Amino-acids and Peptides. Part 41.[1] An Examination of Some New Amino-protecting Groups

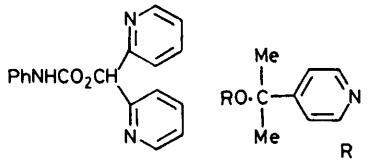
By **Susan Coyle, Oskar Keller, and Geoffrey T. Young,*** The Dyson Perrins Laboratory, Oxford University, Oxford OX1 3QY

In an investigation of the effect of the introduction of basic sites on the stability of alkoxycarbonyl groups to acid the following new amino-protecting groups have been examined: di-2-pyridylmethoxycarbonyl [*e.g.* (1)], 4-pyridylisopropoxycarbonyl [*e.g.* (4)], 3-diethylamino-1,1-diphenylpropoxycarbonyl [*e.g.* (10)], and 1,4-dimethylpiperidin-4-yloxycarbonyl [*e.g.* (13)]. In each case the stability to acid is considerably enhanced. The last-named group provides protection which is more stable to trifluoroacetic acid than is the t-butoxycarbonyl group, is stable to hydrogenolysis, and is cleaved by hydrogen bromide in acetic acid. In an investigation of amino-protecting groups designed to increase the solubility of derivatives by the incorporation of dimethylcarbamoyl substituents, the preparation of 2-dimethylcarbamoylethyl-isopropoxycarbonyl [*e.g.* (18)] and 2-dimethylcarbamoylbenzyloxycarbonyl [*e.g.* (24)] amino-acids is reported.

We have previously noted[2] the stability of 4-picolyl esters to acid, in marked contrast to the analogous benzyl esters, and we have therefore been examining ways in which this stabilisation, which in the case of picolyl esters is combined with a notable increase in the effectiveness of reductive methods of cleavage, may be used to develop useful new protecting groups. A preliminary note has appeared.[3]

RESULTS AND DISCUSSION

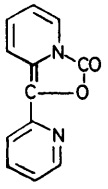
The magnitude of the stabilisation to acid resulting from the introduction of pyridyl residues in place of phenyl was shown by di-2-pyridylmethyl *N*-phenylcarbamate (1), which was prepared by the addition of di-2-pyridylmethanol to phenyl isocyanate. This carbamate was stable to 45% hydrogen bromide in acetic acid for at least 48 h at room temperature, whereas the analogous diphenylmethyl *N*-phenylcarbamate is cleaved within 5 min by trifluoroacetic acid at 0 °C, and by acetic acid saturated with hydrogen chloride at room temperature.[4] An attempt to prepare a mixed carbonate by the reaction of di-2-pyridylmethanol with 2,4,5-trichlorophenyl chloroformate or *p*-nitrophenyl chloroformate resulted in cyclisation to 5-(2′-pyridyl)pyrido-[1,2-*c*]oxazol-2-one (2). Even after the introduction of



(1)

R

(3) 2,4,5—$Cl_3C_6H_2OCO$
(4) NH_2NHCO
(5) Boc—Val—NHNHCO
(6) Boc—Gly—Val—NHNHCO
(7) Boc—Ala—Gly—Val—NHNHCO
(8) Boc—Leu—Ala—Gly—Val—NHNHCO



(2)

a tertiary carbon centre the carbamate is still very stable to acid. αα-Dimethyl-4-pyridylmethanol reacted with 2,4,5-trichlorophenyl chloroformate giving the mixed carbonate (3) which with hydrazine hydrate gave the hydrazide (4); this was stable to trifluoroacetic acid for 18 h at room temperature, but the protecting group was cleaved rapidly by hydrogenolysis. The possible usefulness of 4-pyridylisopropoxycarbonyl as a hydrazide-protecting group was examined by the synthesis of the protected tetrapeptide hydrazide (8);† no difficulty was experienced and in the isolation procedures advantage was taken of the weakly basic ' handle ' afforded by the pyridyl residue.[5,6] The hydrazide-protecting group was removed smoothly by hydrogenolysis and by electrolytic reduction at a mercury cathode. 4-Pyridylisopropoxycarbonyl-L-phenylalanine was prepared as an example of amino-acid protection; it was completely stable to 2M-hydrogen bromide in acetic acid for 48 h at room temperature (whereas the analogous phenylisopropoxycarbonyl group is cleaved within minutes by 1% trifluoroacetic acid in dichloromethane[7]) and was readily cleaved by hydrogenolysis and by electrolytic reduction. It had been hoped that the presence of the tertiary carbon centre might confer some advantageous properties over the 4-pyridylmethoxycarbonyl group,[8] but these were not evident.

