Synthesis and Characterisation of some New Diazopeptides

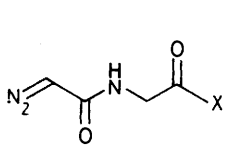
Brian C. Challis *† **and Farida Latif**
Chemistry Department, Imperial College, London SW7 2AZ

The synthesis of eight new diazopeptides by aprotic diazotisation with N_2O_4 is described for glycylglycine, triglycine, pentaglycine, L-leucylglycine and the ethyl esters of L-leucylglycine, L-alanylglycine, L-serylglycine and L-threonylglycine. The diazo derivatives (**7**)–(**10**) of the parent peptides are isolated as calcium salts. The UV-vis., IR, ^1H NMR and MS properties of the new diazopeptides are reported together with those for the diazo derivative of glycylglycine ethyl ester and glycylglycinamide.

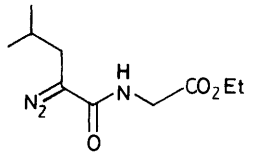
Diazopeptides were first described by Curtius and his colleagues[1–4] at the turn of the century, but generally these compounds are rare and therefore poorly characterised. Much of the early work concerned esters, amides and hydrazides of *N*-(2-diazoacetyl)glycine obtained by diazotisation of the appropriate glycylglycine derivative in aqueous acid.[2–4] With few exceptions, subsequent studies have been confined to improved synthesis of these compounds, such as those using mixed solvents.[5] Neither underivatised *N*-(2-diazoacetyl) peptides nor diazo derivatives of other than glycylpeptides have been reported hitherto.

The diazopeptides synthesised by Curtius and his colleagues and related compounds have attracted sporadic interest following discovery of their antineoplastic properties by Baldini and Brambilla[6] in 1966. Subsequently, it has been shown that many of these compounds are mutagenic,[7–9] immunodepressive,[10] antibacterial[11] and that they exert a complex pattern of cytotoxic properties including tumour induction,[12] tumour suppression,[6,13] and antimetastatic activity.[14]

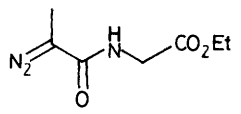
Recently, we reported that diazopeptides are formed readily by dilute gaseous NO_2 in aqueous buffers and human blood from several peptides.[15] A general procedure for the synthesis of these compounds and their characterisation is described below.



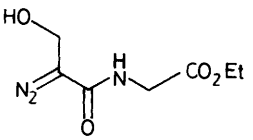
(**1**) **a** X = OEt
b X = NH_2



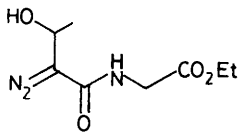
(**2**)



(**3**)



(**4**)



(**5**)

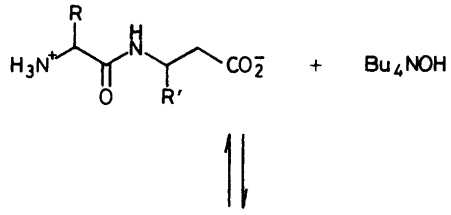
Results and Discussion

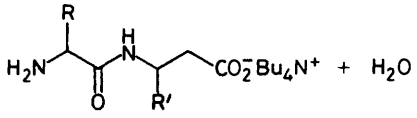
Diazopeptides are relatively labile especially in acidic media.[16] It follows that their synthesis is best carried out under mild, neutral conditions, such as that afforded by nitrosation (diazotisation) using liquid N_2O_4 in an aprotic solvent.

N-(2-Diazoacetyl)glycine ethyl ester (**1a**), however, was obtained readily in *ca.* 50% yield by the procedure of Curtius and his colleagues[2,3] involving the diazotisation of glycylglycine ethyl ester by $NaNO_2$ in an acetic buffer at O °C. Compound (**1a**) crystallised from the reaction mixture due to its low aqueous solubility. The yield of (**1a**) was increased to *ca.* 70% using the two-phase system of Looker and Carpenter[5] in which (**1a**) is extracted into the CH_2Cl_2 layer as formed. Further, compound (**1a**) was readily converted into *N*-(2-diazoacetyl)glycinamide (**1b**) on reaction with an excess of aqueous ammonia. Compound (**1b**) crystallised from the reaction solution in *ca.* 50% yield on standing for 2 h at −20 °C.

