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PREPARATION AND PROPERTIES OF SOME α-AZA-AMINO-ACID DERIVATIVES, THEIR POSSIBLE USE IN PEPTIDE SYNTHESIS

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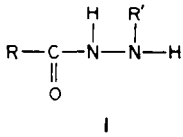
Abstract—Some derivatives of azaglycine and azaphenylalanine are described. Esters of acetyl- and benzoyl-aza-amino-acids rapidly cyclise to stable oxadiazolones and it is concluded that similar derivatives would be unsuitable for aza-peptide synthesis. t-Butyloxycarbonyl-azaglycine azide was too unreactive for use in peptide synthesis. Benzoyl-azaglycylphenylalanine ethyl ester and acetyl-azaphenylalanylphenylalanine ethyl ester were prepared by coupling benzoyl hydrazide and N-acetyl-N′-benzylhydrazide respectively with 2,4-dinitrophenyloxycarbonyl-phenylalanine ethyl ester.

The synthesis of analogues of biologically-active peptides, and the study of their properties, has yielded a great deal of information about structure-function relationships in this class of compounds.[1-3] Most commonly, the analogues have been prepared by replacing one or more amino-acid residues by other similar natural residues,[1-3] by residues of unusual stereochemical configuration,[4,5] or by residues carrying unusual side-chain groups.[6] Recently there has been an increase in interest in aza-amino-acid derivatives, i.e. amino-acid analogues in which the α-carbon is replaced by nitrogen, and in peptides which incorporate such analogues. For example, analogues of angiotensin II,[7] oxytocin[8] and eledoisin[9] have been prepared in which natural amino-acid residues have been replaced by azavaline, azaasparagine and azaglycine residues.

From the work of Gante[10] it is clear that, not unexpectedly, the chemistry of aza-amino-acid derivatives differs in certain important respects from that of derivatives of natural amino-acids. For this reason it was apparent that if peptides containing such analogues were to be prepared, modified synthetic routes might be required.

More recently, Dutta and Morley[11] have reported the preparation of intermediates of use in the introduction into peptides of several aza-amino-acid residues. They found most valuable the t-butyl 3-alkyl- or aralkyl carbazates obtained indirectly from t-butyl carbazate.

Our interest in these amino-acid analogues has been directed more towards the potential of certain of their derivatives as enzyme inhibitors and we have already reported the interaction of chymotrypsin and trypsin with derivatives of azaphenylalanine[12] and azaornithine.[13] In connection with this work we have had occasion to synthesise some new aza-amino-acid derivatives and to investigate some of their properties. This paper reports our results.



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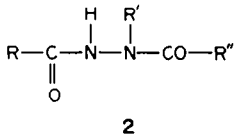
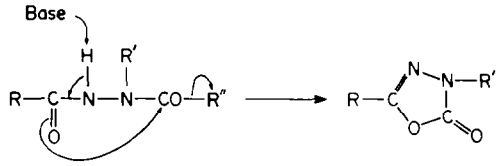


2

α-Aza-amino-acid derivatives (e.g. **2**) are substituted carbazates and as such the free acids (**2**; R″ = OH) cannot be prepared; spontaneous decarboxylation occurs giving the parent hydrazide **1**. The usual method for preparing aza-amino-acid esters therefore is by treatment of the hydrazide **1** with appropriate alkyl or aryl chloroformates under basic conditions. Ethyl, phenyl and benzyl chloroformates are readily available and their use allowed us to prepare the corresponding esters of azaglycine and azaphenylalanine derivatives (see Table 1 and Experimental) in this way. The 2,4-dinitrophenyl ester of benzoyl-azaglycine was prepared in a similar manner. In this case bis(2,4-dinitrophenyl)carbonate was used in place of a chloroformate.

The most striking feature of the chemistry of the acetyl- and benzoyl-aza-amino-acid esters is the ease with which they undergo cyclisation and elimination to give oxadiazolones. Thus, benzoyl-azaglycine phenyl ester (**3**; R = Ph, R′ = H, R″ = OPh) on treatment with ethanolic NaOH readily yielded 2 - phenyl - 1,3,4 - oxadiazol - 5 - one (**4**; R = Ph, R′ = H).



