Polypeptides. Part XIII.[1] Preparation of α-Aza-amino-acid (Carbazic Acid) Derivatives and Intermediates for the Preparation of α-Aza-peptides

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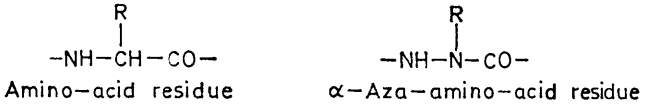
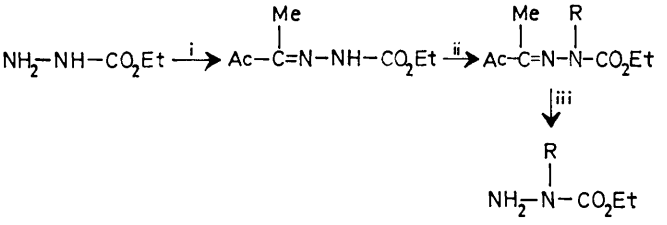
Esters and amides of several α-aza-amino-acids (carbazic acids), and intermediates of use in the introduction into peptides of α-aza-glycyl, -alanyl, -valyl, -leucyl, -isoleucyl, -phenylalanyl, -tyrosyl, -tryptophyl, -prolyl, -aspartyl, -asparaginyl, -glutamyl, -glutaminyl, and -pyroglutaminyl residues are described. t-Butyl 3-alkyl- or -aralkyl carbazates, obtained by catalytic hydrogenation of the corresponding hydrazones, were the most versatile intermediates; they were converted in high yield into α-aza-amino-acid esters and amides, gave α-aza-dipeptide esters when treated with α-isocyanato-esters, and afforded ' active esters ' of *N*-t-butoxycarbonyl-α-aza-amino-acids when treated with 2,4,5-trichlorophenyl chloroformate.

REPLACEMENT in peptides, of the α-CH groups of amino-acid residues by N has little effect on the overall polarity of the molecule and the relative spacing of side-chain residues, but would be expected to induce new chemical and biological properties by virtue of the changed conformational situation at the residue or residues concerned. Our interest over the past six years in analogues



of this type (α-aza-peptides) has led us to establish general methods for the synthesis of α-aza-amino-acid derivatives and the incorporation of α-aza-amino-acid residues into peptides. This aspect of our work is described in the present paper. Subsequent papers in the series will deal with the use of this methodology in the synthesis of α-aza-analogues of biologically active peptides and model enzyme substrates.

α-Aza-amino-acid derivatives and α-aza-peptides have hitherto been little explored. Ethyl esters of α-aza-phenylalanine, -alanine, -leucine, and -methionine were prepared by Ronco *et al.*[2] by alkylation/aralkylation of ethyl carbazate, using biacetyl for amino-protection (Scheme 1). Kurtz and Nieman[3] found the method



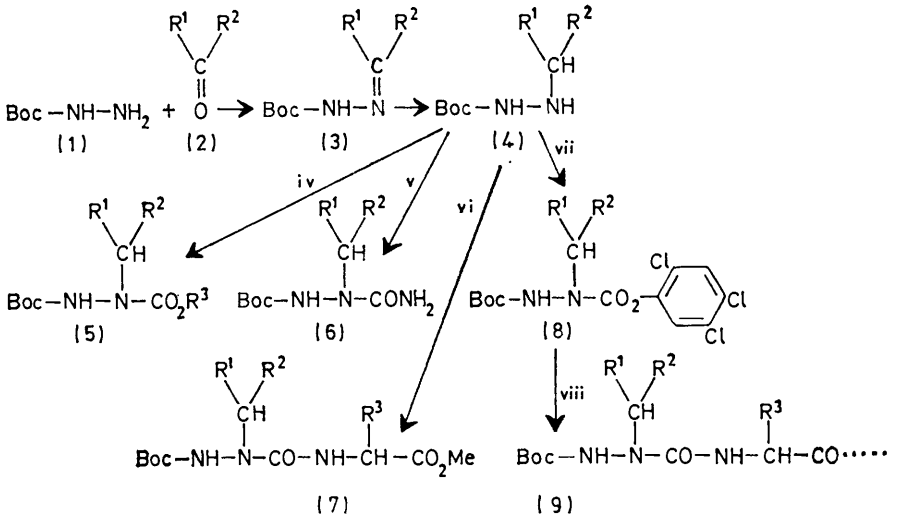
SCHEME 1 Reagents: see caption, Scheme 2

unattractive for the preparation of *N*-acetyl-α-aza-phenylalanine ethyl ester preferring the ethoxycarbonylation of methyl 3-benzylcarbazate. Gante, in extensive studies,[4] developed methods for the incorporation of aza-glycyl residues into peptides, and demonstrated that α-aza-alanine and -phenylalanine may be incorporated by selective acylation of methyl- and benzyl-hydrazine. Hess, Moreland, and Laubach[5] prepared an analogue of bovine angiotensin II in which the 5-valyl residue is replaced by α-aza-valyl; the incorporation of aza-valine involved the reaction of t-butyl-3-isopropylcarbazate with the adjacent amino-acid residue (tyrosine) as its *N*-carbonyl ethyl ester. Niedrich and his co-workers prepared α-aza-asparagine and aza-glycine analogues of oxytocin[6] and of eledoisin[7] octapeptide, and α-aza-alanine analogues of eledoisin hexa- and penta-peptides[8]; the incorporation of α-aza-asparagine and -alanine involved selective acylation of hydrazinoacetamide or methylhydrazine.

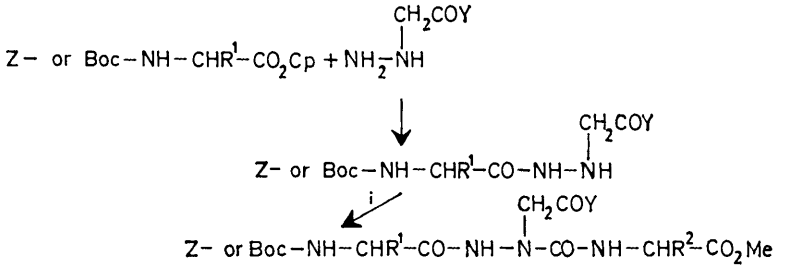
Our own work has shown that the most useful and versatile intermediates are t-butyl 3-alkyl- or -aralkyl-carbazates (4). Carbazates of this type, suitable for the synthesis of aza-valine, -leucine, -isoleucine, -phenylalanine, -tyrosine, and -tryptophan derivatives, and the incorporation of these aza-amino-acids into peptides, are readily accessible by hydrogenation of the hydrazones formed from t-butyl carbazate (1) and the appropriate aldehyde or ketone (2) (Scheme 2). The hydrazones and carbazates so prepared are given in Tables 1 and 2. Hydrogenation of the hydrazone (3; $R^1 = H$, $R^2 = CH_2Ph$) was rapid (*ca.* 20 min) at room temperature over 5% palladium–carbon; indeed care must be taken to avoid over-reduction (see footnote *j*, Table 2); in other cases extended times or more vigorous conditions were necessary. The carbazates (4) reacted smoothly with alkyl chloroformates or potassium cyanate–hydrochloric acid to give *N*-t-butoxycarbonyl-aza-amino-acid esters (5) or amides (6) (Table 3) which, by mild acidic hydrolysis, provided aza-amino-acid esters (Table 4) and amides in high overall yield. Use of the carbazates (4)

[1] Part XII, A. S. Dutta and J. S. Morley, *J. Chem. Soc.* (*C*), 1971, 2896.
[2] K. Ronco and H. Erlenmeyer, *Helv. Chim. Acta*, 1956, **39**, 1045; K. Ronco, B. Prijs, and H. Erlenmeyer, *ibid.*, pp. 1253, 2088.
[3] A. N. Kurtz and C. Niemann, *J. Org. Chem.*, 1961, **26**, 1843.
[4] Reviewed by J. Gante in *Angew. Chem. Internat. Edn.*, 1970, **9**, 813.
[5] H. J. Hess, W. T. Moreland, and G. D. Laubach, *J. Amer. Chem. Soc.*, 1963, **85**, 4040.
[6] H. Niedrich, *J. prakt. Chem.*, 1972, **314**, 769.
[7] H. Niedrich and C. Oehme, *J. prakt. Chem.*, 1972, **314**, 759.
[8] H. Niedrich, C. Oehme, and J. Bergmann, *J. prakt. Chem.*, 1974, **316**, 741.