Diazotisation in aqueous acetic acid buffer at 0 °C, both with and without an organic phase, gave low yields of an impure diazo product for substrates other than glycyl peptide esters. *N*-(2-Diazo-4-methylvaleroyl)glycine ethyl ester (**2**), *N*-(2-diazopropanoyl)glycine ethyl ester (**3**), *N*-(2-diazo-3-hydroxypropanoyl)glycine ethyl ester (**4**), and *N*-(2-diazo-3-hydroxybutanoyl)glycine ethyl ester (**5**) were obtained, however, by aprotic diazotisation of the corresponding dipeptide ethyl ester in CH_2Cl_2 with liquid N_2O_4 at −40 °C. Triethylamine was added to keep the reaction solutions non-acidic, and anhydrous Na_2SO_4 to absorb the water produced by the reaction. After suitable work-up, the diazopeptide products were purified by column chromatography on silica and obtained as pale yellow oils (40–50%). This procedure seems suitable for all but alanyl- and phenylalanyl-peptide esters. A slightly different aprotic diazotisation procedure was devised for the synthesis of the parent diazopeptides (*i.e.* compounds with an underivatised terminal carboxylic acid). An initial problem was the insolubility of the highly polar peptide in aprotic solvents. This was solved by neutralising an aqueous solution of the peptide with tetrabutylammonium hydroxide (Scheme 1) to give the tetrabutylammonium salt (**6**). After being freeze-dried, the salt (6) was dissolved in dry CH_2Cl_2 containing 1 mol equiv. of triethylamine and an excess of anhydrous Na_2SO_4. Diazotisation was effected at −40 °C by the addition of liquid N_2O_4 dissolved in CH_2Cl_2. Following an appropriate work-up and column

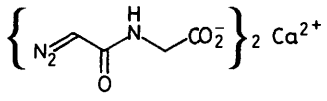
† *Present address:* Chemistry Department, The Open University, Walton Hall, Milton Keynes, MK7 6AA





(6)

Scheme 1.



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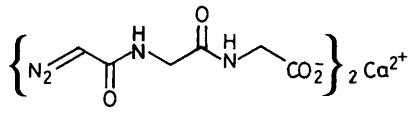

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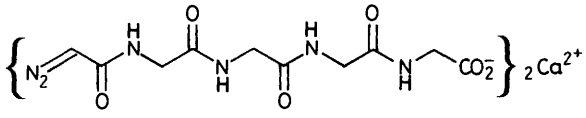

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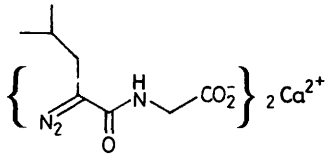
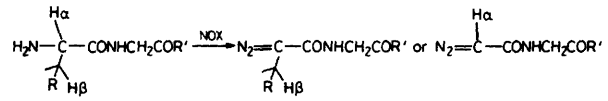


(10)

chromatography on silica using MeOH–EtOH as eluant, evaporation of the solvent gave a yellow hygroscopic paste which proved to be the triethylammonium rather than the tetrabutylammonium salt of the diazopeptide. This was evident from the characteristic ethyl signals in the ^{1}H NMR spectrum of the product. The diazopeptide was obtained as the calcium salt in 20–30% yields by precipitation from EtOH using $CaCl_2$. Four diazopeptides, namely *N*-(2-diazoacetyl)glycine (**7**), *N*-(2-diazoacetyl)glycylglycine (**8**), *N*-(2-diazoacetyl)triglycylglycine (**9**) and *N*-(2-diazoacetyl-4-methylvaleroyl)glycine (**10**) were obtained as calcium salts by this procedure. All were highly soluble in water and slightly soluble in alcohols. The diazopeptides (**1**)–(**5**) and (**7**)–(**10**) were characterised spectroscopically and in most cases by microanalysis. Despite repeated purification, however, satisfactory microanalyses were not obtained for the four least stable compounds (**3**), (**5**), (**7**), and (**10**). The percentage of nitrogen present was invariably low.

Table. ^{1}H NMR chemical shifts (δ ppm) relative to Me_4 Si for α-H and β-H for diazopeptides and parent peptides.[a]



R^1 = OEt, NH_2, O^- or glycyl moiety

Diazopeptide	δH_α	δH_β	$\Delta\delta H_\alpha$	$\Delta\delta H_\beta$
(**1a**)	4.83 (4.00)		0.83	
(**1b**)	5.40		—	
(**2**)		2.19 (1.80)		0.39
(**3**)		2.02 (1.62)		0.40
(**4**)		4.54 (4.14)		0.40
(**5**)		4.87 (4.45)		0.42
(**7**)	5.60 (4.10)		1.50	
(**8**)	5.45 (4.05)		1.40	
(**9**)	5.45 (4.05)		1.40	
(**10**)		2.50 (1.90)		0.60

[a] δ in parentheses for parent peptide.