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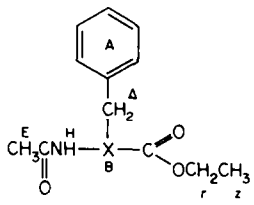
The same product, and not the expected amide, was obtained when the 2,4-dinitrophenyl ester of benzoyl-azaglycine [**3**; R = C_6H_5, R′ = H, R″ = $OC_6H_3(NO_2)_2$] was treated with ammonia. Indeed, this dinitrophenyl ester appeared to be so unstable that it decomposed to the oxadiazolone even on repeated attempts at recrystallisation and column chromatography. In the mass spectrometer the oxadiazolone showed largest mass peak at 162, as anticipated. It is interesting that benzoyl-azaglycine phenyl ester itself showed this same mass peak and none higher in its mass spectrum indicating that decomposition and cyclisation occurred even during the heating of the sample in the instrument.

Similarly the oxadiazolone derived from acetyl-azaphenylalanine (2 - methyl - 4 - benzyl - 1,3,4 - oxadiazol - 5 - one, **4**; R = CH_3, R′ = CH_2Ph) was obtained when

Table 1. Chemical shifts of absorptions in the NMR spectra of N - acetyl - L - phenylalanine ethyl ester (X=CH) and N - acetyl - α - azaphenylalanine ethyl ester (X=N)



Hydrogen	Chemical shift (τ-scale; $CDCl_3$) X=CH	X=N
A	2.8	2.7
B	5.15	—
Γ	5.86	5.85
Δ	6.91	5.35
E	8.07	8.12
Z	8.77	8.76
H	3.91	2.08

acetyl-azaphenylalanine phenyl ester was treated with hydroxide, with hydroxylamine and also even when this ester was incubated at pH 7.0 at 37° for several days. Attempts by Kurtz and Niemann[18] to distil the ethyl ester of acetyl-azaphenylalanine also gave rise to this same oxadiazolone.

1 - Phenyl - 4 - benzyl - 1,3,4, - oxadiazol - 5 - one (**4**; $R = C_6H_5$, $R' = CH_2C_6H_5$) was produced when benzoyl-azaphenylalanine phenyl ester (**2**; $R = C_6H_5$, $R' = CH_2C_6H_5$, $R'' = OC_6H_5$) was treated with bases, including benzylamine or ammonia which were used in attempts to prepare the corresponding amides.

The formation of oxadiazolones from aza-amino-acid derivatives is analogous to that of azlactones (oxazol-5-ones) from natural amino-acid derivatives. These oxazolones have been shown[14,15] to be intermediates in a number of reactions involving amino-acid derivatives and peptides, and a significant feature of their chemistry is that they rapidly undergo ring opening in the presence of nucleophiles which attack at the 5-carbonyl position. The oxadiazolones by contrast are extremely stable. For example, 2 - phenyl - 1,3,4 - oxadiazol - 5 - one (**3**; R = Ph, R' = H) did not undergo ring opening even on refluxing with ethanolic sodium hydroxide. Gante,[10] who described attempts to use oxadiazolones as intermediates in the synthesis of peptides containing aza-amino-acid analogues, reported that temperatures of 145° were required for nucleophilic attack by amines to take place.

The greater stability of oxadiazolones relative to oxazolones is attributable to the presence of the α-nitrogen which renders the carbonyl group less susceptible to nucleophilic attack. This factor alone, however, might be expected to make the formation of the cyclic structure correspondingly more difficult too. In this respect another effect is worth noting. The cyclisation process, for both oxazolone and oxadiazolone, requires abstraction of the hydrogen carried by the nitrogen adjacent to the α-position (see **3**). Comparison of the NMR spectrum of N - acetyl - α - azaphenylalanine ethyl ester with that of N - acetyl - L - phenylalanine ethyl ester (Table 1) shows that the chemical shifts are most different in the cases of the protons attached to atoms adjacent to the α-atom. The effect of the α-nitrogen is to draw electrons from these positions resulting in deshielding and lower chemical shifts. This withdrawal of electrons would be expected to increase the acidity of the N–H and thus lead to cyclisation more readily than might otherwise be expected. Further, in the case of the aza-analogue it was observed that this N–H exchanged rapidly with D_2O, while in the case of the α-carbon compound, exchange was extremely slow. This finding confirms that the N–H is more acidic in the aza-analogue than in the natural derivative. However from a close examination of the NMR spectrum of N-acetyl-azaphenylalanine ethyl ester which had been recorded in the presence of D_2O, no evidence was found for oxadiazolone formation accompanying the exchange, suggesting that loss of this proton and cyclisation are two separate steps, and that a good leaving group (e.g. O-phenyl) must be present for rapid cyclisation under these mild conditions.