in the incorporation of aza-amino-acid residues into peptides will be illustrated in subsequent papers. Two general methods have been employed (see Scheme 2). As illustrated by Hess, Moreland, and Laubach [5] in the incorporation of α-aza-valyl, they may be treated with an α-isocyanato-ester at room temperature to give t-butoxycarbonyl-aza-dipeptide esters (7), from which the peptide chain may be extended at the N- or C-terminus by normal methods. Alternatively, they may be converted into 2,4,5-trichlorophenyl carbamates (8) by reactions involving hydrazinoacetic acid, its methyl, benzyl, and t-butyl esters, and hydrazinoacetamide. In such cases, acylation of the unsubstituted nitrogen atom predominated, providing the basis of a route of choice for the incorporation of α-aza-aspartic acid and its esters, and of α-aza-asparagine (Scheme 3). Thus, for example, *N*-t-butoxycarbonylmethionine succinimido ester with methylhydrazine gave a mixture of *N*-t-butoxycarbonylmethionine 2-methylhydrazide and 1-methylhydrazide, whereas with t-butyl hydrazinoacetate it gave exclusively



SCHEME 2 Reagents: i, $(MeCO)_2$; ii, RCl; iii, reflux in 50% aqueous ethanol; iv, $ClCO_2R^3$; v, KCNO–HCl; vi, $O{=}C{=}N{\cdot}CHR^3{\cdot}CO_2Me$; vii, 2,4,5-trichlorophenyl chloroformate; viii, $NH_2{\cdot}CHR^3{\cdot}CO\cdots$



SCHEME 3 Incorporation of α-aza-aspartic acid and α-aza-asparagine (Y = OH, OMe, OEt, OBu^t, or NH_2) Reagent: i, $O{=}C{=}N{\cdot}CHR^2{\cdot}CO_2Me$

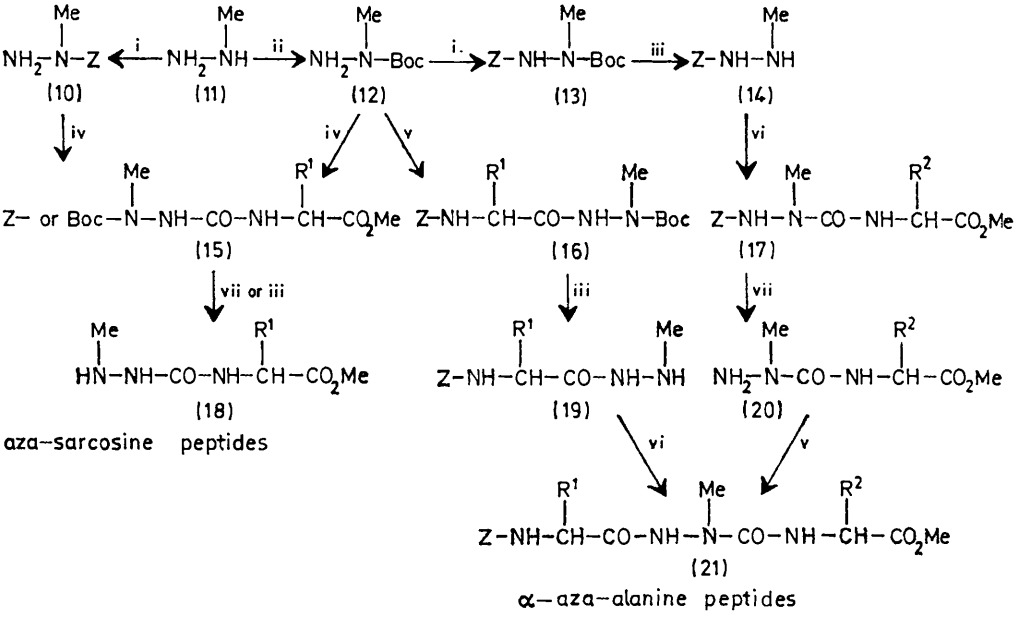
reaction with 2,4,5-trichlorophenyl chloroformate; the activated carbamate (8) may then be treated with an amino-acid derivative or pepride to give an extended aza-peptide (9).

The direct acylation of relevant mono-alkyl- or -aralkyl-hydrazines by *N*-protected amino-acids was also investigated as a possible route to aza-peptides. In this route it is necessary that acylation occurs predominantly on the unsubstituted nitrogen atom of the hydrazine, and in general this was not achieved. The site of acylation was markedly dependent on reaction conditions and steric factors (substituent on the hydrazine; nature of acylation agent) but acylation on the substituted nitrogen atom was usually favoured. Exceptions were *N*-t-butoxycarbonylmethionine 2-(t-butoxycarbonylmethyl)hydrazide. These findings agree with those of Niedrich and his co-workers; from the reaction of *N*-benzyloxycarbonylglycine cyanomethyl ester with t-butyl hydrazinoacetate,[9] and the carbodi-imide mediated reactions of *N*-benzyloxycarbonylglycine with ethyl carbazate,[10] γ-methyl hydrogen *N*-benzyloxycarbonylglutamate with methyl hydrazinoacetate,[9] and *N*-benzyloxycarbonylglycine and N^{α}, N^{ϵ}-bis-t-butoxycarbonyl-lysine *p*-nitrophenyl ester with hydrazinoacetamide,[9,10] only 1,2-disubstituted hydrazines were isolated.

[9] H. Niedrich, *Chem. Ber.*, 1969, **102**, 1557.
[10] W. Knoblock and H. Niedrich, *J. prakt. Chem.*, 1962, **17**, 273.

Although we have much more limited experience with esters and 3-hydrazinopropionamide, it seems that acylation of these derivatives also occurs mainly on the unsubstituted nitrogen atom, providing an attractive route to peptides containing aza-glutamic acid and aza-glutamine peptides.

Under favourable conditions, the acylation of unhindered mono-alkyl- or -aralkyl-hydrazines may occur exclusively on the substituted nitrogen atom. Niedrich *et al.*[8] found that methylhydrazine and *N*-t-butoxycarbonylalanine *p*-nitrophenyl ester gave only a 1,1-disubstituted hydrazine (comparison of the course of this reaction with the above cited reaction of methylhydrazine with *N*-t-butoxycarbonylmethionine *N*-hydroxysuccinimido ester illustrates the influence of the acylating component on the site of acylation), and both Gante [11] and Niedrich *et al.*[8] have used the selectivity of reactions of methylhydrazine and benzylhydrazine with *N*-activated (*N*-carbonyl and *N*-p-nitrophenyloxycarbonyl) amino-acid esters in the preparation of aza-alanine and -phenylalanine peptides. We have found that t-butyl 2,4,5-trichlorophenyl carbonate and benzyl chloroformate also react with methylhydrazine mainly at the substituted nitrogen atom; providing t-butyl 2-methylcarbazate (12) and benzyl 2-methylcarbazate (10) in high yield. These two carbazates and benzyl 3-methylcarbazate (14) [prepared by treating the carbazate (12) with benzyl chloroformate to give (13), followed by cleavage with hydrogen chloride–ethyl acetate] have proved valuable intermediates in the preparation of peptides containing α-aza-alanine and aza-sarcosine (Scheme 4). Thus, for the incorporation of α-aza-alanine at the C-terminus, t-butyl 2-methylcarbazate (12) may be treated with a benzyloxycarbonyl-amino-acid to provide condensation products of type (16); selective acidolysis of these provides benzyloxycarbonyl-amino-acid 2-methylhydrazides (19), which may be converted into α-aza-alanine dipeptide methyl esters or amides by reaction with ethyl chloroformate or hydrogen cyanate, or into α-aza-alanine tripeptide derivatives (21), by reaction with an α-isocyanato-ester. Extended peptides may be prepared by the use of *N*-protected peptides instead of a benzyloxycarbonyl-amino-acid in the reaction with the 2-methylcarbazate (12), or by extension by normal methods at the N-terminus of the intermediate (16) or (21). For the incorporation of α-aza-alanine at the N-terminus, the 3-methylcarbazate (14) may be treated with an α-isocyanato-ester to provide



SCHEME 4 Incorporation of α-aza-alanine and aza-sarcosine. Reagents: i, ZCl; ii, BocOCp; iii, HCl–EtOAc; iv, O=C=N·CHR¹·CO₂Me; v, ZNH·CHR¹CO₂Cp; vi, O=C=N·CHR²CO₂Me; vii, H_2, Pd–C. Cp = 2,4,5-trichlorophenyl.