UV-vis. Spectra.—All the diazopeptides showed strong absorbance at λ_{max} *ca.* 250–260 nm in water or ethanol (log ε 4–4.35) corresponding to the π–π* transition. They also showed a much weaker absorbance at λ_{max} *ca.* 380 nm (log ε 0.94–1.36) corresponding to the n–π* transition, which accounts for their pale yellow colouration. The extinction coefficient at λ_{max} *ca.* 250–260 nm is a useful guide to purity, being noticeably larger for the more stable, microanalytically pure compounds.

IR Spectra.—As Nujol mulls, all the diazopeptides gave a characteristic strong absorbance at ν_{max} *ca.* 2 100 cm^{-1} corresponding to the N=N stretching vibration. Other common absorbances were at ν_{max} *ca.* 3 280 (NH amide), 1 740 (C=O ester) and 1 600 br cm^{-1} (C=O amide and C=N=N). For the Ca salts of the parent diazopeptides, the C=O (acid) absorbance was also at ν_{max} *ca.* 1 600 cm^{-1}.

^{1}H *NMR Spectra.*—These were obtained in $CDCl_3$ for the peptide ethyl esters and their diazo derivatives, and in D_2O and $(CD_3)_2$ SO, respectively, for the native peptides and the Ca salts of their diazo derivatives. The most obvious change on diazotisation (apart from the expected reduction in signal intensity) was pronounced deshielding of protons both α and β to the diazo group. The deshielding, summarised in the Table, ranged from 0.83–1.50 ppm for the α-H of the *N*-(2-diazoacetyl) peptides and from 0.39–0.60 ppm for the β-H of other diazopeptides.

Mass Spectra.—Because of the ready loss of N_2, the molecular ion was either extremely weak or unobservable in the electron-impact spectra of the diazopeptides. Good results were obtained, however, by fast atom bombardment (FAB) techniques in glycerol–MeOH or glycerol–water matrices. The positive ion mode was used for the diazopeptide ethyl esters and the negative ion mode for the calcium salts. In each case, either the MH^+ or the $M - H^+$ peak was intense (*ca.* 80%). A typical FAB (positive ion) spectrum for compound (**2**) is shown in the Figure and characteristic fragment ions for (**2**) and the other diazopeptides are reported in the Experimental section. Both

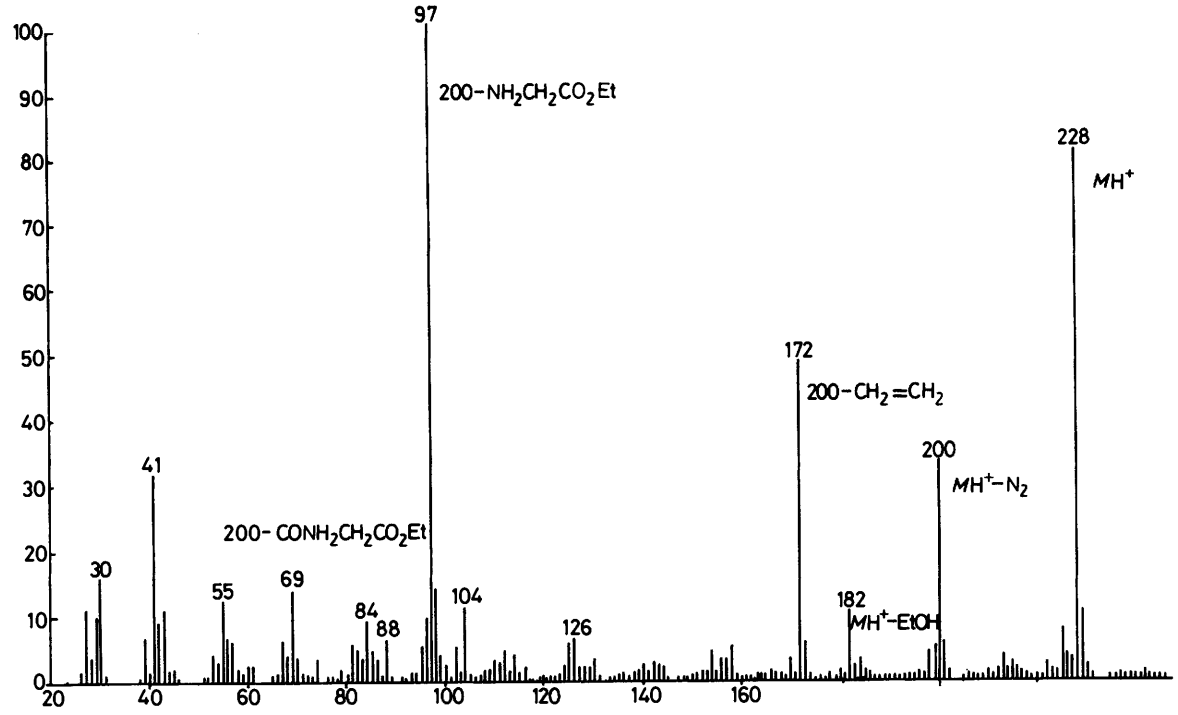
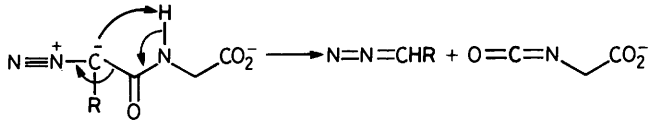