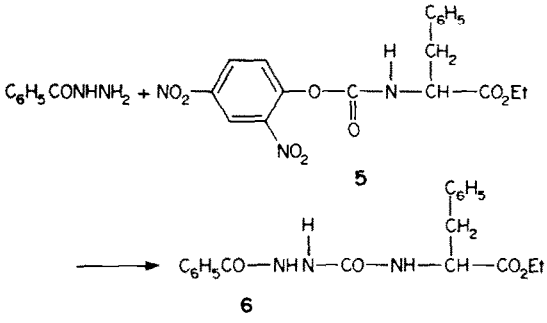
The ready, and apparently unavoidable, formation of oxadiazolones which occurs when these acyl-aza-amino-acid esters are treated with even mild base means that they are likely to be totally unsatisfactory as intermediates in peptide synthesis. Indeed an attempt to prepare benzoyl - α - azaglycylphenylalanine ethyl ester from phenylalanine ethyl ester and benzoyl-azaglycine 2,4-dinitrophenyl ester failed completely; the oxadiazolone (**4**; R = Ph, R' = H) was produced and phenylalanine ethyl ester recovered.

Oxazolone formation is prevented in derivatives of natural amino acids when the acyl derivative is of the urethan (i.e. aryl- or alkyl-oxycarbonyl) type. Thus benzyloxycarbonyl- and t-butyloxycarbonylamino acid esters are resistant to racemisation (since they do not undergo oxazolone formation).[14] The urethan group appears also to prevent ring formation in aza-amino-acid derivatives. For example, t-butyloxycarbonyl-azaglycine ethyl ester is stable to 1 M sodium hydroxide, being recovered unchanged even after prolonged exposure. Hydrazine also had no effect on this ethyl ester. The presence of the nitrogen atom in the α-position makes this ester very stable to nucleophilic attack and hydrolysis, as expected. The corresponding phenyl ester is considerably more stable than the phenyl ester of a natural amino acid (for example, no amide was produced on treatment with concentrated aqueous ammonia at room temperature). However, it is sufficiently reactive to undergo replacement reactions under more forcing conditions; treatment with ammonia solution under reflux gave t - butyloxycarbonyl - azaglycineamide [**2**; $R = (CH_3)_3C\text{–}O$, R' = H, $R'' = NH_2$]. Similarly hydrazine gave rise to the hydrazide [**2**; $R = (CH_3)_3C\text{–}O$, R' = H, $R'' = NHNH_2$].

Since the azide method has long been successfully used in peptide synthesis, we considered whether aza-amino-acid azides might be useful intermediates for the preparation of aza-peptides. t-Butyloxycarbonyl-azaglycine azide [**2**; $R = (CH_3)_3C\text{–}O$, R' = H, $R'' = N_3$] was prepared by treatment of the corresponding hydrazide with amyl nitrite in dioxan containing hydrogen chloride. However it became clear that the azide was not sufficiently reactive to be useful for peptide synthesis. For example, attempts to perform coupling reactions with diethylamine, phenylalanine ethyl ester, benzylamine and glycine t-butyl ester under the mild conditions usually associated with peptide synthesis were totally unsuccessful, unreacted azide being recovered. Benzylamine did react, over 2 h at 70°, to give the benzylamide [**2**, $R = (CH_3)_3C\text{–}O$, R' = H, $R'' = NHCH_2Ph$].

For our work on enzyme inhibitors we wished to

prepare benzoyl - azaglycylphenylalanine ethyl ester. It has been stated above that this dipeptide analogue could not be prepared by coupling phenylalanine ethyl ester with an activated benzoyl-azaglycine ester. An alternative method was therefore used. This involved treatment of phenylalanine ethyl ester with bis(2,4 - dinitrophenyl)carbonate to give 2,4 - dinitrophenyloxycarbonylphenylalanine ethyl ester **5**. Condensation of **5** with benzoyl hydrazide readily gave the required protected peptide ester **6**.



In a similar manner, N - acetyl - N′ - benzylhydrazide reacted with **5** to give acetyl-azaphenylalanyl-phenylalanine ethyl ester.

This method is similar to the use by Hess *et al.*,[7] Gante,[10] and Dutta and Morley,[11] of α-isocyanato derivatives and the use by Gante[10] of *p*-nitrophenyloxycarbonyl derivatives, and we found it to be extremely convenient, especially in view of the easy preparation of the bis - (2,4 - dinitrophenyl)carbonate fom a readily-accessible starting material.