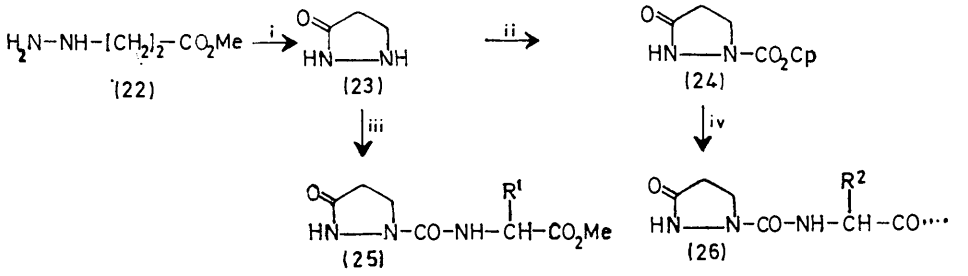
benzyloxycarbonyl-α-aza-alanine dipeptide esters (17), which may be extended at the C-terminus by normal methods. Alternatively, α-aza-alanine dipeptide esters (20), obtained by hydrogenolysis of the benzyloxycarbonyl derivatives (17), may be extended at the N-terminus to provide tripeptide analogues (21) or analogues of greater length. For the incorporation of aza-sarcosine, benzyl 2-methylcarbazate (10) or t-butyl 2-methylcarbazate (12) may be treated with an α-isocyanatoester to provide benzyloxycarbonyl- or t-butoxycarbonyl-aza-sarcosine dipeptide esters (15), and thence aza-sarcosine dipeptide esters (18), or 3-methylcarbazate (14) may be treated with *N*-protected amino-acids or peptides to provide derivatives of peptides containing *C*-terminal aza-sarcosine.

Peptides containing α-aza-pyroglutamyl may be prepared from the readily available pyrazolidin-3-one

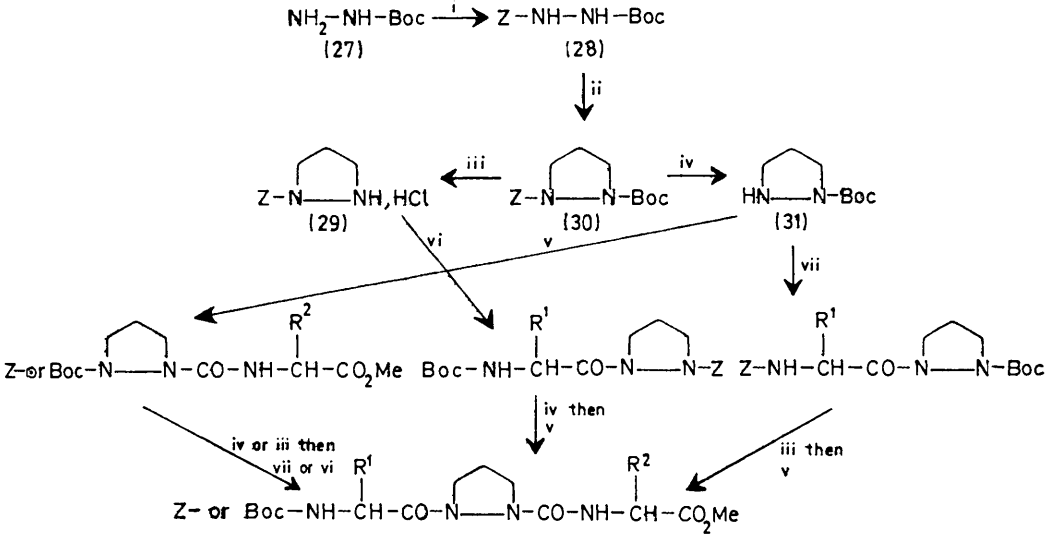
[11] J. Gante, *Chem. Ber.*, 1965, **98**, 540, 3340.

(23);[12] this reacts directly with α-isocyanato-esters to provide dipeptide analogues (25), or, *via* its 2,4,5-trichlorophenyl ester (24), with amino-components of normal peptide synthesis, to provide extended analogues (26) (Scheme 5).

For the incorporation of α-aza-proline, we required 1-benzyloxycarbonylpyrazolidine (29) and 1-t-butoxycarbonylpyrazolidine (31). Initial attempts to prepare these intermediates by the reaction of 1,3-dibromopropane with benzyl carbazate or t-butyl carbazate (27) afforded little of the required product; the reaction of 1,3-dibromopropane with ethyl carbazate was also



SCHEME 5 Incorporation of α-aza-pyroglutamic acid. Reagents: i, reflux in MeOH; ii, dicyclohexylcarbodi-imide–CpOH; iii, O=C=N·CHR¹·CO₂Me; iv, NH₂·CHR²·CO · · ·. Cp = 2,4,5-trichlorophenyl.



SCHEME 6 Incorporation of α-aza-proline. Reagents: i, ZCl; ii, Br[CH₂]₃Br–NaH; iii, HCl–EtOAc; iv, H_2, Pd–C; v, O=C=N·CHR²CO₂Me; vi, BocNH·CHR¹CO₂Cp; vii, ZNH·CHR¹·CO₂Cp. Cp = 2,4,5-trichlorophenyl.

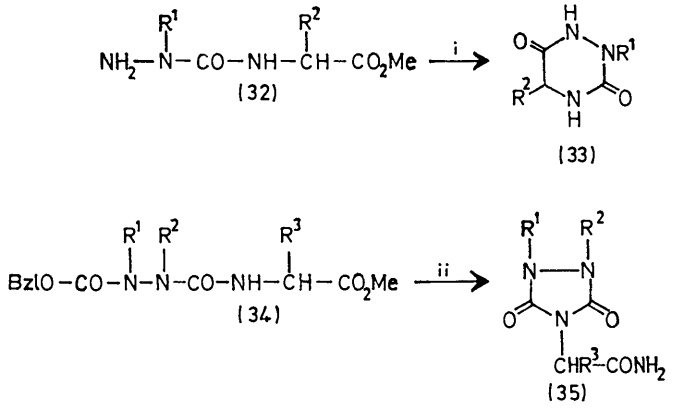
1-t-butoxycarbonylpyrazolidine (31) by hydrogenolysis. The ways in which we have used these two intermediates in the preparation of aza-proline peptides are summarised in Scheme 6.

Two cyclisation reactions of α-aza-dipeptide derivatives were encountered in our work and are conveniently described in this paper. First, α-aza-dipeptide esters (32) gave 1,2,4,5-tetrahydro-1,2,4-triazine-3,6-diones (33). This reaction, analogous to the formation of piperazine-3,6-diones from dipeptide esters,[13] has also been encountered by Niedrich *et al.*[8] As is the case in the analogous piperazinedione formation, the ease of

investigated, but 1-ethoxycarbonylpyrazolidine (α-aza-proline ethyl ester), purified by g.l.c., was obtained in only low yield. However, 1,3-dibromopropane, 1-benzyloxycarbonyl-2-t-butoxycarbonylhydrazine (28) [prepared from t-butyl carbazate (27) and benzyl chloroformate], and sodium hydride in dimethylformamide gave, at ambient temperature, 1-benzyloxycarbonyl-2-t-butoxycarbonylpyrazolidine (30) in high yield, from which the required 1-benzyloxycarbonylpyrazolidine (29) was obtained, as hydrochloride, by treatment with hydrogen chloride–ethyl acetate, and triazinedione formation was influenced by steric factors. Thus, α-aza-phenylalanyl-L-leucine methyl ester hydrochloride (32; $R^1 = CH_2Ph$, $R^2 = Bu^i$) changed into the triazine (33; $R^1 = CH_2Ph$, $R^2 = Bu^i$) on storage at 20—22 °C, whereas α-aza-leucyl-L-leucine methyl ester (32; $R^1 = R^2 = Bu^i$) and α-aza-leucyl-L-valine methyl ester (32; $R^1 = Pr^i$, $R^2 = Bu^i$) were unchanged even at 90—92 °C. These more stable esters were, however,

[12] J. C. Howard, G. Gever, and P. H. L. Wei, *J. Org. Chem.*, 1963, **28**, 868.
[13] E. Fischer and H. Scheibler, *Annalen*, 1908, **363**, 136.

smoothly converted into triazines (**33**; $R^1 = R^2 =$ Bu^i, and R^1 = Pr^i, R^2 = Bu^i) in hot aqueous 5% acetic acid. Secondly, benzyloxycarbonyl-α-aza-dipeptide esters (**34**), when treated with methanolic ammonia, gave 1,2,4-triazolidine-3,5-diones (**35**). This reaction, analogous to the formation of hydantoins from benzyloxycarbonyl-dipeptide esters,[14] was rapid (complete in 16 h at 4 °C) in the case of *N*-benzyloxycarbonyl-α-aza-prolylglycine methyl ester (**34**; $R^1R^2 = [CH_2]_3$, R^3 = H); of three other cases examined, the reaction was slower (complete in 5—7 days at ambient temperature) with *N*-benzyloxycarbonyl-α-aza-alanylglycine (**34**; $R^1 = R^3$ = H, R^2 = Me) and -*N*-benzyl-aza-glycylglycine (**34**; $R^1 = CH_2Ph$, $R^2 = R^3$ = H) methyl esters,



SCHEME 7 Cyclisation reactions of α-aza-dipeptide derivatives; i, storage at ambient temperature; ii, MeOH–NH_3

and slowest (complete in 15 days at ambient temperature) with *N*-benzyloxycarbonyl-*N*-benzyl-aza-glycylphenylalanine methyl ester (**34**; $R^1 = R^3 = CH_2Ph$, R^2 = H).