The effect of a basic site further separated from the potential carbonium ion was examined by the preparation of 3-diethylamino-1,1-diphenylpropoxycarbonyl derivatives *via* the mixed carbonate (9) and the

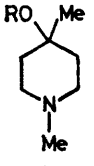
$RO{\cdot}CPh_2{\cdot}CH_2{\cdot}CH_2{\cdot}NEt_2$
R
(9) p-$O_2NC_6H_4OCO$
(10) NH_2NHCO
(11) Z–Val–NHNHCO

hydrazide (10), which was condensed with benzyloxycarbonyl-L-valine giving the protected valyl hydrazide (11). The hydrazide-protecting group required the action of trifluoroacetic acid for 1 h at room temperature for complete removal; Sieber and Iselin[9] found that the

† Abbreviations follow the I.U.P.A.C.–I.U.B. rules, reprinted in the Chemical Society Specialist Periodical Report 'Amino-acids, Peptides, and Proteins,' ed. G. T. Young, The Chemical Society, London, 1972, vol. 4, p. 441. Amino-acids are of the L-configuration.

analogous 1,1-diphenylpropoxycarbonylglycine ethyl ester is cleaved by 80% acetic acid at 22—25 °C with a half-life of 52 min.

The effect of a basic site on the stability of a derivative in which cleavage would involve a tertiary alkyl carbonium ion was examined by the preparation of 1,4-dimethylpiperidin-4-yloxycarbonyl derivatives, for comparison with 1-methylcyclohexyloxycarbonyl analogues.[10] 1,4-Dimethyl-4-hydroxypiperidine reacted with *p*-nitrophenyl chloroformate giving the mixed carbonate (12), from which the hydrazide (13) was prepared. The latter



R

(12) p-$O_2NC_6H_4OCO$
(13) NH_2NHCO
(14) Z—Val—NHNHCO
(15) Z—Gly—Val—NHNHCO
(16) Z—Ala—Gly—Val—NHNHCO

was condensed with benzyloxycarbonyl-L-valine giving the protected hydrazide (14), stable to trifluoroacetic acid during 1 h at room temperature; in contrast, 1-methylcyclohexyloxycarbonylphenylalanine is cleaved within 1 min at 25 °C. Both protecting groups were removed from compound (14) by 45% hydrogen bromide in acetic acid during 1 h at room temperature. The 1,4-dimethylpiperidin-4-yloxycarbonyl group is, as expected, stable to hydrogenolysis, and therefore the benzyloxycarbonyl group could be removed selectively from compound (14) in this way, allowing the peptide chain to be extended, giving *N*-benzyloxycarbonyl-L-alanylglycyl-L-valyl-*N'*-1,4-dimethylpiperidin-4-yloxycarbonyl hydrazide (16); the basic ' handle ' was used for isolation at each step. This protecting group might with advantage replace the t-butoxycarbonyl group when greater stability to acid is needed.

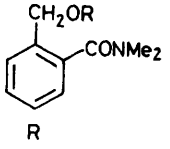
A major difficulty encountered at times during peptide synthesis is the insolubility of protected intermediates even in such a powerful solvent as dimethylformamide. We are therefore seeking to develop protecting groups specifically designed to increase solubility. For this purpose we have introduced a dimethylcarbamoyl substituent into groups of the t-butoxycarbonyl and benzyloxycarbonyl types. 3,3-Dimethylbutyrolactone reacted with dimethylamine giving 4-hydroxy-4-methylvaleric acid dimethylamide, which with *p*-nitrophenyl chloroformate gave the mixed carbonate (17). From this was prepared the 2-dimethylcarbamoylethyl-isopropoxycarbonyl derivatives of phenylalanine (18), glycine (19), isoleucine (20), *N*(α)-benzyloxycarbonyllysine (21) (the last three were isolated as their dicyclohexylammonium salts) and *N*(α)-benzyloxycarbonylornithine (22). The new protecting group was readily removed by trifluoroacetic acid during 1 h at room temperature. A convenient route for the substitution of a dimethylcarbamoyl group into protection of the benzyloxycarbonyl type is afforded by the reaction of