Figure 1. FAB (positive ion) mass spectrum of diazopeptide (**2**).

the M^+ and MH^+ ions, with the exception of $M - H^+$ for the largest diazopeptide (**9**), showed the loss of N_2. For all the diazopeptide ethyl esters, further loss of ethene (characteristic for ethyl esters *via* the McLafferty rearrangement) was apparent and both β-hydroxydiazopeptide ethyl esters (**4**) and (**5**) showed the loss of water characteristics of alcohols. For the Ca salts of diazopeptides, the fragment ion produced by elimination of diazoalkane from the $M - H^+$ ion (Scheme 2)



Scheme 2.

was especially characteristic. For all the diazopeptides, however, the fragment ion produced by cleavage of the peptide linkage (Figure) was strong and often the base peak.

Experimental

M.p.s were taken with a Gallenkamp hot-stage apparatus and are uncorrected. IR spectra were measured for Nujol mulls using a Perkin-Elmer 298 spectrometer, and UV visible spectra were measured for solutions in water or ethanol using a Cecil CE599 spectrophotometer. NMR spectra were taken with either a JEOL FX-90Q or a Bruker WM250 spectrometer in the solvents indicated with tetramethylsilane as internal standard. Mass spectra were obtained with a VG7070E instrument. Peptides, amino acids, benzyl chloroformate, ethyl chloroformate and tetrabutylammonium hydroxide were obtained from commercial suppliers (unless otherwise stated) and used as supplied. N_2O_4 (99%, B.O.S.G.) was also used without further purification. Triethylamine was heated under reflux with potassium hydroxide, then distilled and stored over 4 A molecular sieves. Solvents were purified and dried where necessary by standard procedures.[17] Light petroleum refers to the fraction of b.p. 40–60 °C.

N-Benzyloxycarbonyl Amino Acids.—For L-alanine and L-leucine, these were prepared by a literature procedure[18] involving reaction of the amino acid with benzyl chloroformate in aqueous alkaline solution. On completion of the reaction, the *N*-benzyloxycarbonyl amino acid was precipitated by acidifying the solution to pH 2. Yields were *ca.* 70–80%.

For L-serine and L-threonine, a more recent literature procedure[19] was used involving reaction of the amino acid with benzyl chloroformate in the presence of magnesium oxide with a cold two-phase aqueous–ether solvent. The *N*-benzyloxycarbonyl amino acid was also precipitated by acidifying the aqueous phase to pH 2 on completion of the reaction. Yields were *ca.* 80–90%.

Dipeptide Ethyl Ester Hydrochlorides.—These were prepared from the appropriate *N*-benzyloxycarbonyl amino acid and amino acid ethyl ester by mixed anhydride coupling using ethyl chloroformate. The following procedure for L-leucylglycine ethyl ester hydrochloride was typical. Thus, *N*-benzyloxycarbonyl-L-leucine ethyl ester hydrochloride (4 g, 15 mmol) and triethylamine (2.1 cm^3, 15 mmol) in dry tetrahydrofuran (THF) (25 cm^3) were cooled to −10 °C. Ethyl chloroformate (1.6 g, 15 mmol) in dry THF (25 cm^3) was added dropwise with stirring and the temperature was maintained at −10 °C. The solution was stirred at −10 °C for 30 min. A solution of ethyl glycinate in dry THF (20 cm^3) prepared from the hydrochloride salt (3 g, 21.5 mmol) by the addition of triethylamine (3 cm^3, 21.5 mmol) was added dropwise to the mixed anhydride, and the mixture was then stirred for 1 h at room temperature. The solution was filtered and the oil obtained after evaporation of the filtrate was dissolved in dichloromethane (50 cm^3). The solution was washed with 0.1M HCl (10 cm^3) and aqueous $NaHCO_3$ (10 cm^3) and then dried over anhydrous sodium sulphate. Evaporation of the solvent gave the *N*-benzyloxycarbonyl-L-leucylglycine ethyl ester as a solid, which was recrystallised from ethyl acetate.