EXPERIMENTAL

Ascending, thin-layer chromatograms were run on Keiselgel G with butan-1-ol–acetic acid–water (4 : 1 : 5 v/v) (R_{FA}), butan-1-ol–acetic acid–water–pyridine (15 : 3 : 12 : 10) (R_{FB}), butan-2-ol–3% ammonium hydroxide (3 : 1) (R_{FC}), acetonitrile–water (3 : 1) (R_{FD}), acetone–chloroform (1 : 1) (R_{FE}), ethanol–chloroform (4 : 1) (R_{FF}), cyclohexane–ethyl acetate–methanol (1 : 1 : 1) (R_{FH}), or chloroform–methanol–water (11 : 8 : 2) (R_{FK}). Spots were revealed with ninhydrin, fluorescamine,[15] sodium hypochlorite–potassium iodide, or potassium ferricyanide–ferric chloride reagent.[16]

Hydrazones from t-Butyl Carbazate (*t-Butyl 3-Alkylidene- and Arylmethylene-carbazates*) (*Table* 1).—The appropriate aldehyde or ketone was added at 20—25 °C over 5—10 min to a solution of t-butyl carbazate [17](1 mol. equiv.) in tetrahydrofuran or ether. In most cases, the hydrazone separated and was collected after 2—4 h. In other cases, the solution was evaporated *in vacuo* and the residue was crystallised from the solvent indicated in Table 1. The products had R_{FD} 0.6—0.8, R_{FE} 0.6—0.7, R_{FF} 0.5—0.7, R_{FH} 0.5—0.7, R_{FK} 0.8—0.95.

t-Butyl 3-Alkyl- and -Aralkyl-carbazates (*Table* 2).—The appropriate hydrazone (Table 1) in tetrahydrofuran was hydrogenated at 25 °C and 25—30 lb in^{-2} over 5% palladised charcoal (0.05 g per mmol of hydrazone); the time required for uptake of 1 mol. equiv. of hydrogen is shown in Table 2. In the two cases where uptake was incomplete after 60 h, 30% palladised charcoal and more severe conditions (usually 35 °C and 100 lb in^{-2}) were used. After filtration, the solution was evaporated, and the residual oil was distilled *in vacuo*. The products had R_{FD} 0.68—0.74, R_{FE} 0.6—0.71, R_{FF} 0.65—0.72, R_{FH} 0.6—0.75, R_{FK} 0.84—0.97.

N-*t-Butoxycarbonyl-α-aza-amino-acid Ethyl Esters* (*Table* 3).—Ethyl chloroformate (50 mmol) was added dropwise to a stirred solution of the appropriate carbazate (Table 2) (50 mmol) and triethylamine (50 mmol) in chloroform (100 ml) at −10 °C. The mixture was stirred at −10 °C for 30 min and then overnight at ambient temperature. It was then evaporated *in vacuo*. A solution of the residue in ethyl acetate was washed successively with water, aqueous 20% citric acid, saturated aqueous sodium hydrogen carbonate, and water, and then dried (Na_2SO_4) and evaporated. The products obtained by distillation of the residue *in vacuo* had R_{FD} 0.71—0.80, R_{FE} 0.63—0.68, R_{FF} 0.61—0.70, R_{FH} 0.66—0.74, R_{FK} 0.83—0.95.

N-*t-Butoxycarbonyl-α-aza-amino-acid Amides* (*Table* 3).—Potassium cyanate (2 mol. equiv.) was added over 30 min to a solution of the appropriate carbazate (Table 2) and 4N-hydrochloric acid (1 mol. equiv.) in water or dioxan–water at 20—25 °C. More 4N-hydrochloric acid (1 mol.

TABLE 1

Hydrazones (BocNH·N=R) [a] from carbazates (BocNH·NH_2) and aldehydes or ketones

R	Yield (%)	M.p. (°C)	Solvent [b]	Found (%) C	Found (%) H	Found (%) N	Formula	Required (%) C	Required (%) H	Required (%) N
CMe_2	95.5	104—105	C_6H_{12}	55.8	9.3	16.4	$C_8H_{16}N_2O_2$	55.8	9.3	16.3
CH·CHMe$_2$	92	89—90	C_6H_{12}	58.3	9.7	15.1	$C_9H_{18}N_2O_2$	58.0	9.7	15.0
CMeEt	92	78	C_6H_{12}	57.9	9.8	14.9	$C_9H_{18}N_2O_2$	58.0	9.7	15.0
CHPh	73	185 [c]	MeOH	65.5	7.5	12.7	$C_{12}H_{16}N_2O_2$	65.4	7.3	12.7
CH·C_6H_4·OH-*p*	74	182	THF–C_6H_{12}	60.9	6.7	12.0	$C_{12}H_{16}N_2O_3$	61.0	6.8	11.8
CH·C_6H_4·OBu^t	71	192—193	THF	65.7	8.6	9.8	$C_{16}H_{24}N_2O_3$	65.7	8.3	9.8
CH·C_6H_4Cl-*p*	75	170—171	MeOH	55.8	5.7	10.8	$C_{12}H_{15}ClN_2O_2$	55.9	5.8	10.8
CH·$C_6H_3(OMe)_2$-3,4	78	159—160	THF–Et_2O	59.9	7.1	10.1	$C_{14}H_{20}N_2O_4$	59.9	7.2	9.9
Indol-3-ylmethylene	96	103—105	THF	64.8	6.8	15.9	$C_{14}H_{17}N_3O_2$	64.8	6.6	16.2

[a] All compounds new except where R = CHPh. [b] C_6H_{12} = cyclohexane; THF = tetrahydrofuran. [c] L. A. Carpino, A. A. Santilli, and R. W. Murray (*J. Amer. Chem. Soc.*, 1960, **82**, 2728) give m.p. 185—187°.

[14] J. A. MacLaren, *Austral. J. Chem.*, 1958, **11**, 360.

[15] S. Udenfriend, S. Stein, P. Böhlen, W. Dairman, W. Leimgruber, and M. Weigele, *Science*, 1972, **178**, 871.

[16] 'Thin-layer Chromatography. A Laboratory Handbook,' ed. E. Stahl, Springer-Verlag, Berlin, 1969, p. 876.

[17] L. A. Carpino, B. A. Carpino, C. A. Giza, R. W. Murray, A. A. Santilla, and P. H. Terry, *Org. Synth.*, 1964, **44**, 22.