$Me_2N{\cdot}CO{\cdot}CH_2{\cdot}CH_2{\cdot}CMe_2{\cdot}OR$

R

(17) $CO_2C_6H_4NO_2$-p
(18) CO—Phe
(19) CO—Gly
(20) CO—Ile
(21) Z—Lys(CO)
(22) Z—Orn(CO)

phthalide with dimethylamine; the 2-dimethylcarbamoylbenzyl alcohol so formed was converted into the mixed carbonate (23), which gave 2-dimethylcarbamoylbenzyloxycarbonyl derivatives of phenylalanine (24),



R

(23) $CO_2C_6H_4NO_2$-p
(24) CO—Phe
(25) CO—Gly
(26) CO—Ile

glycine (25), and isoleucine (26), as their dicyclohexylammonium salts. The protection was stable to trifluoroacetic acid at room temperature for 3 h but was removed by 45% hydrogen bromide in acetic acid in 3 h at room temperature and by hydrogenolysis over palladium–charcoal. However, in general a 4-substituent is probably preferable to a 2-substituent, which might in some cases interfere with desired reactions, and we have proceeded further with derivatives having a 4-dimethylcarbamoylbenzyl group.[11]

EXPERIMENTAL

Thin-layer chromatograms were run on unbaked Kieselgel HF 254/366 plates; the solvents used were (proportions are by volume): A2, n-butanol–acetic acid–water (10 : 1 : 3, upper layer); E4, methanol–chloroform (1 : 9); G3, ethyl acetate–pyridine–acetic acid–water (60 : 11 : 3 : 6); and J, acetonitrile–water (3 : 1). Spots were detected by u.v. illumination, by ninhydrin, by iodine vapour, and by chlorine and starch–iodide. M.p.s were determined with a Kofler hot-stage apparatus. Evaporation was by rotary evaporator below 35 °C and solutions in organic solvents were dried over magnesium sulphate. N.m.r. and i.r. spectra are reported only in selected cases. Physical data for mixed carbonates are given in Table 1, for protected hydrazides in Table 2, and for *N*-protected amino-acids in Table 3.

Di-2-pyridylmethyl N-Phenylcarbamate (1).—Di-2-pyridylmethanol [12] (186 mg, 1.0 mmol) and phenyl isocyanate (0.11 ml, 1.0 mmol) in pyridine (5 ml) were set

TABLE 1

Mixed carbonates [a]

Compound	Yield (%)	M.p. (°C)	Analysis: Found (%) C	H	N	Formula	Required (%) C	H	N
(3) [a]	65	99—102	49.85	3.25	4.05	$C_{15}H_{12}NO_3Cl_3$	49.95	3.35	3.9
(9)·HCl [b]	70	84—86	64.1	6.3	5.5	$C_{26}H_{29}N_2O_5Cl$	64.4	6.0	5.8
(12)·HCl [c]	91	155 (decomp.)	50.6	5.8	8.3	$C_{14}H_{19}N_2O_5Cl$	50.8	5.8	8.5
(17) [d]	88	56—58	55.5	6.1	8.5	$C_{15}H_{20}N_2O_6$	55.55	6.2	8.6
(23) [e]	68	81—83	59.05	4.8	8.3	$C_{17}H_{16}N_2O_6$	59.3	4.7	8.1

[a] All the compounds are new. The preparation of compound (3) is typical and is described in the text. In all other cases 4-nitrophenyl chloroformate replaced trichlorophenyl chloroformate. [b] Compound (9) was prepared from 3-diethylamino-1,1-diphenylpropan-1-ol (D. W. Adamson, *J. Chem. Soc.*, 1949, S144) in dichloromethane without added base; the product was precipitated from the concentrated reaction mixture by the addition of ethyl acetate and was recrystallised from acetone. [c] Compound (12) was prepared as for compound (9) from 1,4-dimethyl-4-hydroxypiperidine (S. M. McElvain and R. S. Berger, *J. Amer. Chem. Soc.*, 1955, **77**, 2848); the product was precipitated from the reaction mixture by ether and recrystallised from chloroform–acetone. [d] Compound (17) was prepared as for compound (9) from 4-hydroxy-4-methylvaleric acid dimethylamide (described in the text) with the addition of 1 molar proportion of 1-methylmorpholine; di-*p*-nitrophenyl carbonate was removed by dissolution of the crude reaction product in isopropyl alcohol. [e] Compound (23) was prepared as for compound (9) from 2-dimethylcarbamoylbenzyl alcohol (described in the text); di-*p*-nitrophenyl carbonate was removed by dissolution of the crude product in methanol; the yield is overall from phthalide.