The *N*-benzylcarbonyl-L-leucylglycine ethyl ester (4.5 g, 13 mmol) dissolved in ethanol (50 cm^3) and 5M HCl (2.7 cm^3, 13.5 mmol) was hydrogenated in a gas burette at room temperature and atmospheric pressure in the presence of a catalytic amount

of 5% palladium on charcoal. Hydrogenation was complete overnight. Filtration and vacuum evaporation of the filtrate gave L-leucylglycine ethyl ester hydrochloride as a white crystalline solid. Yield 2.73 g (80%) (Found: C, 47.5; H 8.3; N, 11.05. $C_{10}H_{21}ClN_2O_3$ requires C, 47.57; H 8.32; N, 11.09%). The hydrochlorides of L-alanylglycine ethyl ester, L-serylglycine ethyl ester and L-threonylglycine ethyl ester were prepared similarly. All of the compounds gave microanalyses and spectroscopic properties expected for their structure.

N-(2-*Diazoacetyl*)*glycine Ethyl Ester* (**1a**).—Aqueous $NaNO_2$ (7M, 10 cm^3) was added to a solution of glycylglycine ethyl ester hydrochloride (10 g, 0.05 mol) in 2M sodium acetate (20 cm^3) at 5 °C. Dichloromethane (250 cm^3) and glacial acetic acid (2 cm^3) were added, and the mixture was stirred for 5 h at 5 °C. The dichloromethane layer was separated, washed in 5% aqueous $NaHCO_3$ until neutral (100 cm^3), dried with anhydrous sodium sulphate, concentrated to 30 cm^3 and then cooled to yield yellow crystals of *N*-(2-diazoacetyl)glycine ethyl ester (**1a**). Yield 6 g (70%); m.p. 105 °C (lit.,[2] m.p. 107 °C) (Found: C, 42.1; H, 5.2; N, 24.55. Calc. for $C_6H_9N_3O_3$: C, 42.11; H, 5.26; N, 24.56%); $\lambda_{max}(H_2O)$ 250 (ε 20, 870 dm^3 mol^{-1} cm^{-1}) and 374 nm (ε 18 dm^3 mol^{-1} cm^{-1}), ν_{max}(Nujol) 3 280 (NH amide), 2 110 (C=N=N), 1 740 (C=O ester), and 1 600 br cm^{-1} (C=O, N=N); $\delta_H(CDCl_3)$ 1.27 (3 H, t, *J* 7 Hz, Et) 4.06 (2 H, d, *J* 5.5 Hz, $-CH_2NH-$), 4.2 (2 H, q, *J* 7 Hz, Et), and 4.82 (1 H, s, $-CHN_2$); *m/z* (FAB positive ion) 172 (MH^+), 144 ($MH^+ - N_2$), and 116 ($MH^+ - N_2 - CH{=}CH_2$).

N-(2-*Diazoacetyl*)*glycinamide* (**1b**).—*N*-(2-Diazoacetyl)glycine ethyl ester (2 g, 0.012 mol) was suspended in water (6 cm^3) at room temperature and 35% ammonia solution (10 cm^3, *ca.* 0.2 mol) was added. The suspension was stirred until the diazo ester had dissolved. On standing at −20 °C for 2 h, a mass of yellow crystalline *N*-(2-diazoacetyl)glycinamide precipitated. The crystals were filtered then washed with acetone. Yield 0.85 g (50 %); m.p. 160 °C (lit.,[3] m.p. 160 °C) (Found: C, 33.8 H, 4.2; N, 39.5. Calc. for $C_4H_6N_4O_2$, C, 33.80; H, 4.23; N, 39.44%); $\lambda_{max}(H_2O)$ 255 (ε 27, 056 dm^3 mol^{-1} cm^{-1}), 380 nm (ε 23.1 dm^3 mol^{-1} cm^{-1}); ν_{max}(Nujol) 3 300, 3 175 (N–H amide), 2 100 (C=N=N), 1 650 (C=O, amide), 1 610 (C=O, peptide), and 1 550 cm^{-1} (N–H, C–N amide); δ_H ([$^2H_6$]DMSO) 3.72 (2 H, d, $-NHCH_2-$) and 5.40 (1 H, s, N_2CH-); *m/z* (FAB positive ion) 143 (MH^+) and 115 ($MH^+ - N_2$).