EXPERIMENTAL

TLC was carried out by using Merck ("Kieselgel G nach Stahl" with the following solvent systems; A: EtOH:$CHCl_3$ (2:98); B, EtOH:$CHCl_3$ (5:95); C, hexane:EtOAc (20:80); D, hexane:EtOAc (50:50); E, hexane:EtOAc (70:30); F, hexane:EtOAc (75:25); G, Et_2O:EtOAc (75:25) or MN Silica Gel G with the following solvent systems; H, water:n-butanol:acetic acid (5:4:1); I, EtOH:$CHCl_3$ (10:90); J, EtOH:$CHCl_3$ (5:95); K, EtOH:$CHCl_3$ (30:70). Components were detected by using iodine vapour, ninhydrin or the chlorine spray of Pan and Dutcher.[16] M.ps are uncorrected and are given in the Table 2, along with R_f values and analyses where appropriate.

General method for preparation of aza-amino-acid ester derivatives. Substituted hydrazide **1** in EtOAc containing triethylamine (1 molar proportion) was treated at 0° with a solution of alkyl or aryl chloroformate (1.05 molar proportion) in EtOAc. The mixture was stirred at 0° for 1–2 h then washed with water, 0.1 M Na_2CO_3, 0.5 M citric acid and water, dried and evaporated. Where appropriate products were chromatographed and recrystallised.

Benzoyl-azaglycine 2,4-dinitrophenyl ester (**2**; R = C_6H_5, R′ = H, R″ = $OC_6H_3(NO_2)_2$)

Benzoylhydrazide (3.0 g) in EtOAc (120 ml) was added to a stirred slurry of bis - (2,4 - dinitrophenyl)carbonate (8.7 g) in EtOAc (30 ml). The mixture was then stirred for 75 min and then filtered. Petrol (b.p. 40–60°) was added to the filtrate and the resulting precipitate was filtered off and recrystallised from hexane-ethyl acetate (yield, 1.0 g).

t - Butyloxycarbonyl - azaglycylhydrazide (**2**; R = $(CH_3)_3C$–O, R′ = H, R″ = $NHNH_2$). This was obtained by treatment of the corresponding phenyl ester (2.0 g) with hydrazine hydrate (2.5 ml) in methanol (35 ml) at 60°. After standing overnight the mixture was evaporated to dryness and the residue kept *in vacuo* over conc. H_2SO_4. The residue was washed with ether and subsequently extracted with warm EtOAc (25 ml). Addition of hexane to the solution gave a precipitate which was recrystallized from hexane-ethyl acetate (yield 0.52 g).

t - Butyloxycarbonyl - azaglycine azide (**2**; R = $(CH_3)_3$–C–O, R′ = H, R″ = N_3). HCl in dioxan (0.71 M, 0.74 ml), followed by amyl nitrite (0.07 ml), was added to t - butyloxycarbonyl - azaglycylhydrazide (0.1 g) in dioxan (25 ml) stirred at 11°. After 15 min water (75 ml) was added. The solution was extracted with CH_2Cl_2 (4 × 25 ml) and the extracts dried ($MgSO_4$) and evaporated to give an oil (R_f : 0.59). The IR spectrum (liquid film) showed among others an absorption at 2150 cm^{-1} (azide).

Table 2. Derivatives of azaglycine (**2**; R′ = H) and azaphenylalanine (**2**; R′ = $CH_2C_6H_5$)