TABLE 2

t-Butyl 3-alkyl- or -aralkyl-carbazates (BocNH·NHR) [a,b]

R	Intermediate for incorporation of [c]	Conditions [d]	Yield (%) [e]	B.p. or m.p. (°) [mmHg]	Found (%) C	H	N	Formula	Required (%) C	H	N
	Azval	60—68 h [f]	85	75—80 [0.6] [g]	55.1	10.2	15.8	$C_8H_{18}N_2O_2$	55.1 [h]	10.4 [h]	16.0 [h]
$CH_2{\cdot}CHMe_2$	Azleu	40—45 h [i,j,k]	88	75—80 [0.7]	57.2	10.6	14.8	$C_9H_{20}N_2O_2$	57.4	10.7	14.8
CHMeEt	Azile	5 h [i]	90	84—88 [0.32]	57.4	10.7	14.6	$C_9H_{20}N_2O_2$	57.4	10.7	14.8
CH_2Ph	Azphe	20 min [l]	82	136—140 [2.3]	64.7	8.1	12.4	$C_{12}H_{18}N_2O_2$	64.8	8.1	12.6
$CH_2{\cdot}C_6H_4{\cdot}OH$-*p*	Aztyr	3.5 h	91	162—164 [m]	60.3	7.5	11.7	$C_{12}H_{18}N_2O_3$	60.4	7.6	11.7
$CH_2{\cdot}C_6H_4{\cdot}OBu^t$-*p*	Aztyr(Bu^t)	2.5 h	85	160—162 [0.9] [l]	65.1	8.7	9.3	$C_{16}H_{26}N_2O_3$	65.3	8.8	9.5
$CH_2{\cdot}C_6H_4Cl$-*p*		16 h	82	68—70 [n]	56.2	6.3	10.7	$C_{12}H_{17}ClN_2O_2$	56.1	6.6	10.9
$CH_2{\cdot}C_6H_3(OMe)_2$-3,4		16 h	80	180—185 [1.1]	59.5	7.8	9.7	$C_{14}H_{22}N_2O_4$	59.6	7.8	9.9
Indol-3-ylmethylene	Aztrp	40—60 h	78	106—107 [o]	64.2	7.4	16.2	$C_{14}H_{19}N_3O_4$	64.4	7.3	16.1

[a] All prepared by hydrogenation of related hydrazones (Table 1) by the general method described. [b] All compounds new except BocNH·NH·$CHMe_2$. [c] Intermediates for the incorporation of Azala, Azpro, Azpyr, and Azsar are described in the Experimental section. [d] Unless otherwise stated, time required for uptake of 1 mol H_2 at 30 lb in^{-2} at 25 °C over 5% Pd–C. [e] Yields refer to product with stated constants and analysis. [f] Use of 30% Pd–C and 100 lb in^{-2} H_2 gave a mixture containing little of the desired product. [g] M.p. 49—51° (ref. 5 gives 47—51°). [h] Calculated. [i] Time required for uptake of 1 mol H_2 at 100 lb in^{-2} at 35 °C over 30% Pd–C. [j] In one of four experiments, uptake of 1 mol H_2 was complete in 16 h and the product contained BocNH·NHCH(Pr^i)$_2$. [k] Uptake of 1 mol H_2 at 30 lb in^{-2} at 25 °C over 5% Pd–C required >3 days. [l] After 5 h, BocNH·NH_2 was formed in near quantitative yield. [m] Recrystallised from ethyl acetate–light petroleum (b.p. 60—80°). [n] Low-melting solid. [o] Recrystallised from ether–light petroleum (b.p. 60—80°).

TABLE 3

N-t-Butoxycarbonyl-α-aza-amino-acid ethyl esters [a] and amides [a]

Compound [b]	R	Yield (%) [c]	B.p. or m.p. (°) [mmHg]	Found (%) C	H	N	Formula	Required (%) C	H	N
BocNH·NR·CO_2Et	$CHMe_2$	85	112—116 [1.3]	53.8	9.0	11.4	$C_{11}H_{22}N_2O_4$	53.6	9.0	11.3
	$CH_2{\cdot}CHMe_2$	77	100—102 [0.4]	55.2	9.3	10.8	$C_{12}H_{24}N_2O_4$	55.4	9.2	10.7
	CHMeEt	84	96—100 [0.4]	55.3	9.3	10.4	$C_{12}H_{24}N_2O_4$	55.4	9.2	10.7
	CH_2Ph	91	160—162 [0.7]	60.9	7.5	9.8	$C_{15}H_{22}N_2O_4$	61.2	7.5	9.5
	$CH_2{\cdot}C_6H_4{\cdot}OBu^t$-*p*	90	170—172 [0.1]	62.1	8.0	7.4	$C_{19}H_{30}N_2O_5$	62.3	8.2	7.6
	$CH_2{\cdot}C_6H_4Cl$-*p*	90	165—167 [0.4]	54.7	5.3	7.3	$C_{15}H_{21}ClN_2O_4$	54.8	5.6	7.3
	$CH_2{\cdot}C_6H_3(OMe)_2$-3,4	92	196—200 [1.1]	57.3	7.5	7.8	$C_{17}H_{26}N_2O_6$	57.6	7.4	7.9
BocNH·NR·CO·NH_2	$CHMe_2$	72	140—141 [d]	49.4	8.9	19.4	$C_9H_{19}N_3O_3$	49.7	8.8	19.3
	$CH_2{\cdot}CHMe_2$	69	141—142 [d]	52.1	8.9	18.0	$C_{10}H_{21}N_3O_3$	51.8	9.1	18.1
	CHMeEt	82	98—99 [e]	51.6	8.9	18.4	$C_{10}H_{21}N_3O_3$	51.8	9.1	18.1
	CH_2Ph	71	124—125 [e]	59.0	7.4	15.5	$C_{13}H_{19}N_3O_3$	58.8	7.2	15.8
	$CH_2{\cdot}C_6H_4Cl$-*p*	68	161—162 [e]	52.3	6.2	14.2	$C_{13}H_{18}ClN_3O_3$	52.1	6.0	14.0
	$CH_2{\cdot}C_6H_3(OMe)_2$-3,4	75	163—164 [d]	55.1	7.3	13.0	$C_{15}H_{23}N_3O_5$	55.3	7.1	12.9

TABLE 4

α-Aza-amino-acid ethyl esters [f]

Compound [b]	R	Abbreviation [g]	Yield (%) [c]	M.p. (°)	Found (%) C	H	N	Formula	Required (%) C	H	N
NH_2·NR·CO_2Et,HCl	Me [h]	Azala-OEt,HCl	92	94—96	31.2	7.0	18.2	$C_4H_{11}ClN_2O_2$	31.0	7.1	18.1
	$CHMe_2$	Azval-OEt,HCl	85	Oil	39.3	8.5	15.2	$C_6H_{15}ClN_2O_2$	39.6	8.3	15.3
	$CH_2{\cdot}CHMe_2$ [i]	Azleu-OEt,HCl	79	82—83	42.5	8.8	14.4	$C_7H_{17}ClN_2O_2$	42.7	8.7	14.2
	CHMeEt	Azile-OEt,HCl	80	75—77	42.5	8.9	13.9	$C_7H_{17}ClN_2O_2$	42.7	8.7	14.2
	CH_2Ph [j]	Azphe-OEt,HCl	83	141—143	52.1	6.6	12.1	$C_{10}H_{15}ClN_2O_2$	52.0	6.5	12.1
	$CH_2{\cdot}C_6H_4{\cdot}OH$-*p*	Aztyr-OEt,HCl	70	152—155	48.5	6.1	11.6	$C_{10}H_{15}ClN_2O_3$	48.6	6.1	11.3
	$CH_2{\cdot}C_6H_4Cl$-*p*		80	140—142	45.1	5.4	10.3	$C_{10}H_{14}Cl_2N_2O_2$	45.1	5.4	10.3
	$CH_2{\cdot}C_6H_3(OMe)_2$-3,4		80	176—178	49.4	6.6	9.8	$C_{12}H_{19}ClN_2O_4$	49.5	6.5	9.6
Ethyl 3-oxopyrazolidine-1-carboxylate [k]		Azpyr-OEt	85	63—64	45.7	6.5	17.4	$C_6H_{10}N_2O_3$	45.6	6.3	17.7

[a] Prepared from the corresponding t-butyl 3-alkyl- or -aralkyl-carbazate (Table 2) by the general method described in the Experimental section. [b] All compounds are new (the bases corresponding to Azala-, Azleu-, and Azphe-OEt,HCl in Table 4 have been described previously. [c] Yield refers to product with stated constants and analysis. [d] Recrystallised from ethyl acetate–ether. [e] Recrystallised from ethyl acetate–light petroleum (b.p. 60—80°). [f] Except for Azpyr-OEt, all prepared from the corresponding t-butoxycarbonyl-α-aza-amino-acid ethyl ester by the general method described in the Experimental section. [g] Use of abbreviations follows the practice of H. Niedrich *et al.* in ' Peptides 1969,' ed. E. Scoffone, North Holland, Amsterdam, 1971, p. 370. [h] K. Ronco, B. Prijs, and H. Erlenmeyer (*Helv. Chim. Acta*, 1956, **39**, 1253) describe base, b.p. 40° at 0.05 mmHg. [i] K. Ronco, B. Prijs, and H. Erlenmeyer (*Helv. Chim. Acta*, 1956, **39**, 2088) describe base, b.p. 40° at 0.08 mmHg. [j] K. Ronco and H. Erlenmeyer (*Helv. Chim. Acta*, 1956, **39**, 1045) describe base, b.p. 80—85° at 0.01 mmHg. [k] Prepared by ethoxycarbonylation of 3-oxopyrazolidine (J. C. Howard, G. Gever, and P. H. L. Wei, *J. Org. Chem.*, 1963, **28**, 868) by the general method used to prepare *N*-t-butoxycarbonyl-amino-acid ethyl esters (Table 3) (see Experimental section).

equiv.) was added and the mixture was gently stirred overnight. After removal of the dioxan *in vacuo*, the solution was extracted with ethyl acetate. The extracts were washed successively with water, aqueous 20% citric acid, and water, dried (Na_2SO_4), and evaporated, and the solid residue was recrystallised from the solvent indicated in Table 3. The products had R_{FD} 0.62—0.69, R_{FE} 0.24—0.51, R_{FF} 0.6—0.72, R_{FH} 0.54–0.66, R_{FK} 0.73—0.92.