N-(2-*Diazo*-4-*methylvaleroyl*)*glycine Ethyl Ester* (**2**).—L-Leucylglycine ethyl ester hydrochloride (2 g, 7.9 mmol) was dissolved in dichloromethane (20 cm^3). Triethylamine (2.2 cm^3, 15.8 mmol) was added along with anhydrous sodium sulphate (1 g), and the solution was cooled to −40 °C. N_2O_4 (0.5 cm^3, 8 mmol) in dichloromethane (20 cm^3) cooled to −70 °C was added gradually to the solution with stirring. The resulting yellow-green solution was allowed to warm to room temperature, washed with water (5 cm^3) and aqueous $NaHCO_3$ 5 cm^3) and then dried over anhydrous sodium sulphate. The solution was filtered and the filtrate evaporated to give a yellow oil. The product was purified by column chromatography on silica using 1:1 (v/v) light petroleum–ether (sodium dried) as eluant to give N-(2-*diazo*-4-*methylvaleroyl*)*glycine ethyl ester* as a yellow oil. Yield 0.72 g (40%) (Found: C, 52.7; H, 7.5; N, 18.45. $C_{10}H_{17}N_3O_3$ requires C, 52.86; H, 7.49; N, 18.50%); $\lambda_{max}(H_2O)$ 260 nm (ε 15 000 dm^3 mol^{-1} cm^{-1}) and 380 nm (ε 13 dm^3 mol^{-1} cm^{-1}); ν_{max}(Nujol) 2 050 (C=N=N), 1 740 (C=O ester), 1 610 (C=O peptide), and 1 200 cm^{-1} (C–O ester); $\delta_H(CDCl_3)$ 1.0 [6 H, d, $-CH(CH_3)_2$], 1.29 (3 H, t, *J* 7 Hz, Et), 1.85 [1 H, m, $-CH(CH_3)_2$], 2.19 [2 H, d, $CH_2CH(CH_3)_2$], 4.11 (2 H, d, $-H_2CNH-$), and 4.22 (2 H, q, *J* 7 Hz, Et); *m/z* (FAB positive ion) 228 (MH^+), 200 ($MH^+ - N_2$) 172, ($MH^+ - N_2 - CH{=}CH_2$), and 97.

N-(2-*Diazopropanoyl*)*glycine Ethyl Ester* (**3**).—This was synthesised in a similar manner to that for (**2**) using L-alanylglycine ethyl ester hydrochloride (1.67 g, 7.9 mmol), NEt_3 (2.2 cm^3, 15.8 mmol), Na_2SO_4 (1 g) and N_2O_4 (0.5 cm^3, 8 mmol) in dichloromethane (20 cm^3) at −40 °C. The eluant for silica column chromatography was 1:1 (v/v) light petroleum–ether (sodium dried). Yield 0.58 g (40%) (Found: C, 46.0, H, 6.0; N, 18.0. $C_7H_{11}N_3O_3$ requires C, 45.51; H, 5.95; N, 22.70%); λ_{max}(ethanol) 260 (ε 10 000 dm^3 mol^{-1} cm^{-1}) and 380 nm (8.63 dm^3 mol^{-1} cm^{-1}); ν_{max}(Nujol) 2 100 (C=N=N), 1 745 (C=O ester), 1 630 (C=O peptide), and 1 200 cm^{-1} (C–O ester); $\delta_H(CDCl_3)$ 1.30 (3 H, t, *J* 7 Hz, Et), 2.02 (3 H, s, N_2CCH_3), 4.10 (2 H, d, $-H_2C$ NH–), and 4.24 (2 H, q, *J* 7 Hz, Et); *m/z* (FAB positive ion) 186 (MH^+), 158 ($MH^+ - N_2$), 130 ($MH^+ - N_2 - CH{=}CH_2$), and 55.

N-(2-*Diazo*-3-*hydroxypropanoyl*)*glycine Ethyl Ester* (**4**).—This was synthesised in the same way as (**2**) using L-serylglycine ethyl ester hydrochloride (1.8 g, 7.9 mmol), NEt_3 (2.2 cm^3, 15.8 mmol), Na_2SO_4 (1 g), and N_2O_4 (0.5 cm^3, 8 mmol) at −40 °C. The eluant for silica column chromatography was 1:1 (v/v) chloroform–ethylacetate. Yield 0.63 g (45%) (Found: C, 41.9; H 5.8; N, 18.55. $C_7H_{11}N_3O_4$ requires C, 41.79, H, 5.47; N, 20.90%); λ_{max}(ethanol) 254 (ε 13 000 dm^3 mol^{-1} cm^{-1}) and 380 nm (ε 13.2 dm^3 mol^{-1} cm^{-1}); ν_{max}(Nujol) 2 100 (C=N=N), 1 745 (C=O ester), 1 625 (C=O peptide), and 1 205 cm^{-1} (C–O ester); $\delta_H(CDCl_3)$ 1.30 (3 H, t, *J* 7 Hz, Et), 4.10 (2 H, d, $-H_2C$ NH–), 4.20 (2 H, q, *J* 7 Hz, Et), and 4.54 (2 H, s, $-H_2COH$); *m/z* (FAB positive ion) 202 (MH^+), 174 (MH^+-N_2), 156 ($MH^+ - N_2 - H_2O$), and 128 ($MH^+ - N_2 - H_2O - CH{=}CH_2$).

