Synthesis and Characterisation of Some New *N*-Nitrosodipeptides

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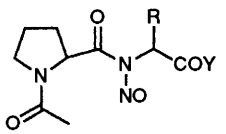
The synthesis of 11 new *N*-nitrosodipeptides by aprotic nitrosation with N_2O_4 is described for *N*-(*N'*-acetyl-L-prolyl)glycine, -L-alanine, -L-phenylalanine; and *N*-phthalimidoacetylglycine peptides and their (benzyl or ethyl) esters. The UV–vis, IR, ^1H NMR and MS properties of the new *N*-nitrosodipeptides are reported and their structural significance is analysed.

The nitrosation of proteins and peptides is of interest in connection with dietary-related cancers. Nitrosation reactions proceed in the stomach[1] and gastric contents often contain small amounts of unidentified *N*-nitroso compounds[2] which may well derive from proteins and peptides, the commonest dietary *N*-compounds. Further, there is a correlation between the incidence of colon cancer and dietary protein intake.[3]

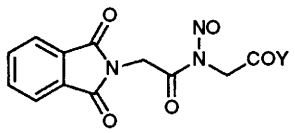
Diazotisation of the terminal primary amino groups of proteins and peptides by nitrous acid has been known for over 80 years,[4] but evidence for the nitrosation of peptide *N*-atoms to give *N*-nitroso derivatives has proven more elusive. Bonnett and his colleagues found no ^{15}N NMR evidence for *N*-nitrosopeptides using aqueous HNO_2,[5] but were able to isolate *N*-acetyl-*N*-nitrosomethionine methyl ester.[6] The *N*-nitroso derivatives of *N*-acyl amino acid esters are readily obtained, however, by aprotic nitrosation.[7] Chow *et al.* prepared some *N*-acyl-*N*-nitroso-α-amino acids both aprotically[8] and in aqueous solution,[9] but all were too unstable to isolate.

Preliminary reports of the synthesis of *N*-nitrosopeptides by aprotic nitrosation and their characterisation were published in 1984.[7,10] These refer to both *N*-acylpeptides and their ester analogues. Subsequently, it has been shown that one of these compounds, *N*-(*N'*-acetyl-L-prolyl)-*N*-nitrosoglycine (**1f**), exhibits a broad spectrum of genotoxic properties.[11–14] The synthesis and formation kinetics of several *N*-nitrosoprolyl peptides have also been described, but these compounds are α-substituted *N*-nitrosamines.[15]

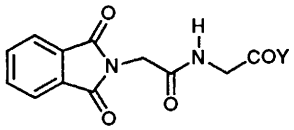
Here the synthesis and characterisation of five *N*-(*N'*-acetyl-L-prolyl)-*N*-nitrosopeptide esters (**1a–e**), three *N*-(*N'*-acetyl-L-prolyl)-*N*-nitrosopeptides (**1f-h**) and three *N*-phthalimidoacetyl-*N*-nitrosoglycine derivatives (**2a–c**) are described in detail along with three parent un-nitrosated *N*-(*N'*-acetyl-L-prolyl) peptides (**5f–h**).



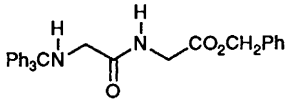
(1) **a**; R = H, Y = OCH_2Ph
b; R = CH_3, Y = OCH_2Ph
c; R = CH_2Ph, Y = OCH_2Ph
d; R = H, Y = OEt
e; R = CH_3, Y = OEt
f; R = H, Y = OH
g; R = CH_3, Y = OH
h; R = CH_2Ph, Y = OH



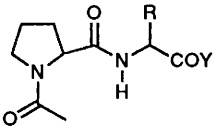
(2) **a**; Y = OEt
b; Y = OCH_2Ph
c; Y = OH



(3) **a**; Y = OEt
b; Y = OCH_2Ph



(4)



(5) **a**; R = H Y = OCH_2Ph
b; R = CH_3, Y = OCH_2Ph
c; R = CH_2Ph, Y = OCH_2Ph
d; R = H, Y = OEt
e; R = CH_3, Y = OEt
f; R = H, Y = OH
g; R = CH_3, Y = OH
h; R = CH_2Ph, Y = OH