α-Aza-amino-acid Ethyl Ester Hydrochlorides (*Table* 4).—A solution of the *N*-t-butoxycarbonyl-α-aza-amino-acid ethyl ester (Table 3) and anhydrous hydrogen chloride (3 mol. equiv.) in ethyl acetate was kept at 20—24 °C for 1 h. Most of the solvent was removed *in vacuo*, and ether was then added. The hydrochlorides were collected and recrystallised from methanol–ether; they had R_{FD} 0.57—0.73, R_{FE} 0.22—0.60, R_{FF} 0.36—0.68, R_{FH} 0.38—0.65, R_{FK} 0.70—0.92.

1-*Benzyloxycarbonyl*-2-*t-butoxycarbonylhydrazine*.—Benzyl chloroformate (68 g, 400 mmol) was added at 0 °C to a vigorously stirred mixture of t-butyl carbazate (52.8 g, 400 mmol), chloroform (600 ml), sodium hydroxide (18 g, 450 mmol), and water (200 ml). After overnight stirring at ambient temperature, the chloroform layer was separated, washed with water and aqueous 20% citric acid, dried, and evaporated. Crystallisation of the residue from ether–light petroleum gave the *hydrazide* (65 g, 62%), m.p. 79—80°, R_{FD} 0.77, R_{FE} 0.62, R_{FF} 0.74, R_{FH} 0.68, R_{FK} 0.82 (Found: C, 58.7; H, 6.7; N, 10.6. $C_{13}H_{18}N_2O_4$ requires C, 58.6; H, 6.8; N, 10.5%).

1-*Benzyloxycarbonyl*-2-*t-butoxycarbonylpyrazolidine*.—Sodium hydride (4.8 g, 200 mmol) was suspended in dimethylformamide (100 ml) under nitrogen, and a solution of 1-benzyloxycarbonyl-2-t-butoxycarbonylhydrazine (26.6 g, 100 mmol) in dimethylformamide (250 ml) was added slowly with continuous stirring. The mixture was further stirred under nitrogen for 30 min, then a solution of 1,3-dibromopropane (20.2 g, 100 mmol) was added and stirring was continued overnight. The solvent was removed *in vacuo*, and the residue, in ethyl acetate, was washed with water, citric acid solution, saturated aqueous sodium hydrogen carbonate, and water. After removal of the ethyl acetate, the *diacylpyrazolidine* was obtained as an oil (25.5 g, 83.3%), R_{FA} 0.90, R_{FB} 0.83, R_{FC} 0.80, R_{FD} 0.87, R_{FE} 0.87, R_{FH}0.82 (Found: C, 62.9; H, 7.1; N, 9.2. $C_{16}H_{22}N_2O_4$ requires C, 62.7; H, 7.1; N, 9.1%).

1-*Benzyloxycarbonylpyrazolidine Hydrochloride*.—The preceding oil (45.9 g, 150 mmol) was treated with 2N-hydrogen chloride–ethyl acetate (200 ml, 400 mmol) for 45 min at 20—22 °C. The solvent was removed *in vacuo*, and the *hydrochloride* (16 g, 50%) was collected in ether and crystallised from methanol–ether; m.p. 162—164°, R_{FA} 0.61, R_{FB} 0.67, R_{FC} 0.60, R_{FD} 0.64, R_{FE} 0.36, R_{FF} 0.50, R_{FH} 0.50, R_{FK} 0.87 (Found: C, 54.1; H, 6.4; N, 11.5. $C_{11}H_{15}N_2O_2$,HCl requires C, 54.2; H, 6.6; N, 11.5%).

1-*t-Butoxycarbonylpyrazolidine*.—1-Benzyloxycarbonyl-2-t-butoxycarbonylpyrazolidine (30.6 g, 100 mmol) was hydrogenated in 80% aqueous methanol (200 ml) over 5% palladised charcoal (4 g) for 5 h. The catalyst was removed and the filtrate was evaporated, to yield 1-*t-butoxycarbonylpyrazolidine* (10.2 g, 59.3%), b.p. 88—94° at 1.8 mmHg, R_{FD} 0.56, R_{FE} 0.31, R_{FF} 0.43, R_{FH} 0.45, R_{FK} 0.80 (Found: C, 55.7; H, 9.3; N, 16.3. $C_8H_{16}N_2O_2$ requires C, 55.9; H, 9.2; N, 16.5%).

t-Butyl 2-*Methylcarbazate*.—A solution of methylhydrazine (18.4 g, 400 mmol), t-butyl 2,4,5-trichlorophenyl carbonate [18] (118.8 g, 400 mmol), and triethylamine (63.4 ml, 400 mmol) in t-butyl alcohol (150 ml) was stirred overnight at 45—50 °C. Fractional distillation yielded the *carbazate* (35.1 g, 60%), b.p. 80—90° at 6.0 mmHg, R_{FD} 0.62, R_{FE} 0.54, R_{FF} 0.57, R_{FH} 0.54, R_{FK} 0.85 (Found: C, 49.3; H, 9.5; N, 19.1. $C_6H_{14}N_2O_2$ requires C, 49.3; H, 9.5; N, 19.3%)

Benzyl 2-*Methylcarbazate*.—Benzyl chloroformate (68 g, 400 mmol) was added dropwise to an ice-cold solution of methylhydrazine (18.4 g, 400 mmol) and triethylamine (58 ml, 400 mmol) in chloroform (300 ml). The mixture was stirred at 20—22 °C for 2 h, then the chloroform layer was separated, washed with water, dried, and evaporated, to yield the *carbazate* (45 g, 62%), b.p. 98—102° at 0.3 mmHg, R_{FD} 0.65, R_{FE} 0.52, R_{FF} 0.51, R_{FH} 0.52, R_{FK} 0.81 (Found: C, 59.7; H, 6.2; N, 15.4. $C_9H_{12}N_2O_2$ requires C, 60.0; H, 6.6; N, 15.5%).

1-*Benzyloxycarbonyl*-2-*methyl*-2-*t-butoxycarbonylhydrazine*.—To vigorously stirred t-butyl 2-methylcarbazate (36.5 g, 250 mmol), chloroform (250 ml), and sodium hydroxide (10 g, 250 mmol) in water (250 ml), benzyl chloroformate was added dropwise at 0—5 °C. The mixture was stirred overnight at room temperature, then the chloroform layer was separated, washed with water, dried, and evaporated, to provide the *diacylmethylhydrazine* (45 g, 64%), b.p. 162° at 0.8 mmHg, R_{FD} 0.60, R_{FE} 0.55, R_{FH} 0.56, R_{FK} 0.80 (Found: C, 60.2; H, 7.0; N, 9.3. $C_{14}H_{20}N_2O_4$ requires C, 60.0; H, 7.1; N, 9.6%).

Benzyl 3-*Methylcarbazate Hydrochloride*.—A solution of the preceding diacylmethylhydrazine (42 g, 150 mmol) in 2N-hydrogen chloride–acetic acid (225 ml, 450 mmol) was kept at 20—22° for 45 min. Excess of ether was added and the *hydrochloride* (30 g, 93%) was collected, washed with ether, and dried; m.p. 172°, R_{FD} 0.65, R_{FE} 0.43, R_{FF} 0.57, R_{FH} 0.60, R_{FK} 0.81 (Found: C, 50.1; H, 6.4; N, 13.1. $C_9H_{12}N_2O_2$,HCl requires C, 49.9; H, 6.5; N, 12.9%).