				Found (%)				Required (%)		
R	R″	m.p.°	R_f	C	H	N	Formula	C	H	N
Azaglycine (R′ = H)										
C_6H_5	OC_6H_5	147–148	0.28$_J$	65.9	4.6	10.7	$C_{14}H_{12}N_2O_3$	65.6	4.7	10.9
C_6H_5	$OC_6H_3(NO_2)_2$	90–93	0.29$_A$	49.4	3.2	15.4	$C_{14}H_{10}N_4O_7$	48.6	2.9	16.2
C_4H_9O†	OC_2H_5	92–93	0.50$_J$	47.2	7.6	13.8	$C_8H_{16}N_2O_4$	47.1	7.8	13.7
C_4H_9O	OC_6H_5	123–124	0.56$_J$	56.9	6.2	11.3	$C_{13}H_{16}N_2O_4$	57.1	6.35	11.1
C_4H_9O	$OCH_2C_6H_5$	73–75	0.65$_J$	59.1	6.5	10.4	$C_{13}H_{18}N_2O_4$	58.6	6.8	10.5
C_4H_9O	NH_2	150–152	0.17$_A$	41.0	7.2	23.7	$C_6H_{13}N_3O_3$	41.1	7.4	24.0
C_4H_9O	$NHNH_2$	130–132	0.13$_K$	38.2	7.2	29.7	$C_6H_{14}N_4O_3$	37.9	7.4	29.5
C_4H_9O	N_3	oil	0.59$_I$							
C_4H_9O	$NHCH_2C_6H_5$	114–115	0.62$_I$	59.0	7.3	15.6	$C_{13}H_{19}N_3O_3$	58.9	7.2	15.8
C_6H_5	$NH{-}\underset{\displaystyle CH_2C_6H_5}{\underset{\vert}{CH}}{-}CO_2Et$	137–138	0.46$_C$	64.1	5.9	12.0	$C_{19}H_{21}N_3O_4$	64.2	5.9	11.8
Azaphenylalanine (R′ = $CH_2C_6H_5$)										
CH_3	OC_6H_5[12]	109–110	0.79$_G$	67.6	5.8	10.2	$C_{16}H_{16}N_2O_3$	67.6	5.6	9.9
CH_3	OC_2H_5[18]	oil	0.60$_G$							
C_6H_5	OC_6H_5	134–136	0.50$_J$	72.9	5.1	8.4	$C_{21}H_{18}N_2O_3$	72.8	5.2	8.1
C_6H_5	OC_2H_5	88–89	0.43$_J$	68.7	6.2	9.6	$C_{17}H_{18}N_2O_3$	68.4	6.0	9.4
C_4H_9O	OC_6H_5[12]	109–111	0.66$_J$	66.6	6.7	8.5	$C_{19}H_{22}N_2O_4$	66.7	6.4	8.2
C_4H_9O	OC_2H_5	61–63	0.50$_J$	61.2	7.4	9.6	$C_{15}H_{22}N_2O_4$	61.2	7.5	9.5
CH_3	$NH{-}\underset{\displaystyle CH_2C_6H_5}{\underset{\vert}{CH}}{-}CO_2Et$	118–120	0.58$_C$	65.4	6.8	10.6	$C_{21}H_{25}N_3O_4$	65.8	6.5	11.0

†C_4H_9O refers to t-butyloxy.

t - Butyloxycarbonyl - azaglycine benzylamide (2; R = $(CH_3)_3$–C–O, R′ = H, R″ = $NHCH_2C_6H_5$). The azide as above was prepared using 0.5 g of hydrazide. To the CH_2Cl_2 solution was added benzylamine (1.0 ml) and the mixture heated at 70° for 1 h, then at 80° for 1 h. Water (100 ml) was added and the mixture extracted with CH_2Cl_2 (4 × 50 ml). The extracts were washed with 1 M citric acid (4 × 50 ml) and water (4 × 50 ml), dried ($MgSO_4$) and evaporated to give a solid which was recrystallised to give the amide from hexane–benzene (yield 0.33 g), R_{f_1} 0.62.

2 - *Methyl* - 4 - *benzyl* - 1,3,4 - *oxadiazol* - 5 - *one* (4; R = CH_3, R′ = $CH_2C_6H_5$). (a) NaOH solution (0.56 M, 2.5 ml) was added to a solution of acetyl - azaphenylalanine phenyl ester (0.40 g) in methanol (20 ml) and the solution left at room temp. for 75 min. HCl solution (0.56 M, 2.5 ml) was added and the solution evaporated to small volume and then extracted with ether (3 × 10 ml). The extracts were dried ($MgSO_4$) and evaporated to give a brown oil which was then dissolved in ether (25 ml) and the ether solution washed with NaOH solution (0.1 M, 2 × 25 ml) and water (25 ml), dried and evaporated to give 2 - methyl - 4 - benzyl - 1,3,4 - oxadiazol - 5 - one as an oil. TLC R_{f_E} 0.38 (UV and iodine positive, chlorine negative. MN Kieselgel G/UV, R_{f_D} 0.66, R_{f_B} 0.63. IR γ_{max} (CCl_4) 1800 (C=O) and 1640 cm^{-1} (C=N) (Kurtz and Niemann[18] quote γ_{max} (CCl_4) 1800 and 1640 cm^{-1}), γ_{max} (CH_2Cl_2) 1770 and 1630 cm^{-1}. There was no absorption corresponding to N–H. NMR ($CDCl_3$) 2.7 (multiplet, 5 protons: aromatic), 5.22 (singlet, 2 protons: CH_2–Ph) and 7.8τ (singlet, 3 protons: CH_3–CO).