Results and Discussion

The lapse of 80 years between the first synthesis of diazopeptides and *N*-nitrosopeptides suggests that the terminal primary amino groups of peptides nitrosate much more readily than peptide *N*-atoms. It follows that *N*-nitrosopeptides are best obtained from terminal *N*-protected compounds. Preliminary experiments showed that *N*-nitroso derivatives of glycylglycine, for example, could not be obtained from either protic or aprotic nitrosations which readily produce the *N*-nitroso derivatives of simple amides. Our initial studies therefore involved the nitrosation of *N*-phthalimidoacetylglycine esters (**3a**) and (**3b**). These readily gave the corresponding *N*-nitroso derivatives (**2a**) and (**2b**) by protic and aprotic nitrosation. Compounds (**2a**) and (**2b**) were distinguished by a characteristic visible absorption triplet at λ_{max} = 380–430 nm and shifts in ^1H NMR and IR bands due to electron-withdrawal by the *N*-nitroso substituent. Attempts to remove selectively the *N*-phthalimide and ester functions from compounds (**2a**) and (**2b**) were unsuccessful with one exception. Thus, treatment of esters (**2a**) and (**2b**) with various acids and bases and the catalytic hydrogenolysis of (**2a**) invariably removed the *N*-nitroso group more rapidly than the protection or caused massive decomposition. Catalytic hydrogenolysis of ester (**2b**), however, gave acid (**2c**) in good yield, which showed that hydrogenolysis of the benzyl ester was more facile than the *N*-nitroso group. Other initial studies involving peptides bearing more labile terminal *N*-protection [*e.g.* trityl compound (**4**)] resulted in extensive removal of the *N*-protection and subsequent di-

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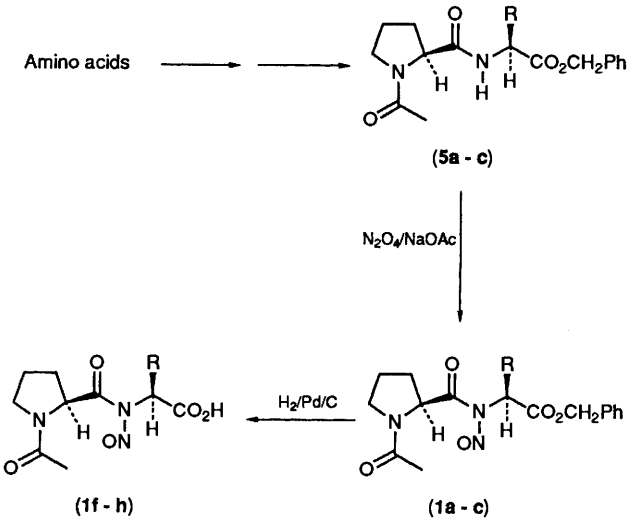
azotisation in the course of both protic and aprotic nitrosation. These findings suggested that the synthesis of authentic *N*-nitrosopeptides could be achieved for substrates (**5a–c**) where diazotisation is blocked and the benzyl ester facilitates solubility in organic solvents for aprotic nitrosation yet is removable by hydrogenolysis under mild, neutral conditions.

The successful procedure for the synthesis of compounds (**1f–h**) is outlined in the Scheme. The peptide benzyl esters (**5a–c**) were synthesised by conventional procedures. Nitrosation was carried out aprotically at −10 °C using liquid N_2O_4 in CH_2Cl_2 with a 4-fold excess of NaOAc to neutralise HNO_3 by-product. Other solvents were investigated, but CH_2Cl_2 proved best for reasons of solubility and volatility. After an appropriate aqueous work-up, the *N*-nitroso benzyl esters (**1a–c**) were obtained as chromatographically homogenous, yellow oils in yields >90% and typically quantitative (see Experimental section). They were debenzylated by hydrogenolysis without further purification.



Scheme.