Benzyloxycarbonyl-α-aza-prolylglycine Methyl Ester.—A solution of 1-benzyloxycarbonylpyrazolidine hydrochloride (7.3 g, 30 mmol), triethylamine (4.4 ml, 30 mmol). and methyl isocyanatoacetate (3.45 g, 30 mmol) in chloroform (75 ml) was kept at 20—22 °C for 16 h, then washed with water, dried, and evaporated. The residue was crystallised from ethyl acetate–light petroleum, to yield the *aza-dipeptide derivative* (7.7 g, 80%), m.p. 119—120°, R_{FA} 0.83, R_{FD} 0.64, R_{FE} 0.49, R_{FF} 0.62, R_{FH} 0.57, R_{FK} 0.92 (Found: C, 56.2; H, 5.8; N, 13.1. $C_{15}H_{19}N_3O_5$ requires C, 56.0; H, 5.9; N, 13.0%).

2,3,6,7-*Tetrahydro*-1,3-*dioxo*-1H,5H-*pyrazolo*[1,2-a]-[1,2,4]*triazol*-2-*ylacetamide*.—The preceding aza-dipeptide derivative (6.42 g, 20 mmol) in saturated methanolic ammonia (50 ml) was kept overnight at 4 °C. The solvent was removed *in vacuo*, and the residue was crystallised from methanol–ether to yield the *triazole* (3.2 g, 80.8%), m.p. 178—180°, R_{FA} 0.27, R_{FB} 0.50, R_{FC} 0.25, R_{FD} 0.54, R_{FE} 0.07, R_{FF} 0.33, R_{FH} 0.26, R_{FK} 0.67 (Found: C, 42.4; H, 5.2; N, 28.1. $C_7H_{10}N_4O_3$ requires C, 42.4; H, 5.1; N, 28.3%).

N-*Benzyloxycarbonyl-α-aza-alanyl-L-leucine Methyl Ester*.—Methyl 2-isocyanato-4-methylpentanoate (3.42 g, 20 mmol) (b.p. 60—62° at 0.8 mmHg; prepared by the general method of Goldschmidt and Wick [19]) was added to benzyl

[18] W. Broadbent, J. S. Morley, and B. E. Stone, *J. Chem. Soc.* (*C*), 1967, 2632.
[19] S. Goldschmidt and M. Wick, *Annalen*, 1952, **575**, 217.

3-methylcarbazate hydrochloride (4.32 g, 20 mmol) and triethylamine (2.9 ml, 20 mmol) in chloroform (50 ml), and the mixture was kept at 20—22 °C for 16 h. The solvent was removed *in vacuo* and the residue, in ethyl acetate, was washed with water, aqueous 20% citric acid, and water again. The *azadipeptide derivative* (5 g, 85%) was obtained as an oil after removal of the ethyl acetate; R_{FD} 0.62, R_{FE} 0.55, R_{FF} 0.56, R_{FH} 0.58, R_{FK} 0.85 (Found: C, 52.6; H, 5.5; N, 14.1. $C_{13}H_{17}N_3O_5$ requires C, 52.8; H, 5.7; N, 14.2%).

4-*Acetamido*-1-*methyl*-1,2,4-*triazolidine*-3,5-*dione*.— A solution of the preceding azadipeptide derivative (2.95 g, 10 mmol) in saturated methanolic ammonia (50 ml) was kept at 20—22 °C for 5 days. The solvent was removed *in vacuo*, and the residue was crystallised from methanol–ether, to yield the *triazolidine* (1.2 g, 69.9%), m.p. 183—184°, R_{FA} 0.30, R_{FB} 0.50, R_{FC} 0.31, R_{FD} 0.29, R_{FE} 0.11 (Found: C, 34.8; H, 4.5; N, 32.5. $C_5H_8N_4O_3$ requires C, 34.8; H, 4.6; N, 32.5%).

1-*Benzyl*-1-*benzyloxycarbonyl*-2-*t*-*butoxycarbonylhydrazine*. —Benzyl chloroformate (17 g, 100 mmol) was added at 0 °C to a vigorously stirred mixture of t-butyl 3-benzylcarbazate (22.2 g, 100 mmol), chloroform (50 ml), and sodium hydroxide (4.0 g, 100 mmol) in water (25 ml). The resulting mixture was stirred at 20—22 °C for 4 h, then the chloroform layer was separated, washed with water, aqueous 20% citric acid and water, dried, and evaporated, to provide the *benzylhydrazide* (28.5 g, 80%) (from ethyl acetate–light petroleum), m.p. 71—72°, R_{FA} 0.86, R_{FB} 0.78, R_{FC} 0.83, R_{FD} 0.76, R_{FE} 0.73, R_{FF} 0.72, R_{FH} 0.78 (Found: C, 67.5; H, 6.8; N, 8.0. $C_{20}H_{24}N_2O_4$ requires C, 67.4; H, 6.7; N, 7.8%).

Benzyl 2-*Benzylcarbazate Hydrochloride*.—2N-Hydrogen chloride–ethyl acetate (90 ml, 180 mmol) was added to the preceding benzylhydrazide (21.36 g, 60 mmol) in ethyl acetate (75 ml). Anhydrous ether (250 ml) was added after 1 h, and the *hydrochloride* (12.4 g, 71%) was collected, washed with ether, and dried; m.p. 148—149°, R_{FA} 0.75, R_{FB} 0.84, R_{FC} 0.74, R_{FD} 0.70, R_{FE} 0.59, R_{FF} 0.60, R_{FH} 0.72 (Found: C, 61.3; H, 5.8; N, 9.7. $C_{15}H_{16}N_2O_2$,HCl requires C, 61.5; H, 5.8; N, 9.5%).

N-(3-*Benzyl*-3-*benzyloxycarbonylcarbazoyl*)*glycine Methyl Ester*.—A solution of benzyl 2-benzylcarbazate hydrochloride (2.92 g., 10 mmole), methyl isocyanoacetate [20] (1.15 g, 10 mmol), and triethylamine (1.42 ml, 10 mmol) in chloroform (50 ml) was kept at 20—22 °C for 16 h. The chloroform was removed *in vacuo* and the residue, in ethyl acetate, was washed with aqueous 20% citric acid and water. The solution was dried and evaporated, and the residue was crystallised from ethyl acetate–light petroleum, to give the *aza-dipeptide derivative* (3.5 g, 94.5%), m.p. 97—98°, R_{FA} 0.78, R_{FB} 0.72, R_{FC} 0.77, R_{FD} 0.70, R_{FE} 0.59, R_{FF} 0.65, R_{FH} 0.70 (Found: C, 61.3; H, 5.7; N, 11.3. $C_{19}H_{21}N_3O_5$ requires C, 61.5; H, 5.6; N, 11.3%).

4-*Acetamido*-1-*benzyl*-1,2,4-*triazolidine*-3,5-*dione*.— The preceding aza-dipeptide derivative (3.71 g, 10 mmol) in saturated methanolic ammonia was kept at 20—22 °C for 7 days. The solvent was removed *in vacuo*, and the residue was crystallised from methanol–ether to yield the *triazolidine* (1.8 g, 72.6%), m.p. 162—163°, R_{FA} 0.66, R_{FB} 0.64, R_{FC} 0.28, R_{FD} 0.43, R_{FF} 0.15, R_{FK} 0.78 (Found: C, 53.1; H, 5.1; N, 22.8. $C_{11}H_{12}N_4O_3$ requires C, 53.2; H, 4.8; N, 22.5%).

N-(3-*Benzyl*-3-*benzyloxycarbonylcarbazoyl*)-L-*phenylalanine Methyl Ester*.—A solution of benzyl 2-benzylcarbazate hydrochloride (2.92 g, 10 mmol), triethylamine (1.42 ml, 10 mmol), and L-methyl 2-isocyanato-3-phenylpropionate [21] (2.05 g, 10 mmol) in chloroform (25 ml) was kept at 20—22 °C for 16 h. Ethyl acetate (200 ml) was added and the solution was washed with aqueous 20% citric acid and water, dried, and evaporated. Recrystallisation of the residue from ethyl acetate–light petroleum gave the *aza-dipeptide derivative* (3.9 g, 85%), m.p. 81—82°, R_{FA} 0.85, R_{FB} 0.76, R_{FC} 0.77, R_{FD} 0.78, R_{FE} 0.67, R_{FF} 0.59, R_{FH} 0.68 (Found: C, 67.4; H, 5.9; N, 9.2. $C_{26}H_{27}N_3O_5$ requires C, 67.6; H, 5.9; N, 9.1%).