TABLE 2

Protected hydrazides [a]

Compound	Yield (%)	M.p. (°C)	$[\alpha]_D^{20}$ [b]/°	Analysis: Found (%) C	H	N	Formula	Required (%) C	H	N
(4) [a]	88	69—70.5		55.35	6.75	21.5	$C_9H_{13}N_3O_2$	55.35	6.7	21.5
(5) [a]	65	amorphous	−23	57.6	7.55	13.95	$C_{19}H_{30}N_4O_5$	57.85	7.65	14.2
(6) [a]	80	103—104	−34	55.65	7.5	15.2	$C_{21}H_{33}N_5O_6$	55.85	7.35	15.5
(7) [a,c]	91	114—116	−19	54.15	7.15	15.65	$C_{24}H_{38}N_6O_7 \cdot 0.5\ H_2O$	54.2	7.4	15.8
(8) [a,c,d]	90	200—201	−19	56.95	7.6	15.2	$C_{30}H_{49}N_7O_8$	56.65	7.75	15.4
(10)·HCl [e]	55	171—173 [f]		60.2	7.7	10.5	$C_{20}H_{28}N_3O_2Cl \cdot 1.25\ H_2O$	60.0	7.7	10.5
(11) [g]	80	109—114	−25	67.6	7.4	9.4	$C_{33}H_{42}N_4O_5 \cdot 0.5\ H_2O$	67.9	7.4	9.6
(13)·HCl [h]	92	163—165		42.8	8.2	18.6	$C_8H_{18}N_3O_2Cl$	42.95	8.1	18.8
(14) [i]	88	80—85	−30	59.65	7.6	13.2	$C_{21}H_{32}N_4O_5$	60.0	7.7	13.3
(15) [j]	66	100—105	−23	55.9	7.3	14.2	$C_{23}H_{35}N_5O_6 \cdot H_2O$	55.7	7.5	14.1
(16) [k]	75	112—115	−20	56.9	7.4	15.1	$C_{26}H_{40}N_6O_7$	56.9	7.35	15.3

[a] All the compounds are new. The preparations of compounds (4) and (5) are described in the text. Compounds (6)—(8) were prepared by removing the t-butoxycarbonyl group from the preceding compound in the Table by means of trifluoroacetic acid as usual and coupling the appropriate t-butoxycarbonyl-amino-acid to the amino-component by means of dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole, as described for compound (5). In these cases the yields given are calculated on the preceding t-butoxycarbonyl compound. Isolation was by extraction into 0.7M-citric acid. [b] Optical rotations were measured in dimethylformamide solution (*c* 1.0) except for compounds (6) and (16), for which chloroform was the solvent, and (11), (14), and (15), for which ethanol was the solvent. [c] The solvent for the coupling reaction was dimethylformamide. [d] Found after acid hydrolysis (110 °C, 24 h); Leu, 1.05; Ala, 1.00; Gly, 1.00; Val, 0.96. [e] In the preparation of compound (10), the reaction of the mixed carbonate (9) with hydrazine hydrate proceeded in methanol during 20 min at 0 °C and 40 min at room temperature. Addition of ether to the concentrated solution gave an oil which solidified after trituration with ethyl acetate–chloroform; the hydrazide was recrystallised from isopropanol. [f] Sintering at 75—80 °C. [g] Compound (11) was prepared similarly to compound (5) but the solvent for the coupling reaction was dichloromethane. [h] Compound (13) was prepared as for compound (10), but the hydrazide was precipitated from the concentrated reaction solution by addition of a large volume of ether; recrystallisation was from isopropanol. [i] Compound (14) was prepared similarly to compound (5) but the solvent for the coupling reaction was dimethylformamide. [j] In the preparation of compound (15), compound (14) was hydrogenolysed (Pd–C) in aqueous methanol to remove the benzyloxycarbonyl group and the crude product was coupled with benzyloxycarbonylglycine as described for compound (5), with dimethylformamide as solvent. The product crystallised after trituration with ether and light petroleum. [k] In the preparation of compound (16) compound (15) was hydrogenolysed as usual and the crude product was coupled with benzyloxycarbonylalanine as described for compound (5), with dimethylformamide as solvent.