In contrast to the *N*-nitrosation step, the hydrogenolysis of (**1a–c**) required precise conditions for success. Removal of the benzyl group without appreciable N–NO cleavage could only be achieved in either EtOH or AcOEt with palladium (5–10% on charcoal) catalyst. Hydrogenolysis was slow unless relatively large amounts of catalyst were used, and it was advantagous to follow the extent of reaction by the uptake of hydrogen to minimise unintentional N–NO cleavage. Polar solvents (*e.g.*, HOAc, H_2O) not only increased the rate of hydrogenolysis but also decomposition of the *N*-nitrosopeptide product, as did Pd–$BaSO_4$. Homogeneous catalysts were not examined because the work-up procedures seemed likely to decompose the *N*-nitrosopeptides. The major impurity after hydrogenolysis was the parent dipeptide, which exemplifies the lability of the N–NO bond. After appropriate work-up, the *N*-nitrosopeptides were purified by recrystallisation (see Experimental) and obtained as yellow crystalline solids in yields of 40–50%. Compounds (**1f**) and (**1g**) were microanalytically pure, but (**1h**) could not be separated from *ca.* 10% of the parent dipeptide.

Apart from the elemental analyses, compounds (**1f–h**) were characterised by UV–vis spectrophotometry, IR and ^{1}H NMR spectrometry and their FAB (+ve ion) mass spectra. These results are best analysed by comparison with the parent (unnitrosated) dipeptide.

UV–vis Spectra.—Compounds (**1f–h**) in EtOH showed triplet absorbance bands between λ_{max} 380 and 430 nm (log ε *ca.* 2) which are characteristic of *N*-nitrosoamides (but absent in the parent peptides) and related to forbidden n–π^* transitions. This property has been noted by Shuker *et al.*[16] and is consistent with data for *N*-nitroso-α-amino acid esters reported by Djerassi *et al.*[17] and Bonnett and Nicolaidou.[6] Compounds (**1f–h**) also showed absorbances at λ_{max} 238 nm (log ε *ca.* 3.7–3.8) corresponding to an allowed π–π^* transition. These are consistent with the *N*-nitrosopeptide structure but not characteristic because the Ph substituent of acid (**1h**) should also absorb strongly at this wavelength.

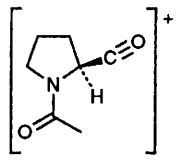
IR Spectra.—As Nujol mulls, compounds (**1f–h**) show no peptide NH stretch and absence of the amide CO and II bands compared with the parent dipeptides. The C=O bands of *N*-nitrosamides are generally found at 1 730 cm^{-1},[18] which coincides with the carboxylic acid C=O of compounds (**1f–h**); this contrasts with an absorption at 1 660 cm^{-1} for the parent dipeptides and manifests extensive electron withdrawal by the N–NO group. The N=O absorption is apparent at 1 510 cm^{-1} for compounds (**1f–h**), which is also typical of *N*-nitrosoamides[18] compared to < 1 500 cm^{-1} for *N*-nitrosoamines.[19] The shift to higher frequencies also reflects electron withdrawal, in this instance by the C=O function. The *N*-acetyl C=O stretch is observed at 1 590–1 610 cm^{-1} in compounds (**1f–h**) and the parent dipeptides.

^{1}H *NMR Spectra.*—The lack of a suitable, common solvent complicates the comparison of the ^{1}H NMR spectra of the *N*-nitroso compounds (**1f–h**) with the parent dipeptides. Thus, compounds (**1f–h**) were too unstable in $(CD_3)_2SO$ to record their ^{1}H NMR spectra, whereas $CDCl_3$ and $(CD_3)_2CO$ were suitable. Conversely, the parent dipeptides were insoluble in $CDCl_3$ and $(CD_3)_2CO$, but dissolved in $(CD_3)_2SO$. $CDCl_3$ was a suitable common solvent, however, for the ^{1}H NMR measurements of the benzyl and ethyl esters (**5a–e**) and their *N*-nitroso derivatives (**1a–e**). Results summarised in the Table show that the protons α to the peptide CO are deshielded by *ca.* 1 ppm on *N*-nitrosation and the protons α to the peptide *N*-atom are deshielded by *ca.* 0.6 ppm. For a few results in the Table where overlapping signals are unresolved at 60 MHz, the uncertainties in the chemical shift differences are as high as 0.3 ppm. In most cases, however, the uncertainty was eliminated by correlating signal multiplicities. A pair of compounds, where precise assignment proved impossible, was *N*-(*N'*-acetyl-L-prolyl)-*N*-nitroso-L-phenylalanine benzyl ester (**1c**) and its parent peptide (**5c**). Nonetheless, the results in the Table show that ^{1}H NMR deshielding effects in compounds (**1a–h**) relative to the parent dipeptide are in good agreement with data for α-amino acid esters reported by Shuker *et al.*[16] and by Bonnett and Nicolaidou.[6]