1-*Benzyl*-4-(2-*benzylacetamido*)-1,2,4-*triazolidine*-3,5-*dione*.—The preceding aza-dipeptide derivative (2.3 g, 5 mmol) in saturated methanolic ammonia (50 ml) was kept at 20—22 °C for 15 days. The solvent was removed *in vacuo* and the residue was crystallised from methanol–ether, to yield the *triazolidine* (1.1 g, 65%), m.p. 190—192°, R_{FA} 0.75, R_{FB} 0.69, R_{FC} 0.38, R_{FD} 0.59, R_{FF} 0.30, R_{FH} 0.25, R_{FK} 0.86 (Found: C, 63.7; H, 5.5; N, 16.8. $C_{18}H_{18}N_4O_3$ requires C, 63.9; H, 5.3; N, 16.5%).

N-*t*-*Butoxycarbonyl*-α-*aza-dipeptide Methyl Esters*.—A solution of t-butyl 3-benzylcarbazate (11.1 g, 50 mmol) and L-methyl 2-isocyanato-4-methylpentanoate (8.55 g, 50 mmol) in tetrahydrofuran (70 ml) was kept at 20—22 °C for 16 h. The solvent was removed *in vacuo* and the residue, in ethyl acetate, was washed with aqueous 20% citric acid and water. The solid obtained by evaporation was crystallised from ethyl acetate–light petroleum, to yield N-*t*-*butoxycarbonyl*-α-*aza-phenylalanyl*-L-*leucine methyl ester* (18 g, 91.8%), m.p. 91—92°, $[\alpha]_D^{25}$ −23.2° (*c* 2.3 in MeOH), R_{FD} 0.80, R_{FE} 0.68, R_{FF} 0.70, R_{FH} 0.70, R_{FK} 0.93 (Found: C, 61.2; H, 7.8; N, 10.6. $C_{20}H_{31}N_3O_5$ requires C, 61.1; H, 7.9; N, 10.6%). Likewise, use of t-butyl 3-isobutylcarbazate gave N-*t*-*butoxycarbonyl*-α-*aza-leucyl*-L-*leucine methyl ester* (81%), m.p. 72—73° (from ether–light petroleum), R_{FD} 0.63, R_{FE} 0.57, R_{FF} 0.58, R_{FH} 0.61 (Found: C, 54.5; H, 9.2; N, 11.6. $C_{17}H_{33}N_3O_5$ requires C, 54.5; H, 9.0; N, 11.5%), and t-butyl 3-isobutylcarbazate and methyl 2-isocyanato-3-methylbutyrate [22] with ether as solvent gave N-*t*-*butoxycarbonyl*-α-*aza-leucyl*-L-*valine methyl ester* (79%), m.p. 77—78° (from ether–light petroleum), R_{FD} 0.76, R_{FE} 0.68, R_{FF} 0.70, R_{FH} 0.70 (Found: C, 55.5; H, 9.2; N, 12.2. $C_{16}H_{31}N_3O_5$ requires C, 55.6; H, 9.0; N, 12.1%).

1,2,4,5-*Tetrahydro*-1,2,4-*triazine*-3,6-*diones*.—(*a*) *N*-t-Butoxycarbonyl-α-aza-phenylalanine-L-leucine methyl ester (3.93 g, 10 mmol) in 2N-hydrogen chloride–ethyl acetate (15 ml, 30 mmol) was kept for 1 h at 20—25 °C. The solvent was removed *in vacuo* to leave a solid foam. After 4 months at room temperature, this had changed into a white solid, and t.l.c. indicated complete conversion into a second product. Recrystallisation from methanol–ether gave 2-*benzyl*-1,2,4,5-*tetrahydro*-5-*isobutyl*-1,2,4-*triazine*-3,6-*dione* (1.8 g, 69.2%), m.p. 211—212°, R_{FD} 0.60, R_{FE} 0.40, R_{FF} 0.56, R_{FH} 0.51, R_{FK} 0.95 (Found: C, 64.2; H, 7.0; N, 15.8. $C_{14}H_{19}N_3O_2$ requires C, 64.3; H, 7.3; N, 16.0%).

(*b*) *N*-t-Butoxycarbonyl-α-aza-leucyl-L-leucine methyl ester (0.72 g, 2 mmol) in 2N-hydrogen chloride–ethyl acetate (3 ml, 6 mmol) was kept for 1 h at 20—25 °C. Ether was added, and the hydrochloride was collected and then shaken with ethyl acetate and aqueous sodium hydrogen carbonate.

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The organic layer was separated, washed with water, dried, and evaporated, to yield oily α-aza-leucyl-L-leucine methyl ester, R_{FD} 0.70, R_{FE} 0.59, R_{FF} 0.63, R_{FH} 0.61. A solution of this ester in aqueous 5% acetic acid was heated on a steam-bath for 10 h. The solvent was removed *in vacuo*, and the residue was crystallised from methanol, to give *1,2,4,5-tetrahydro-2,5-di-isobutyl-1,2,4-triazine-3,6-dione* (0.32 g, 70%), m.p. 242—243°, R_{FD} 0.69, R_{FE} 0.42, R_{FF} 0.75, R_{FH} 0.63, R_{FK} 0.91 (Found: C, 58.2; H, 9.2; N, 18.5. $C_{11}H_{21}N_3O_2$ requires C, 58.1; H, 9.3; N, 18.5%). Likewise, *N*-t-butoxycarbonyl-α-aza-leucyl-L-valine methyl ester gave *1,2,4,5-tetrahydro-2-isobutyl-5-isopropyl-1,2,4-triazine-3,6-dione* (72%), m.p. 233—234°, R_{FD} 0.67, R_{FE} 0.35, R_{FF} 0.68 R_{FH} 0.61 (Found: C, 56.2; H, 9.1; N, 19.6. $C_{10}H_{19}N_3O_2$ requires C, 56.4; H, 8.9; N, 19.7%).

3,6-Di-isobutylpiperazine-2,5-dione.— *N*-Benzyloxycarbonyl-L-leucyl-L-leucine methyl ester [23] (196 mg, 0.5 mmol) in methanol (25 ml) was hydrogenated at room temperature and pressure over 5% palladised carbon (50 mg) for 4 h. The solution was filtered and the filtrate was heated under reflux for 5 h. T.l.c. indicated a single, ninhydrin-positive component (L-leucyl-L-leucine methyl ester) before the refluxing, and complete conversion into a ninhydrin-negative component afterwards (under similar conditions, α-aza-leucyl-L-leucine methyl ester was unchanged). The methanol was removed, and the residue was recrystallised from methanol–ether, to give the piperazine (80 mg, 71%), m.p. 277—278° (lit.,[24] 278°), R_{FA} 0.69, R_{FB} 0.71, R_{FC} 0.67, R_{FD} 0.62, R_{FE} 0.30, R_{FH} 0.59 (Found: C, 63.5; H, 9.6; N, 12.5. Calc. for $C_{12}H_{22}N_2O_2$: C, 63.7; H, 9.7; N, 12.3%).

α-Aza-pyroglutamic Acid 2,4,5-Trichlorophenyl Ester.— 2,4,5-Trichlorophenyl chloroformate (5.28 g, 20 mmol) was added to pyrazolidin-3-one hydrochloride (2.44 g, 20 mmol) and triethylamine (5.8 ml, 40 mmol) in chloroform (50 ml) at 0—4 °C. The solution was stirred overnight at 0—4 °C, then washed with water, dried, and evaporated. The solid residue was recrystallised from ethyl acetate–light petroleum, to yield the *trichlorophenyl ester* (5.1 g, 82.5%), m.p. 157—158° (Found: C, 39.0; H, 2.2; Cl, 34.4; N, 9.1. $C_{10}H_7Cl_3N_2O_3$ requires C, 38.8; H, 2.2; Cl, 34.3; N, 9.0%).

N-t-Butoxycarbonyl-α-aza-phenylalanine 2,4,5-Trichlorophenyl Ester.—2,4,5-Trichlorophenyl chloroformate (5.28 g, 20 mmol) was added to t-butyl 3-benzylcarbazate (4.44 g, 20 mmol) and triethylamine (2.9 ml, 20 mmol) in chloroform (50 ml) at 0—4 °C. The solution was stirred overnight at 0—4 °C, then washed with aqueous citric acid and water, dried, and evaporated. Recrystallisation of the residue from cyclohexane gave the *trichlorophenyl ester* (6.8 g, 76.4%), m.p. 136—137° (Found: C, 51,1; H, 4.1; Cl, 23.6; N, 6.2. $C_{19}H_{19}Cl_3N_2O_4$ requires C, 51.2; H, 4.2; Cl, 23.8; N, 6.2%).

[5/598 *Received*, 2*nd April*, 1975]

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