aside at 30—40 °C for 4 d, when t.l.c. (solvent, ether) showed the reaction to be complete. The solution was evaporated and the residue was recrystallised from ethyl acetate–light petroleum, giving the *carbamate* (1) (251 mg, 82%), m.p. 153—155 °C; R_F 0.55 (E4) and 0.86 (G3) (Found: C, 70.4; H, 4.95; N, 13.85. $C_{18}H_{15}N_3O_2$ requires C, 70.8; H, 4.95; N, 13.75%).

T.l.c. (solvent E4) detected no change in a solution of the carbamate in trifluoroacetic acid at room temperature during 27 h (samples were evaporated to dryness and the residue was applied to the plate in dichloromethane solution after the addition of triethylamine). In a similar experiment using 45% hydrogen bromide in acetic acid at room temperature, samples of the solution were evaporated to dryness, the residue was taken up in water and acid was removed by Amberlite IR-45 resin; t.l.c. (solvents E4 and G3) detected no change during 48 h. The carbamate was recovered unchanged (m.p., i.r., and n.m.r. evidence) in 93% yield from a solution in 2M-hydrogen bromide after 4 h at room temperature. Hydrogenolysis (Pd–C) in ethanol solution cleaved the carbamate within 2 h, and zinc dust with aqueous acetic acid effected cleavage within 15 min.

The Reaction of Di-2-pyridylmethanol with 2,4,5-Trichlorophenyl Chloroformate.—A solution of 2,4,5-trichlorophenyl chloroformate (1.63 g, 6.27 mmol) in dichloromethane (10 ml) was added to a solution of di-2-pyridylmethanol [12] (1.16 g, 6.23 mmol) in dichloromethane (10 ml) at room temperature; stirring was continued for 24 h, when t.l.c. (solvent E4) indicated that reaction was complete. The

solution was diluted with dichloromethane (50 ml), washed (sodium hydrogencarbonate and brine), dried, and evaporated. The residue was recrystallised from carbon tetrachloride–light petroleum, giving 5-(2′-*pyridyl*)*pyrido*[1,2-c]-*oxazol*-2-*one* (2) (1.02 g, 77%), m.p. 178—179 °C; R_F 0.71 (E4) and 0.91 (Et_2O); $\nu_{max.}$($CHCl_3$) 1 755, 1 650, 1 602, and 1 476 cm^{-1}; $\lambda_{max.}$(EtOH) 244 (ε 6 500), 314 (11 600), 330 (13 100), 342 (13 400), and 404 nm (9 100); in 1M-hydrochloric acid, the last peak moved to 441 nm (ε 13 900); τ($CDCl_3$) 1.51 (1 H, m, pyridyl 6-H), 2.10—2.75 (4 H, complex, pyridyl 3- and 4-H, C*H*:CH·CH:C*H*), 2.99 (1 H, m, pyridyl 5-H), 3.39 (1 H, ddd, *J* 10, 6, and 1 Hz, CH:C*H*·CH:CH), and 3.90 (1 H, ddd, *J* 8, 6, and 1 Hz, CH:CH·C*H*:CH); *m/e* 212 (M^+, 54%), 156 (72), 78 (100), and 51 (57) (Found: C, 67.7; H, 3.95; N, 13.15. $C_{12}H_8N_2O_2$ requires C, 67.9; H, 3.8; N, 13.2%). The same product was obtained from the reaction of di-2-pyridylmethanol with *p*-nitrophenyl chloroformate.