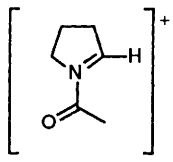
Mass Spectra.—In common with simple *N*-nitrosamines, the molecular ion is unobservable in the electron-impact spectra of the *N*-nitrosopeptides (**1f–h**) because of the ready fragmentation of the N–NO bond. Good results were obtained for compounds (**1f**) and (**1g**), however, by fast atom bombardment (FAB) techniques in the positive ion mode using a glycerol–water matrix. The most important features from a structural confirmation standpoint were relatively weak (7%) MH^+ ions but stronger ions resulting from the loss of both the NO and CH_2CO groups. Four other ions with *m/z* 140, 112, 70 (100%), and 43 were prominent in both the FAB (positive ion) mass spectra of acids (**1f**) and (**1g**) and the FAB and electron impact spectra of the parent dipeptides and their ester derivatives. These are assigned the structures (**6**) to (**9**), respectively. Our findings agree with recently published results[20] for compound (**1f**).[19]

Table. Effect of *N*-nitrosation on the ^{1}H NMR deshielding of protons adjacent to the peptide C=O group (δH_A) and *N*-atom (δH_B): $\Delta\delta H$ = δH (*N*-nitrosopeptide) − δH (peptide).

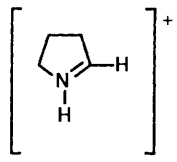
Compound	Solvent	δH_A	δH_B	$\Delta\delta H_A$	$\Delta\delta H_B$
(5f)	$(CD_3)_2SO$	4.11–4.39	3.55–3.82	1.45	0.92
(1f)	$(CD_3)_2C{=}O$	5.5–5.9	4.6		
(5d)	$CDCl_3$	4.33–4.63	3.81–3.92	1.16	0.54
(1d)	$CDCl_3$	5.49–5.78	4.4		
(5a)	$CDCl_3$	4.5–4.8	3.95–4.10	1.05	0.58
(1a)	$CDCl_3$	5.6–5.8	4.6		
(5g)	$(CD_3)_2SO$	4.0–4.4	4.0–4.4	1.09	0.32
(1g)	$CDCl_3$	5.29	4.52		
(5e)	$CDCl_3$	4.57	4.43	1.13	0.76
(1e)	$CDCl_3$	5.70	5.19		
(5b)	$CDCl_3$	4.1–4.8	4.1–4.8	1.13	0.74
(1b)	$CDCl_3$	5.58	5.19		
(5h)	$(CD_3)_2SO$	3.98–4.70	3.98–4.70	1.21	0.26
(1h)	$CDCl_3$	5.31–5.78	4.37–4.83		
(5c)	$CDCl_3$	4.31–4.94	4.31–4.94	0.87	0.87
(1c)	$CDCl_3$	5.30–5.69	5.30–5.69		



(6) *m/z* 140



(7) *m/z* 112



(8) *m/z* 70

$[CH_3C{\equiv}O]^+$

(9) *m/z* 43

Experimental

M.p.s were measured on a Gallenkamp hot-stage and are uncorrected. IR spectra were recorded on a Perkin-Elmer 298 grating spectrometer and calibrated against polystyrene. ^{1}H NMR spectra were recorded on Varian EM-360A and JEOL FX-90Q spectrometers in the solvent indicated, with tetramethylsilane as internal standard. UV and visible spectra were recorded on Pye–Unicam SP8-500, Cecil CE599, and Perkin-Elmer 555 spectrophotometers. Mass spectra were obtained with a VG7070 instrument. Amino acids, peptides, benzyl chloroformate, ethyl chloroformate, acetic anhydride, and toluene-4-sulphonic acid were obtained from commercial sources and used as supplied. N_2O_4 (99% Matheson) was also used without further purification. Other reagents and solvents were purified by standard procedures.[21]

