperidyl ester.* The ester (0·504 g.) and triethylamine (0·30 ml.) were dissolved in purified dichloromethane (to give 50 ml.); $[\alpha]_D^{22\pm2}$ −147·8° ± 0·1°

[31] M. Zaoral and J. Rudinger, *Coll. Czech. Chem. Comm.*, 1959, **24**, 1993.
[32] C. Ressler and H. Ratzkin, *J. Org. Chem.*, 1961, **26**, 3356.
[33] B. Liberek, *Bull. Acad. polon. Sci., Ser. Sci. chim.*, 1962, **10**, 227.

was unchanged after 119 hr.; under the same conditions, the analogous *p*-nitrophenyl ester is completely racemised.[17]

(*d*) S-*Benzyl*-N-*benzyloxycarbonyl*-L-*cysteine* 1-*piperidyl ester*. The ester (0·168 g.) and triethylamine (0·12 ml.) were dissolved in purified acetone (to give 10 ml.); $[\alpha]_D^{22\pm2}$ −45·1° ± 0·2° was unchanged after 24·5 hr.; Liberek's data [12] for the analogous *p*-nitrophenyl ester show complete racemisation in *ca.* 26 hr.

(*e*) *Benzoyl*-L-*leucine* 1-*piperidyl ester*. The ester (0·250 g.) and triethylamine (0·110 ml.) were dissolved in AnalaR ethyl acetate (to give 10 ml.); $[\alpha]_D^{22\pm2}$ +12·4° ± 0·1° was unchanged after 14 days at 22° ± 2°.

(2) *Oxazolone formation*. Solutions of benzoyl-DL-leucine 1-piperidyl ester (0·318 g., 0·001 mole) in chloroform (5 ml.) containing triethylamine (0·14 ml., 0·001 mole) or triethanolamine (0·133 ml., 0·001 mole) developed no infrared absorption at 1832 cm.$^{-1}$ (oxazolone CO) within 7 days at room temperature. Williams and Young [18] showed that under similar conditions a solution of benzoyl-L-leucine *p*-nitrophenyl ester with 1-methylpiperidine in chloroform developed strong absorption at 1832 cm.$^{-1}$ within 20 min. A similar experiment but with the addition of AnalaR acetic acid (0·06 ml., 0·001 mole) in place of triethylamine gave a dark amber solution after 7 days but no absorption at 1832 cm.$^{-1}$ was found.

Reactions of 1-*Piperidyl Esters with Amines, Ammonia, and Hydrazine*.—(1) *Reactions with amines*. (*a*) *Benzyloxycarbonylglycine* N-*benzylamide*. Benzyloxycarbonylglycine 1-piperidyl ester (1·41 g., 0·005 mole) and benzylamine (0·65 g., 0·006 mole) in ethyl acetate (15 ml.) deposited crystalline amide (1·38 g., 93%) of m. p. 115—117° after 30 min. at room temperature. Recrystallisation from methanol raised the m. p. to 118—119° (lit.,[34] 117—118°) (0·98 g., 66%).

(*b*) *Benzyloxycarbonylsarcosine* N-*benzylamide*. To a solution of benzyloxycarbonylsarcosine 1-piperidyl ester (1·30 g.) in chloroform (5 ml.) was added benzylamine (4 ml.). After 12 hr. at room temperature more chloroform (50 ml.) was added and the solution was washed as usual and dried. Evaporation left crystalline product of m. p. 117—118° (1·32 g., 99%), which after recrystallisation from methanol gave the *benzylamide* of m. p. 119—120° (Found: C, 69·0; H, 6·5; N, 8·8. $C_{18}H_{20}N_2O_3$ requires C, 69·2; H, 6·4; N, 9·0%). Ben-Ishai [35] gives m. p. 116—117° but no analysis for this compound.

(*c*) *Benzyloxycarbonyl*-β-*cyano*-L-*alanine* N-*benzylamide*. Analogous reaction of the corresponding 1-piperidyl ester with benzylamine in ethyl acetate gave crystalline *N*-benzylamide of m. p. 133—137° (95% yield). Recrystallisation gave product of m. p. 136—137° (lit.,[36] 135—136°).