4-*Pyridylisopropyl* 2,4,5-*Trichlorophenyl Carbonate* (3).—A solution of 2,4,5-trichlorophenyl chloroformate (17.4 g, 67 mmol) in tetrahydrofuran (60 ml) was added to a solution of αα-dimethyl-4-pyridylmethanol [13] (9.2 g, 67 mmol) and 1-methylmorpholine (7.35 ml, 67 mmol) in tetrahydrofuran (60 ml) at 22 °C. After 30 min further chloroformate (17.4 g) was added, and after 1 h in all the solution was filtered and then evaporated. The residue was dissolved in chloroform (300 ml), the solution was washed (water and brine), dried, and evaporated. Methanol (30 ml) was added to the residue and the insoluble di-2,4,5-trichlorophenyl carbonate [m.p. 167—168 °C, $\nu_{max.}$($CHCl_3$) 1 790 cm^{-1}] was filtered off. Evaporation of the filtrate and washings gave an oil which was decolourised in ethyl acetate solution with charcoal and crystallised from ethyl acetate–light petroleum, giving the *carbonate* (3) (see Table 1).

4-*Pyridylisopropoxycarbonylhydrazide*.—Hydrazine hydrate (0.1 ml, 2 mmol) was added to a solution of the mixed carbonate (3) (361 mg, 1 mmol) in methanol (3 ml). After 10 min the solvent was evaporated, and the residue was dissolved in chloroform (20 ml), washed, dried, and evaporated. The residue was crystallised from carbon tetrachloride, giving the *hydrazide* (4) (see Table 2).

A solution of the hydrazide in trifluoroacetic acid was evaporated after setting aside for 18 h at room temperature. The residue was dissolved in dichloromethane and neutralised with trimethylamine; t.l.c. (solvents E4 and G3) detected no change. The hydrazide was hydrogenolysed (Pd–C) in ethanol for 1.75 h, when t.l.c. (E4, G3) showed that cleavage was complete.

N-t-*Butoxycarbonyl*-L-*valyl*-N′-4-*pyridylisopropoxycarbonylhydrazide* (5).—A solution of dicyclohexylcarbodiimide (1.99 g, 9.65 mmol) in tetrahydrofuran (10 ml) was added slowly to a solution of t-butoxycarbonyl-L-valine (2.10 g, 9.65 mmol) and 1-hydroxybenzotriazole (1.48 g, 9.65 mmol) in tetrahydrofuran (25 ml) at 0 °C. After 1 h at 0 °C and 1 h at room temperature a solution of 4-pyridylisopropoxycarbonylhydrazide (4) (1.25 g, 6.4 mmol) in tetrahydrofuran (15 ml) was added. After setting aside for a further 4 h the solution was filtered and evaporated, and the residue was partitioned between 0.7M-citric acid (50 ml) and ether (50 ml). The aqueous layer was washed with ether and then made alkaline with solid sodium hydrogencarbonate and the product was extracted into ethyl acetate; the extracts were washed (sodium hydrogencarbonate, water, and brine) and evaporated, giving the *hydrazide* (5) (see Table 2).

A solution of the hydrazide (5) in trifluoroacetic acid was evaporated after setting aside for 30 h, the residue was taken up in dichloromethane and the solution was washed with aqueous sodium hydrogencarbonate; t.l.c. (solvents E4, G3, and J) detected only one component (R_F 0.13, 0.28, and 0.42 respectively), identified as *N*-L-valyl-*N*′-4-pyridylisopropoxycarbonylhydrazide by isolation of its *bis-trifluoroacetate* after reaction of the t-butoxycarbonyl derivative in trifluoroacetic acid for 30 min at room temperature; τ(D_2O) 1.30 (2 H, d, *J* 6 Hz, pyridyl 2- and 6-H), 2.03 (2 H, d, *J* 6 Hz, pyridyl 3- and 5-H), 6.15 (1 H, br d, *J* 7 Hz, C*H*CHMe$_2$), 7.90 (1 H, m, ·C*H*·CHMe$_2$), 8.19 (6 H, s, *Me*$_2$C·py), and 8.96 (6 H, d, *J* 6 Hz, *Me*$_2$CH) (Found: C, 41.15; H, 4.85; N, 10.5. $C_{18}H_{24}N_4O_7F_6$ requires C, 41.4; H, 4.65; N, 10.75%).