N-*Acetyl*-L-*proline* (**10**).—L-Proline (5.76 g, 50 mmol) and toluene-4-sulphonic acid monohydrate (10.46 g, 55 mmol) were suspended in a mixture of benzene (30 cm^3) and benzyl alcohol (30 cm^3). On heating, a pale yellow colour developed. The solution was heated under reflux using a Dean and Stark trap until azeotropic removal of water was complete (*ca.* 10 h). The remaining solution was diluted with ether (300 cm^3), the oily lower phase was isolated by decantation, and dissolved in CH_2Cl_2 (70 cm^3) containing Et_3N (5.06 g, 50 mmol). The solution was cooled to −5 °C, treated with a solution of Ac_2O (5.10 g, 50 mmol) in CH_2Cl_2 (10 cm^3), and then washed successively with dilute HCl (0.01M; 20 cm^3), satd. aq. $NaHCO_3$ (20 cm^3), water (20 cm^3) and saturated brine (20 cm^3) and then dried ($MgSO_4$). Removal of solvent under reduced pressure left *N*-acetyl-L-proline benzyl ester (**11**) as a mobile yellow oil (10.1 g, 82%) which was purified by vacuum distillation, b.p. 100 °C at 7 × 10^{-3} mmHg (Found: C, 67.9; H, 7.2; N, 5.65. $C_{14}H_{17}NO_3$ requires C, 68.00; H, 6.93; N, 5.66%); $\nu_{max}(CCl_4)$ 1 750 (ester CO) and 1 660 cm^{-1} (amide CO); $\delta_H(CDCl_3)$ 2.02 (4 H, m, 3-H_2 and 4-H_2), 2.10 (3 H, s, Ac), 3.60 (2 H, m, 5-H_2), 4.50 (1 H, m, 2-H), 5.18 (2 H, s, CH_2Ph), and 7.29 (5 H, s, Ph); *m/z* (electron impact) 247 (9%, M^+), 112 (61, M-CO_2CH_2Ph), 91 (17, $C_7H_7^+$) and 70 (100, M − $AcCO_2$-CH_2Ph).

A solution of benzyl ester (**11**) (9.40 g, 38 mmol) in absolute EtOH (40 cm^3) containing 5% Pd–C (100 mg) was stirred under 760 mmHg of hydrogen. After cessation of H_2 uptake (*ca.* 1 h), the catalyst was removed by filtration, and the filtrate concentrated under vacuum to give a white solid, which was recrystallized from water to give *N*-acetyl-L-proline (**10**) (4.68 g, 78%); m.p. 89–90 °C (Found: C, 53.05; H, 6.95; N, 9.2. $C_7H_{11}NO_3$ requires C, 53.49; H, 7.04; N, 8.91%); ν_{max}(Nujol) 3 000–2 500 (CO_2H), 1 730 (acid CO), and 1 610 cm^{-1} (amide CO); $\delta_H(CDCl_3)$ 2.10 (4 H, m, 3-H_2 and 4-H_2), 2.19 (3 H, s, Ac), 3.55 (2 H, m, 5-H_2), 4.60 (1 H, m, 2-H), and 9.21 (1 H, br s, CO_2H).

N-(N′-*Acetyl*-L-*prolyl*)-α-*amino Acid Benzyl Esters.*—All were prepared from *N*-acetyl-L-proline (**10**) and the appropriate α-amino acid benzyl ester by mixed anhydride coupling. The following procedure for *N*-(*N*′-acetyl-L-prolyl)glycine benzyl ester (**5a**) is exemplary.