(*d*) *Benzoylation of amines*. 1-Benzoyloxypiperidine has been prepared by a Schotten–Baumann reaction with 1-hydroxypiperidine [37] and by the action of dibenzoyl peroxide on piperidine.[38] Method (B) gave an 86% yield of ester of m. p. 63—64°, chromatographically pure. Recrystallisation from light petroleum gave analytically pure ester of m. p. 63—64° (lit.,[38] 62°), $\nu_{max.}$ 1745 cm.$^{-1}$ (in CCl_4), R_F 0·62.

Thin-layer chromatography showed that 1-benzoyloxypiperidine (2·02 g.) in dioxan (20 ml.) reacted completely (disappearance of ester) with benzylamine (1·1 g.) in 50 hr. The dioxan was evaporated and the solid residue was triturated with 2N-hydrochloric acid, washed, and dried, giving a quantitative yield of crude *N*-benzylbenzamide which, after recrystallisation from ethanol, had m. p. 105—106° (lit.,[39] 105—106°).

Similar conditions using n-butylamine (0·80 g.) gave *N*-n-butylbenzamide as an oil (1·52 g., 91%), identical on thin-layer chromatograms with the product from a Schotten–Baumann benzoylation of n-butylamine.

Under similar conditions using isopropylamine, thin-layer chromatography showed some 1-hydroxypiperidine to be formed after 30 hr., but much ester still to be present after 20 days; using aniline, no change was detected by chromatography until the 10th day, when a trace of 1-hydroxypiperidine was found (a concentration of 0·2% could be detected); using t-butylamine, no change was detected by chromatography in 20 days; using cyclohexylamine, the reaction was still incomplete after 18 days, but during this time some crystals of *N*-benzoylcyclohexylamine separated.

[34] B. Iselin and R. Schwyzer, *Helv. Chim. Acta*, 1956, **39**, 57.
[35] D. Ben-Ishai, *J. Amer. Chem. Soc.*, 1957, **79**, 5736.
[36] B. Liberek, *Chem. and Ind.*, 1961, 987.
[37] E. Maass and R. Wolffenstein, *Ber.*, 1898, **31**, 2687.
[38] S. Gambarjan, *Ber.*, 1925, **58**, 1775.
[39] E. Beckmann, *Ber.*, 1890, **23**, 3331.

Selective benzoylation of benzylamine (1·1 g.) admixed with aniline (1·0 g.) in dioxan (15 ml.) was effected by heating with 1-benzoyloxypiperidine (2·01 g.) at 80° for 4 hr. The dioxan was evaporated, the residue was taken up in chloroform and washed and dried as usual. Thin-layer chromatography detected no benzanilide in the solution, which was then evaporated; trituration of the residue with ether gave *N*-benzylbenzamide (1·45 g., 71%) of m. p. 105°.

(2) *Reactions with ammonia and hydrazine.* (*a*) *Benzyloxycarbonyl-L-leucineamide.*—Benzyloxycarbonyl-L-leucine 1-piperidyl ester (2·0 g.) was added to a saturated solution of ammonia in methanol (50 ml.). After 30 min., chromatography showed the absence of ester; evaporation left a syrup which solidified on acidification with 2N-hydrochloric acid. The solid was collected and washed with water, giving amide (1·50 g., 99%) of m. p. 118—122°. Recrystallisation from aqueous methanol gave amide of m. p. 125—127° (lit.,[40] 122—123°), $[\alpha]_D^{21}$ −12·5° (*c* 1·0 in EtOH).

(*b*) *Benzyloxycarbonyl-L-phenylalanineamide.* Benzyloxycarbonyl-L-phenylalanine 1-piperidyl ester (2·0 g.) was dissolved in ethanol (50 ml.) saturated with ammonia. After 1 hr. at room temperature, thin-layer chromatography showed the reaction to be complete. Some solvent was allowed to evaporate, and the needles which appeared (1·41 g., 92%; m. p. 163—165°) were collected. Recrystallisation from ethanol gave amide of m. p. 166°, $[\alpha]_D^{23}$ +12·6° (*c* 1·0 in $CHCl_3$) {lit., m. p. 167°; [41] m. p. 167°, $[\alpha]_D^{20}$ +12·0° (*c* 1 in $CHCl_3$) [35]}.

(*c*) *Benzoylhydrazide.* 1-Benzoyloxypiperidine (2·25 g.) and hydrazine hydrate (100%; 10·0 ml.) were heated in boiling ethanol (30 ml.) for 30 min. The solution was evaporated at 70°/12 mm., leaving a white solid which after trituration with ether gave benzoylhydrazide (1·50 g., 100%) of m. p. 113—114°. Recrystallisation from aqueous ethanol raised the m. p. to 114—115° (lit.,[42] 112·5°).