A sample (100 mg) of hydrazide (5) in dimethylformamide (DMF) was hydrogenolysed (Pd–C) for 1.5 h; reduced pyridyl derivatives were removed by p.l.c. [eluant, methanol–chloroform (1 : 6)] giving t-butoxycarbonyl-L-valylhydrazide (49 mg, 85%), identical in t.l.c., n.m.r., i.r., and optical rotation with authentic material prepared from t-butoxycarbonylvaline methyl ester (see below).

t-Butoxycarbonyl-L-*valylhydrazide*.— t-Butoxycarbonyl-L-

TABLE 3

N-Protected amino-acids [a]

Compound [b]	M.p. (°C)	$[\alpha]_D^{20}$ [c]/°	Analysis: Found (%) C	H	N	Formula	Required (%) C	H	N
(18)	98—100	−120	62.0	7.3	8.0	$C_{18}H_{26}N_2O_5$	61.7	7.5	8.0
(19) (DCHA salt) [d]	119—121		61.4	9.6	9.3	$C_{23}H_{43}N_3O_5 \cdot 0.5\ H_2O$	61.3	9.8	9.3
(20) (DCHA salt) [e]	120—122	4	65.45	10.2	8.2	$C_{27}H_{51}N_3O_5$	65.2	10.3	8.4
(21) (DCHA salt)	106—108	5	65.3	8.7	8.7	$C_{35}H_{58}N_4O_7$	65.0	9.0	8.7
(22)	102—104	−4	58.7	7.3	9.2	$C_{22}H_{33}N_3O_7$	58.5	7.4	9.3
(24) (DCHA salt)	110—113	19	69.7	8.3	7.7	$C_{32}H_{45}N_3O_5$	69.7	8.2	7.6
(25) (DCHA salt) [d]	150—152		63.9	8.4	8.5	$C_{25}H_{39}N_3O_5 \cdot 0.5\ H_2O$	63.8	8.6	8.9
(26) (DCHA salt) [f]	155—157	+4	67.55	9.1	8.1	$C_{29}H_{47}N_3O_5$	67.3	9.15	8.1

[a] All the compounds are new. The preparation of compound (18) is described in the text. The other derivatives in the Table were prepared analogously (see however notes below); dicyclohexylamine (DCHA) salts were prepared by addition of the amine to solutions of the protected amino-acid in chloroform or ethyl acetate and precipitation by ether or light petroleum, or [compounds (20), (21), (26)] in ether with precipitation by light petroleum. [b] DCHA = dicyclohexylamine. [c] Optical rotations were measured on solutions (*c* 1.0) in dimethylformamide except for compounds (24) and (16), for which the solvent was ethanol. [d] The product was extracted from the aqueous acid by ethyl acetate–n-butanol (1 : 1). [e] The mixed carbonate was added at 0 °C. [f] The mixed carbonate was added in portions; reaction time 18 h.

valine methyl ester and hydrazine hydrate reacted at room temperature giving a glass which crystallised slowly from methanol; the hydrazide (94% yield) had m.p. 116—117 °C, $[\alpha]_D^{20}$ −20° (*c* 1.0 in $CHCl_3$) (Found: C, 51.8; H, 9.0; N, 18.05. $C_{10}H_{21}N_3O_3$ requires C, 51.9; H, 9.15; N, 18.15%).

N-*t-Butoxycarbonyl*-L-*leucyl*-L-*alanylglycyl*-L-*valylhydrazide*.—(*a*) *By hydrogenolysis*. The protected tetrapeptide hydrazide (8) (80 mg, 0.126 mmol) in dimethylformamide was hydrogenolysed (Pd–C) in the usual way during 1 h, giving hydrazide (56 mg, 94%) of m.p. 204—207 °C, $[\alpha]_D^{20}$ −11° (*c* 1.0 in DMF) (lit.,[6] m.p. 198—201 °C; $[\alpha]_D^{20}$ −13°, −11.5°), R_F 0.61 (A2) and 0.64 (G3) (Found: C, 53.0; H, 8.6; N, 17.55. Calc. for $C_{21}H_{40}N_6O_6$: C, 53.35; H, 8.55; N, 17.8%).