N-(N′-*Acetyl*-L-*prolyl*)*glycine Benzyl Ester* (**5a**).—A solution of *N*-acetyl-L-proline [(**10**), 1.57 g, 10 mmol] and Et_3N (1.12 g, 10 mmol) in dry THF (30 cm^3) at −10 °C was treated with a solution of ethyl chloroformate (0.87 g, 8 mmol) in dry THF (6 cm^3). The resulting suspension was stirred at −10 °C for 30 min, then treated with glycine benzyl ester (1.32 g, 8 mmol) and allowed to reach room temperature. After removal of solvent under reduced pressure, the residue was dissolved in CH_2Cl_2 (60 cm^3) and washed successively with dilute HCl (0.01M; 20 cm^3), saturated aqueous $NaHCO_3$ (20 cm^3), water (20 cm^3), and saturated brine (20 cm^3), and then dried ($MgSO_4$). Removal of the solvent under reduced pressure gave *N*-(*N*′-acetyl-L-prolyl)glycine benzyl ester (**5a**) as a colourless oil (2.15 g, 71%); $\nu_{max}(CCl_4)$ 3 305 (NH), 1 750 (ester CO), 1 690 (amide CO), and 1 620 cm^{-1} (amide CO); $\delta_H(CDCl_3)$ 2.10 (3 H, s, Ac), 1.7–2.6 (4 H, m, 3-H_2 and 4-H_2), 3.3–3.9 (2 H, m, 5-H_2), 3.95 and 4.10 (2 H, s, $CH_2CO_2CH_2Ph$), 4.5–4.8 (1 H, m, 2-H), 5.10 (2 H, s, CH_2Ph), 7.30 (5 H, s, Ph), and 7.60 (1 H, br s, NH).

N-(N′-*Acetyl*-L-*prolyl*)*glycine Ethyl Ester* (**5d**). As for (**5a**)

using glycine ethyl ester in place of the benzyl ester. *N*-(*N'*-Acetyl-L-prolyl)glycine ethyl ester (**5d**) was obtained as a colourless oil in 81% yield; $\nu_{max}(CCl_4)$ 3 300 (NH), 1 750 (ester CO), 1 690 (amide CO), and 1 640 cm^{-1} (amide CO); $\delta_H(CDCl_3)$ 1.20 (3 H, t, *J* 8 Hz, $CO_2CH_2CH_3$), 2.06 (3 H, s, Ac), 1.62–2.51 (4 H, m, 3-H_2 and 4-H_2), 3.20–3.77 (2 H, m, 5-H_2), 3.81 and 3.92 (2 H, s, CH_2CO_2Et), 4.08 (2 H, q, *J* 8 Hz, $CO_2CH_2CH_3$), 4.33–4.63 (1 H, m, 2-H), and 7.35 (1 H, br s, NH); *m/z* (electron impact) 242 (5%, M^+) 197 (2, *M* – OEt), 185 (5), 175 (4), 167 (2), 142 (20, *M* – $NHCH_2CO_2Et$), 112 (86, *M* – $CONHCH_2CO_2Et$), 102 (70), 92 (21), 70 (100, *M* – Ac – $CONHCH_2CO_2Et$), and 43 (47, Ac).

N-(N'-*Acetyl*-L-*prolyl*)-L-*alanine Benzyl Ester* (**5b**). From *N*-acetyl-L-proline [(**10**); 0.19 g, 1.2 mmol] and L-alanine benzyl ester (0.08 g, 1 mmol) as for (**5a**) above. *N*-(*N'*-Acetyl-L-prolyl)-L-alanine benzyl ester (**5b**) was isolated as a white solid after recrystallisation from ethyl acetate (270 mg, 85%); m.p. 113 °C (Found: C, 64.1; H, 7.0; N, 8.75. $C_{17}H_{22}N_2O_4$ requires C, 64.13; H, 6.97; N, 8.80%); ν_{max}(Nujol) 3 320 (NH), 1 745 (ester CO), 1 660 (amide CO), 1 630 (amide CO), 1 535 (amide II), 760 and 705 cm^{-1} (Ph); $\delta_H(CDCl_3)$ 1.36 (2 H, m, 5-H_2), 1.6–2.5 (4 H, m, 4-H_2 and 3-H_2), 2.06 (3 H, s, Ac), 3.47 (2 H, m, 5-H_2), 4.1–4.8 (2 H, m, 2-H and C*H*Me), 5.11 (2 H, s, CO_2CH_2Ph), and 7.27 (5 H, s, Ph); *m/z* (electron impact) 318 (5%, M^+), 227 (1, *M* – CH_2Ph), 210 (1), 183 (2, *M* – CO_2CH_2Ph), 168 (1), 151 (4), 140 (57, *M*-$NHCHMeCO_2CH_2Ph$), 112 (57, *M* – $CONHCHMeCO_2CH_2Ph$), 91 (2, $C_7H_7^+$), 70 (100, *M* – Ac – $CONHCHMeCO_2CH_2Ph$), and 43 (16, Ac).