N-(2-*Diazo*-3-*hydroxybutanoyl*)*glycine Ethyl Ester* (**5**).—This was synthesised in the same way as (**2**) using L-threonylglycine ethyl ester hydrochloride (1.92 g, 8 mmol), NEt_3 (2.2 cm^3, 15.8 mmol), Na_2SO_4 (1 g), and N_2O_4 (0.5 cm^3, 8 mmol) at −40 °C. The eluant for silica column chromatography was 1:1 (v/v) chloroform–ethyl acetate. Yield 0.68 g (40%) (Found: C, 46.8; H, 6.55; N, 16.55. $C_8H_{13}N_3O_4$ requires C, 44.70; H, 6.05; N, 19.5%); λ_{max}(ethanol) 254 (ε 13 865 dm^3 mol^{-1} cm^{-1}) and 380 nm (ε 12.1 dm^3 mol^{-1} cm^{-1}); ν_{max}(Nujol) 2 100 (C=N=N), 1 750 (C=O ester), 1 625(C=O peptide), and 1 200 cm^{-1} (C–O ester); $\delta_H(CDCl_3)$ 1.30 (3 H, t, *J* 7 Hz, Et), 1.45 [3 H, d, $-HC(CH_3)OH$], 4.10 (2 H, d, $-H_2CNH-$), 4.20 (2 H, q, *J* 7 Hz, Et), and 4.87 [1 H, q, $-HC(CH_3)OH$]; *m/z* (FAB positive ion) 216 (MH^+), 188 ($MH^+ - N_2$), 170 ($MH^+ - N_2 - H_2O$), and 142 ($MH^+ - N_2 - H_2O - CH{=}CH_2$).

N-(2-*Diazoacetyl*)*glycine, Calcium Salt* (**7**).—A mixture of glycylglycine (0.66 g, 5 mmol) dissolved in water (10 cm^3) and 40% aq. tetrabutylammonium hydroxide (3.25 cm^3, 5 mmol) was evaporated under reduced pressure to a semi-crystalline, colourless oil. The oil was dissolved in dichloromethane (50 cm^3) and then dried with anhydrous $MgSO_4$. After filtration, Et_3N (0.7 cm^3, 5 mmol) and anhydrous Na_2SO_4 (2 g) were added to the peptide solution. After being cooled to −40 °C, a solution of N_2O_4 (0.32 cm^3, 5 mmol) in dry dichloromethane (20 cm^3), precooled to −70 °C, was added slowly with stirring. The resultant yellow/orange solution was warmed to room temperature, evaporated under reduced pressure to a small volume, and then chromatographed on silica with 2:1 (v/v) ethanol–methanol as eluant (R_f 0.3 on silica TLC in EtOH). The fraction containing the alkylammonium salt of the diazopeptide was evaporated to a small volume. Addition of a slight excess of saturated $CaCl_2$ in ethanol precipitated the *calcium salt of* N-(2-*diazoacetyl*)*glycine*. Yield 0.32 g (20%);

m.p. > 200 °C (Found: C, 31.8; H, 3.0; N, 18.0. $C_8H_9N_6O_6Ca$ requires C, 29.63; H, 2.47; N, 25.93%); $\lambda_{max}(H_2O)$ 252 nm (ε 22 130 dm^3 mol^{-1} cm^{-1}) and 380 nm (ε 19 dm^3 mol^{-1} cm^{-1}); ν_{max}(Nujol) 2 110 (C=N=N), and 1 600br cm^{-1} (CO_2^-); δ_H ([$^2H_6$]-DMSO) 3.60 (2 H, d, –NHCH_2), 5.60 (1 H, s, N_2CH–), and 7.50 (1 H, br s, –CONH–); *m/z* (FAB negative ion) 142 ($M - H^+$), 114 ($M - H^+ - N_2$), 100 ($M - H^+ - N_2$=CHR), and 72.