(b) A mixture of acetyl-azaphenylalanine phenyl ester (170 mg), NH_2OH. HCl (50 mg) and triethylamine (1.10 cm^3) in ethanol (2.5 ml) was kept at 37° for 21 h. The solution was evaporated to dryness and the residue dissolved in water (1 ml). This solution was made alkaline with NaOH solution and extracted with $CHCl_3$. The extracts were dried and evaporated to dryness to give an oil (70 mg) which was shown by TLC to contain, among other compounds, phenol. The material was chromatographed on a column (10 mm diameter) of silica gel (8 g). Elution with EtOAc–hexane gave a fraction containing two components (R_{f_D} 0.66 and 0.79) the latter identified as phenol. The slower running component although contaminated by phenol was identified as the oxazolone by comparison of its behaviour on TLC, and its NMR and IR spectra (CH_2Cl_2), with the material from (a) above. The mass spectrum showed a top mass of 190.

(c) Acetyl-azaphenylalanine phenyl ester (10.2 mg) in 0.05 M phosphate buffer, pH 7.0 (1.0 ml) containing methanol (0.1 ml) was incubated at 37° for 16 h. The mixture was then examined by TLC and shown to contain both phenol and the oxadiazolone.

2 - *Phenyl* - 1,3,4 - *oxadiazol* - 5 - *one* (4; R = C_6H_5, R′ = H)

(a) A solution of benzoyl-azaglycine 2,4-dinitrophenyl ester (0.40 g) in ethanol (137 ml) and ammonia solution (s.g. 0.88, 23 ml) was left at room temp. for 45 min and then evaporated to dryness. EtOAc (500 ml) was added and the resulting solution filtered to remove insoluble 2,4-dinitrophenol. The filtrate was evaporated to leaving a yellow residue which was recrystallised from hexane-ethyl acetate (yield 0.06 g, 32%) m.p. 132–135° (lit.[17] 138°) TLC R_{f_P} 0.17 (Cl_2 positive) and R_{f_1} 0.0 (yellow, trace, 2,4-dinitrophenol).

(b) An attempt to purify a sample (281 mg) of crude (m.p. 85–90°) benzoyl-azaglycine 2,4-dinitrophenyl ester by recrystallisation from ethyl acetate-hexane gave a small amount (27 mg) of a solid (m.p. 126–128°), probably the oxadiazolone. Column chromatography of the residue on two columns of Kieselgel 60 using ethyl acetate and ethyl acetate : ethanol (93 : 7) as eluants afforded 88 mg of a solid which was recrystallized from ethyl acetate : hexane to give 2 - phenyl - 1,3,4 - oxadizaol - 5 - one as pale yellow needles, m.p. 134–135° (Found: C, 59.0; H, 3.7; N, 17.6. Calc. for $C_8H_6N_2O_2$: C, 59.3; H, 3.7; N, 17.3%).

(c) NaOH solution (1.0 M, 1.0 ml) was added to a solution of benzoyl-azaglycine phenyl ester (56.7 mg) in ethanol (1 ml). After 1 h at room temp., the solution was acidified. Water (5 ml) was added and the mixture was evaporated to a small volume under reduced pressure whereupon a colourless solid was deposited (19.7 mg, m.p. 134–135°). The IR (Nujol mull) was identical to those from the products of (a) and (b) above.

2 - *Phenyl* - 4 - *benzyl* - 1,3,4 - *oxadiazol* - 5 - *one* (4; R = C_6H_5, R′ = $CH_2C_6H_5$). (a) Benzylamine (0.30 ml) was added to a solution of benzoyl-azaphenylalanine phenyl ester (0.20 g) in ethanol (20 ml) and the mixture left at room temp. for 18 h when it was evaporated to give a residue which was dissolved in $CHCl_3$ (50 ml). This solution was washed with HCl solution, NaOH solution, and water, dried and evaporated to give the oxadiazolone which was recrystallised from hexane-EtOAc, (yield, 0.11 g, 74%) m.p. 114–115°. TLC R_{f_1} 0.90 (iodine positive). (Found: C, 71.6; H, 4.8; N, 11.1. $C_{15}H_{12}N_2O_2$ requires C, 71.4; H, 4.8; N, 11.1).

(b) Benzoyl-azaphenylalanine phenyl ester (0.15 g) was treated with ammonia solution (s.g. 0.88, 0.08 ml), in methanol for 18 h, and yielded 0.1 g of the oxadiazolone shown by TLC and IR to be identical with the above material.