N-(N'-*Acetyl*-L-*prolyl*)-L-*alanine Ethyl Ester* (**5e**). As for (**5b**) using L-alanine ethyl ester in place of the benzyl ester. *N*-(*N'*-Acetyl-L-prolyl)-L-alanine ethyl ester (**5e**) was obtained as a white solid in 79% yield after recrystallisation from EtOAc; m.p. 79–80 °C (Found: C, 56.4; H, 7.9; N, 10.95. $C_{12}H_{20}N_2O_4$ requires C, 56.24; H, 7.86; N, 10.93%); ν_{max}(Nujol) 3 320 and 3 260 (NH), 1 740 (ester CO), 1 675 (amide CO), 1 640 (amide CO), and 1 540 cm^{-1} (amide II); $\delta_H(CDCl_3)$ 1.23 (3 H, t, *J* 7 Hz, $CO_2CH_2CH_3$), 1.34 (3 H, d, *J* 7 Hz, CH*Me*), 2.05 (3 H, s, Ac), 1.8–2.4 (4 H, m, 3-H_2 and 4-H_2), 3.49 (2 H, m, 5-H_2), 4.11 (2 H, q, *J* 7 Hz, CO_2CH_2Me), 4.43 (1 H, q, *J* 7 Hz, C*H*Me), and 4.57 (1 H, m, 2-H); *m/z* (electron impact) 256 (9%, M^+), 140 (14, *M* – $NHCHMeCO_2Et$), 113 (31), 112 (77, *M* – $CONHCHMeCO_2Et$), 70 (100, *M* – Ac – $CONHCHMeCO_2Et$), and 43 (17, Ac).

N-(N'-*Acetyl*-L-*prolyl*)-L-*phenylalanine Benzyl Ester* (**5c**). From *N*-acetyl-L-proline [(**6**); 0.78 g, 5 mmol] and L-phenylalanine benzyl ester (1.02 g, 4 mmol) as for (**5a**) above. *N*-(*N'*-Acetyl-L-prolyl)-L-phenylalanine benzyl ester (**5c**) was obtained as a white solid after recrystallisation from ethyl acetate (1.31 g, 83%), m.p. 104 °C (Found: C, 69.95; H, 6.7; N, 7.05. $C_{23}H_{26}N_2O_4$ requires C, 70.03; H, 6.64; N, 7.10%); ν_{max}(Nujol) 3 210 (amide NH), 1 745 (ester CO), 1 690 (amide CO), 1 620 (amide I), 1 570 (amide II), and 755 and 710 cm^{-1} (Ph); $\delta_H(CDCl_3)$ 1.96 (3 H, s, Ac), 1.53–2.25 (4 H, m, 3-H_2 and 4-H_2), 2.87–3.46 (4 H, m, 5-H_2 and $CHCH_2Ph$), 4.31–4.94 (2 H, m, 2-H and $CHCH_2Ph$), 5.08 (2 H, s, CO_2CH_2Ph), 6.74–7.38 (10 H, m, Ph), and 7.50 (1 H, br s, NH); *m/z* (electron impact) 394 (2%, M^+), 319 (30), 140 [6, *M* – $NHCH(CH_2Ph)CO_2CH_2Ph$], 112 [60, *M* – $CONHCH(CH_2Ph)CO_2CH_2Ph$], 108 (13), 91 (25, $C_7H_7^+$), 77 (16, Ph^+), 70 [100, *M* – Ac – $CONHCH(CH_2Ph)CO_2CH_2Ph$], and 43 (15, Ac).

N-(N'-*Acetyl*-L-*prolyl*) *Peptides* (**5f–h**).—These were prepared from the corresponding benzyl esters (**5a–c**) by hydrogenolysis in absolute EtOH over 5% Pd–C, as described above for the conversion of (**10**) into (**11**).