The Stability of Benzyloxycarbonyl-L-leucine 1-Piperidyl Ester in Ethanol.—A solution of ester (1·93 g.) in dry ethanol (30 ml.) was heated at boiling point for 1·75 hr.; the solution, which was discoloured, was evaporated to dryness, and the residue was taken up in ethyl acetate, which was washed with 2N-hydrochloric acid and water, and dried. The residue (1·72 g., 89% recovery) crystallised after seeding, and had m. p. 62—65°; the infrared absorption was unchanged, and the thin-layer chromatograms in ether and in ether–light petroleum (70 : 30) were unchanged.

Benzyl 1-Piperidyl Carbonate.—Method (B) was used; the final ether solution was concentrated, when crystalline carbonate separated in 71% yield; recrystallisation from light petroleum gave product of m. p. 63·5°, $\nu_{max.}$ 1770 cm.$^{-1}$ (in CCl_4), R_F 0·65; Zinner [20] obtained a 59% yield of product of m. p. 66°, using Schotten–Baumann conditions.

p-*Nitrobenzyl 1-Piperidyl Carbonate.*—This was prepared as for the benzyl analogue, except that complete removal of the ether left crystalline carbonate of m. p. 86—87° (93% yield), which on recrystallisation from ether gave *ester* of m. p. 87—88°, $\nu_{max.}$ 1780 cm.$^{-1}$ (in CCl_4), R_F 0·54, (Found: C, 55·5; H, 5·65; N, 9·5. $C_{13}H_{16}N_2O_5$ requires C, 55·7; H, 5·7; N, 10·0%).

Benzyloxycarbonylation of Glycine Benzyl Ester.—To a solution of glycine benzyl ester hydrochloride (1·21 g.) and benzyl 1-piperidyl carbonate (1·25 g.) in dioxan (15 ml.) was added finely powdered sodium acetate trihydrate (0·83 g.) and the suspension was shaken at room temperature for 24 hr. The dioxan was then evaporated, the residue was taken up in ethyl acetate and water; the organic layer was washed (water, 2N-hydrochloric acid, N-potassium hydrogen carbonate, water) and dried. Evaporation left a white solid of m. p. 66—70° (1·34 g., 90%), which after recrystallisation from ether–light petroleum gave benzyloxycarbonylglycine benzyl ester (0·96 g., 65%) of m. p. 70·5—72° (lit.,[43] 72°).

p-*Nitrobenzyloxycarbonylglycine Ethyl Ester.*—To a solution of *p*-nitrobenzyl 1-piperidyl carbonate (1·04 g.) in dioxan (20 ml.) was added glycine ethyl ester hydrochloride (0·83 g.) and sodium acetate trihydrate (0·82 g.), both finely powdered. After shaking for 17 hr., thin-layer chromatography showed that the carbonate had been consumed; the solution was evaporated and the residue was dissolved in ethyl acetate, which was washed and dried. Evaporation gave the ester as a pale yellow solid (1·22 g., 87%) of m. p. 106—107°, unchanged on recrystallisation from ethyl acetate–ether; $\nu_{max.}$ 1730 cm.$^{-1}$ (in $CHCl_3$) (Found: C, 51·1; H, 4·9; N, 9·9. Calc.

[40] O. K. Behrens and M. Bergmann, *J. Biol. Chem.*, 1939, **129**, 587.
[41] J. S. Fruton and M. Bergmann, *J. Biol. Chem.*, 1942, **145**, 253.
[42] Beilstein, Handbuch der Organischen Chemie, 4th edn., **9**, 319.
[43] D. Ben-Ishai and A. Berger, *J. Org. Chem.*, 1957, **17**, 1564.

for $C_{12}H_{14}N_2O_6$: C, 51·05; H, 5·0; N, 9·9%). We are grateful to Professor H. N. Rydon for informing us that this compound, when prepared by use of the chloroformate, has m. p. 107°.[44]

We thank the D.S.I.R. for a Grant for a Special Research, the Medical Research Council for a Scholarship (held by B. O. H.), and Miss L. Williams for technical assistance.

THE DYSON PERRINS LABORATORY, OXFORD UNIVERSITY. [*Received, June 18th*, 1965.]

[44] R. B. Homer, R. B. Moodie and H. N. Rydon, *J.*, 1965, 4403.