(c) Benzoyl-azaphenylalanine phenyl ester (0.15 g) in ethanol (15 ml) was treated with NaOH solution (0.5 M, 1.0 ml) for 18 h and yielded 0.082 g of the oxadiazolone shown by TLC and IR to be identical with the above material.

Benzoyl-azaglycine phenyl ester (2; R = C_6H_5, R′ = H, R″ = OC_6H_5). A solution of t - butyloxycarbonyl - azaglycine phenyl ester [2; R = $(CH_3)_3C$–O, R′ = H, R″ = OC_6H_5; prepared as described in the general method from t-butyl carbazate and phenyl chloroformate: 2.0 g] in trifluoroacetic acid (14 ml) was left at room temp. for 1 h, and them evaporated to dryness. Water (40 ml) was added and the pH adjusted to 8.0 with $NaHCO_3$ solution. This solution yielded to CH_2Cl_2 (2 × 50 ml) a solid which was reprecipitated from EtOAc by the addition of petrol (b.p. 60–80°). The product, azaglycine phenyl ester (0.63 g) was almost pure on TLC (R_{f_1} 0.36; iodine positive) with a trace of starting material (R_{f_1} 0.53).

Benzoyl chloride (0.28 g) was added slowly to a solution of azaglycine phenyl ester (0.3 g) and triethylamine (0.27 ml) in $CHCl_3$ (40 ml) at 0°. After 1 h at room temp. the solution was washed with HCl solution (5%, 2 × 25 ml), $NaHCO_3$ solution (5%, 2 × 25 ml) and water, dried and evaporated yielding a solid which was recrystallised from aqueous ethanol to give benzoyl-azaglycine phenyl ester, m.p. 147–148°, R_{f_1} 0.28.

t - Butyloxycarbonyl - azaglycineamide [2; R = $(CH_3)_3$–O–, R′ = H, R″ = NH_2]. t - Butyloxycarbonyl - azaglycine phenyl ester [2; R = $(CH_3)_3$–O–, R′ = H, R″ = OC_6H_5: 2.0 g] was heated under reflux with ammonia solution (s.g. 0.88, 10 ml) for 45 min. The mixture was evaporated to dryness and then dissolved in 1 M HCl (75 ml). The solution was extracted with ether (3 × 50 ml) and the aqueous solution evaporated to dryness. This residue was dissolved in ethanol (50 ml), any insoluble material being filtered off, and the solution evaporated to give a solid which was recrystallised from hexane-ethyl acetate (yield 0.4 g).

Bis-(2,4-dinitrophenyl)carbonate. Diphenyl carbonate (20 g) was added to a stirred mixture of conc. HNO_3 (100 ml) and conc. H_2SO_4 (120 ml) at 50–55° over 1 h. The resulting suspension was stirred at 50° for 3 h, left at room temp. for 90 h and then poured onto a large excess of ice. The precipitate was collected, washed with iced water until the washings were neutral and then dried *in vacuo* over P_2O_5 (yield, 31.2 g). Recrystallisation from hexane–benzene gave bis - (2,4 - dinitrophenyl)carbonate as yellow crystals, m.p. 144–146° (lit.[19] 148°). (Found: C, 39.9; H, 1.4; N, 14.1. Calc. for $C_{13}H_6N_4O_{11}$: C, 39.4; H, 1.5; N, 14.2%).

2,4-Dinitrophenyloxycarbonyl-L-phenylalanine ethyl ester. An ice-cold solution of L-phenylalanine ethyl ester hydrochloride (4.0 g) in 0.1 M NaOH solution (250 cm^3) was quickly extracted with ether (6 × 240 cm^3) and the combined extracts dried ($MgSO_4$) filtered and evaporated to approx. 120 cm^3. This solution, containing L-phenylalanine ethyl ester, was added over 35 min to a stirred solution of bis - (2,4 - dinitrophenyl)carbonate (6.0 g) in dry EtOAc (200 cm^3) at room temp. in a vessel fitted with a guard tube containing silica gel. The resulting yellow solution was left for 1 h, heated at 37° for 20 h and then evaporated to approx. 75 cm^3. Petrol (b.p. 40–60°; 500 cm^3) was added and the resulting precipitate collected and dried (yield 3.3 g). The ester was crystallised from EtOAc–hexane and gave m.p. 104–107°, $[\alpha]_{589}^{20} + 65.7°$ (C = 1, $CHCl_3$). (Found: C, 53.4; H, 4.3; N, 10.3. $C_{18}H_{17}N_3O_8$ requires: C, 53.6; H, 4.2; N, 10.4%). TLC R_{f_G} 0.0 (yellow, 2,4-dinitrophenol) and R_{f_G} 0.47 (ninhydrin and Cl_2 positive). IR γ_{max} (Nujol) 1750 (urethan C=O) 1730 (ester C=O), 1535 (NO_2) and 3350 cm^{-1} (N–H). NMR ($CDCl_3$) 1.1–2.6 (multiplet; 3 protons: aromatic), 2.7 (multiplet; 5 protons: aromatic),