(*b*) *By electrolytic reduction*. The protected tetrapeptide hydrazide (8) (64 mg, 0.1 mmol) in 0.05M-sulphuric acid (6 ml) at 0 °C was electrolysed at a mercury cathode [14] (diameter 2.5 cm) for 1 h (current, *ca.* 50 mA). The solution was passed down a column of Amberlite IR-45 resin (free base); the column was washed with 50% aqueous methanol and the eluate was evaporated. The residue was dried and washed with ether, giving hydrazide (43 mg, 91%) of m.p. 200—203 °C, $[\alpha]_D^{20}$ −9° (*c* 1.0 in DMF).

4-*Pyridylisopropoxycarbonyl*-L-*phenylalanine*.—4-Pyridylisopropyl 2,4,5-trichlorophenyl carbonate (7.04 g, 19.5 mmol) reacted with L-phenylalanine (4.95 g, 30 mmol) and triethylamine (10.4 ml, 75 mmol) in t-butanol–water (3 : 2; 60 ml) at 60 °C during 2 h. The solvent was evaporated and the residue was taken up in 0.7M-citric acid solution and washed with ether. The aqueous layer was brought to pH 4 by the addition of sodium hydrogencarbonate and the product was extracted into chloroform. Evaporation of the chloroform left a foam which crystallised under warm ethyl acetate, giving *4-pyridylisopropoxycarbonyl*-L-*phenylalanine* (6.26 g, 98%), m.p. 161—163 °C, $[\alpha]_D^{20}$ −30° (*c* 1.0 in DMF), R_F 0.49 (A2) and 0.60 (G3) (Found: C, 65.4; H, 6.2; N, 8.25. $C_{18}H_{20}N_2O_4$ requires C, 65.85; H, 6.15; N, 8.55%).

Samples of this derivative dissolved in anhydrous trifluoroacetic acid, and in 2M-hydrogen bromide in acetic acid, at room temperature were recovered unchanged after 48 h. Hydrogenolysis (Pd–C) in 60% acetic acid during 1 h gave L-phenylalanine (94% yield). Electrolytic reduction in 1M-hydrochloric acid at 0 °C at a mercury cathode [14] during 30 min (current 200 mA) also gave L-phenylalanine [isolated by use of Amberlite IR-45 resin (acetate form)] in 90% yield.

4-*Hydroxy*-4-*methylvaleric Acid Dimethylamide*.—3,3-Dimethylbutyrolactone (5.7 g, 50 mmol) was added dropwise to anhydrous dimethylamine (6.6 ml, 100 mmol) at −10 °C. The flask was closed and set aside for 3 d, when more dimethylamine (2.0 ml) was added. After 2 weeks the i.r. absorption at 1 770 cm^{-1} had disappeared; the mixture was then evaporated and the residue was distilled, giving the *dimethylamide* (7.0 g, 88%), b.p. 108.5 °C (0.6 mmHg); R_F 0.43 (E4) (Found: C, 60.1; H, 10.8; N, 8.7. $C_8H_{17}NO_2$ requires C, 60.35; H, 10.8; N, 8.8%).

2-(*Dimethylcarbamoylethyl*)*isopropoxycarbonyl*-L-*phenylalanine* (18).—The mixed carbonate (17) (780 mg, 2.4 mmol) reacted with L-phenylalanine (330 mg, 2.0 mmol) and tetramethylguanidine (250 mg, 2.2 mmol) in dimethylformamide (5 ml) during 3 h. The solution was evaporated, the residue was taken up in brine and *p*-nitrophenol was extracted into ether, the solution being saturated with carbon dioxide in later extractions. The aqueous layer was brought to pH 1—2 and the product was extracted into ethyl acetate, giving the protected phenylalanine (18) (see Table 3).

2-*Dimethylcarbamoylbenzyl Alcohol*.—Dimethylamine (40 ml, 0.6 mol) was distilled through a drying tower containing potassium hydroxide pellets and condensed into an ice-cooled two-necked flask containing phthalide (13.4 g, 0.1 mol). After 4 d at room temperature in the closed vessel the mixture was evaporated, leaving the alcohol (17.3 g, 97%), R_F 0.44 (E4) (Found: C, 66.8; H, 7.2; N, 7.9. $C_{10}H_{13}NO_2$ requires C, 67.0; H, 7.3; N, 7.8%), which was suitable for conversion to the mixed carbonate (23) (see Table 1).

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