N-(2-*Diazoacetyl*)*glycylglycine, Calcium Salt* (**8**).—This was synthesised from triglycine (0.95 g, 5 mmol), tetrabutylammonium hydroxide (3.25 cm^3, 5 mmol), Et_3N (0.7 cm^3, 5 mmol), Na_2SO_4 (2 g), and N_2O_4 (0.32 cm^3, 5 mmol) at −40 °C in the same manner as for (**7**). Purification of the triethylammonium salt of the diazopeptide followed by precipitation of the calcium salt was also identical with (**7**). Yield 0.66 g (30%); m.p. > 200 °C (Found: C, 32.75; H, 3.2; N, 25.6. $C_{12}H_{14}N_8O_8Ca$ requires C, 32.87; H, 3.20; N, 25.60%); $\lambda_{max}(H_2O)$ 250 (ε 20 610 dm^3 mol^{-1} cm^{-1}) and 380 nm (ε 17.8 dm^3 mol^{-1} cm^{-1}); ν_{max}(Nujol) 2 110 (C=N=N), and 1 600br cm^{-1} (CO_2^-); δ_H([$^2H_6$]-DMSO) 3.50 (2 H, d), 3.75 (2 H, d), 5.45 (1 H, s, N_2CH–), 7.60 (1 H, br s), and 8.05 (1 H, br s); *m/z* (FAB negative ion) 199 ($M - H^+$), 171 ($M - H^+ - N_2$), 157 ($M - H^+ - N_2$=CHR), and 129.

N-(2-*Diazoacetyl*)*triglycylglycine, Calcium Salt* (**9**).—This was synthesised by the same procedure as that used for (**7**) from pentaglycine (1.52 g, 5 mmol), tetrabutylammonium hydroxide (3.25 cm^3, 5 mmol), Et_3N (0.7 cm^3, 5 mmol), Na_2SO_4 (2 g), and N_2O_4 (0.32 cm^3, 5 mmol) at −40 °C. The alkylammonium salt of the diazopeptide was purified by silica column chromatography using methanol as eluant. The N-(2-*diazoacetyl*)-*triglycylglycine calcium salt* was precipitated as for (**7**). Yield 0.98 g (30%); m.p. > 200 °C (Found: C, 35.5; H, 4.0; N, 25.15. $C_{20}H_{26}N_{12}O_{12}Ca$ requires C, 36.0; H, 3.9; N, 25.22%); $\lambda_{max}(H_2O)$ 250 (ε 18 072 dm^3 mol^{-1} cm^{-1} and 380 nm (ε 15.6 dm^3 mol^{-1} cm^{-1})); ν_{max}(Nujol) 2 110 (C=N=N) and 1 600 br cm^{-1} (CO_2^-); δ_H([$^2H_6$]-DMSO) 3.55 (2 H, d), 2.72 (2 H, d), 3.8 (2 H, d), 3.9 (2 H, d), 5.45 (1 H, s, N_2CH–), 7.6 (1 H, br s), 8.0 (1 H, br s), 8.25 (1 H, br s), and 8.4 (1 H, br s); *m/z* (FAB negative ion) 313 ($M - H^+$), 271 ($M - H^+ - N_2$=CHR), and 243.

N-(2-*Diazo*-4-*methylvaleroyl*)*glycine, Calcium Salt* (**10**).—This was synthesised using L-leucylglycine (0.94 g, 5 mmol), tetrabutylammonium hydroxide (3.25 cm^3), Et_3N (0.7 cm^3, 5 mmol), Na_2SO_4 (2 g) and N_2O_4 (0.32 cm^3, 5 mmol) at −40 °C in a similar manner to that for (**7**). The eluant used for silica column chromatography of the alkylammonium salt was 5:1 (v/v) chloroform–ethanol, and the calcium salt was precipitated as for (**7**). Yield 0.44 g (20%); m.p. > 200 °C; $\lambda_{max}(H_2O)$ 260 (ε 13 000 dm^3 mol^{-1} cm^{-1}) and 380 nm (ε 13 dm^3 mol^{-1} cm^{-1}); ν_{max} 2 110 (C=N=N) and 1 600 br cm^{-1} (CO_2^-); δ_H([$^2H_6$]-DMSO) 1.0 [6 H, d, CH(CH_3)$_2$], 1.80 [1 H, m, –CH(CH$_3$)$_2$], 2.5 [2 H, d, H_2CCH(CH$_3$)$_2$], and 3.65 (2 H, d, –H_2CNH); *m/z* (FAB negative ion) 198 ($M - H^+$), 170 ($M - H^+ - N_2$), 100 ($M - H^+ - N_2$=CHR), and 72.

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