3.98 (doublet; 1 proton: $-N\underline{H}^{a}-CH^{b}-$, $J_{a,b}=9$ Hz), 5.27 (sextet; 1 proton: $-NH^{a}-C\underline{H}_2^{b}-CH_2^{c}$, $J_{b,c}=6$ Hz), 5.72 (quartet; 2 protons: $-O-C\underline{H}_2^{d}-CH_3^{e}$, $J_{de}=7$ Hz), 6.76 (doublet; 2 protons: $-CH-C\underline{H}_2-$Ph) and 8.72τ (triplet; 3 protons: $-O-CH_2-C\underline{H}_3$).

Benzoyl - azaglycyl - L - phenylalanine ethyl ester **6**. A solution containing 2,4 - dinitrophenyloxycarbonyl - L - phenylalanine ethyl ester (1.10 g) and benzoylhydrazide (0.37 g) in EtOAc (30 ml) was left at room temp. for 1 h, kept at 37° for 6 h and then left at room temp. for 15 h. The solution was then filtered and hexane (250 ml) added to the filtrate, giving a precipitate (0.7 g) which was recrystallised from EtOAc–hexane to give benzoyl - azaglycylphenylalanine ethyl ester as colourless crystals, m.p. 137–138°, $[\alpha]_{589}^{20}+59.4$ (C = 1, $CHCl_3$) R_{fc} 0.46 (chlorine positive, ninhydrin negative). NMR ($CDCl_3$) 0.53 (singlet; 1 proton: $-N\underline{H}-$), 1.90 (singlet; 1 proton: $-N\underline{H}-$) 2.2–3.0 (multiplet; 10 protons: aromatic), 3.51 (doublet; 1 proton: $-CO-N\underline{H}-CH$), 5.28 (sextet; 1 proton: $-NH^{a}-C\underline{H}^{b}-CH_2^{c}$, $J_{a,b}=8$, $J_{b,c}=6$ Hz) 5.95 (quartet; 2 protons: $-O-C\underline{H}_2^{d}-CH_3^{e}$ $J_{d,e}=7$ Hz), 6.97 (doublet; 2 protons: $-CH^{b}-C\underline{H}_2^{c}-$Ph), 8.89τ (triplet; 3 protons: $O-CH_2-C\underline{H}_3$).

Acetyl - azaphenylalanyl - L - phenylalanine ethyl ester. A solution of 2,4 - dinitrophenyloxycarbonyl - L - phenylalanine ethyl ester (0.5 g) and 1 - acetyl - 2 - benzylhydrazine (0.20 g) in EtOAc (15 ml) was kept at 37° for 20 h. The solution was filtered and hexane added to the filtrate. The precipitate was collected (0.30 g) and recrystallised from EtOAc–hexane to give acetyl - azaphenylalanyl - L - phenylalanine ethyl ester as colourless crystals, m.p. 118–120°. $[\alpha]_{589}^{20}+17.2°$ (C = 1, $CHCl_3$) R_{fc} 0.58 (chlorine positive, ninhydrin negative). NMR ($CDCl_3$) 2.8τ (multiplet; 11 protons: aromatic + $N\underline{H}$), 4.20 (doublet; 1 proton, $-N\underline{H}^{c}-C\underline{H}-^{d}$, $J_{c,d}=8$ Hz), 5.3 (multiplet; 3 protons, $N-C\underline{H}_2-$Ph and $-N-C\underline{H}-$), 5.82 (quartet; 2 protons: $O-C\underline{H}_2^{a}-CH_3^{b}$, $J_{a,b}=7$ Hz), 6.85 (quartet (?); 2 protons: $-CH-C\underline{H}_2-$Ph), 8.16 (singlet; 3 protons: $C\underline{H}_3-CO$) and 8.76τ (triplet; 3 protons: $-O-CH_2-C\underline{H}_3$).

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