N-(N'-*Acetyl*-L-*prolyl*)*glycine* (**5f**). After complete uptake of hydrogen (*ca.* 1 h), the catalyst was filtered off and washed with water to dissolve out (**5f**), which is only sparingly soluble in alcohol. The combined filtrate and washings were concentrated by vacuum rotary evaporation and the residue was recrystallised from water to give *N*-(*N'*-acetyl-L-prolylglycine (**5f**) as a white, crystalline solid (253 mg, 83%); m.p. 206–207 °C (decomp.) (Found: C, 50.7; H, 6.6; N, 12.95. $C_9H_{14}N_2O_4$ requires C, 50.46; H, 6.59; N, 13.08%); ν_{max}(Nujol) 3 320 (NH), 3 200–2 100 (CO_2H), 1 740 (acid CO), 1 655 (amide CO), 1 610 (amide CO), and 1 540 cm^{-1} (amide II); $\delta_H[(CD_3)_2SO]$ 1.86 and 1.94 (3 H, s, Ac), 1.60–2.13 (4 H, m, 3-H_2 and 4-H_2), 3.08–3.55 (2 H, m, 5-H_2), 3.55–3.82 (2 H, m, CH_2CO_2H), 4.11–4.39 (1 H, m, 2-H), and 8.19 (1 H, br s, NH).

N-(N'-*Acetyl*-L-*prolyl*)-L-*alanine* (**5g**). From *N*-(*N'*-acetyl-L-prolyl)-L-alanine benzyl ester (**5b**) (640 mg, 2 mmol) to give, after recrystallisation from EtOH, *N*-(*N'*-acetyl-L-prolyl)-L-alanine (**5g**) as a white solid (320 mg, 70%); m.p. 255–257 °C (Found: C, 52.65; H, 7.15; N, 12.15. $C_{10}H_{16}N_2O_4$ requires C, 52.62; H, 7.07; N, 12.27%); ν_{max}(Nujol) 3 350 and 3 310 (NH), 3 200–2 400 (CO_2H), 1 735 (acid CO), 1 660 (amide CO), 1 610 (amide CO), 1 560 cm^{-1} (amide II); $\delta_H[(CD_3)_2SO]$ 1.26 and 1.28 (3 H, d, *J* 5 Hz, CH*Me*), 1.83 and 1.95 (3 H, s, Ac), 1.6–2.3 (4 H, m, 3-H_2 and 4-H_2), 3.1–3.7 (2 H, m, 5-H_2), 4.0–4.4 (2 H, m, 2-H and C*H*Me), 8.11–8.35 (1 H, d, *J* 6 Hz, NH), and 11.8–12.9 (1 H, s, CO_2H).

N-(N'-*Acetyl*-L-*prolyl*)-L-*phenylalanine* (**5h**). From *N*-(*N'*-acetyl-L-prolyl-L-phenylalanine benzyl ester (**5c**) (395 mg, 1 mmol) to give, after recrystallisation from a mixture of MeOH and EtOH, *N*-(*N'*-acetyl-L-prolyl)-L-phenylalanine (**5h**) as a white solid (270 mg, 82%); m.p. 201–203 °C (Found: C, 63.1; H, 6.5; N, 9.15. $C_{16}H_{20}N_2O_4$ requires C, 63.14; H, 6.62; N, 9.20%); ν_{max}(Nujol) 3 320 (NH), 3 100–2 200 (CO_2H), 1 735 (acid CO), 1 660 (amide CO), 1 595 (arom. C=C), 1 550 (amide II), and 705 cm^{-1} (Ph); $\delta_H[(CD_3)_2SO]$ 1.41–2.05 (7 H, m, Ac, 3-H_2, and 4-H_2), 2.70–3.68 (4 H, m, 5-H and CH_2Ph), 3.98–4.70 (2 H, m, 2-H and $CHCH_2Ph$), and 7.23 (5 H, s, Ph).

N-(N'-*Acetyl*-L-*prolyl*)-N-*nitroso Peptides* (**1a–h**).—These were synthesised by aprotic nitrosation (using N_2O_4) of the appropriate ester (**5a–e**), followed by removal of the benzyl group by hydrogenolysis over Pd–C catalyst to obtain (**1f–h**).

N-(N'-*Acetyl*-L-*prolyl*)-N-*nitrosoglycine* (**1f**). To a mixture of *N*-(*N'*-acetyl-L-prolyl)glycine benzyl ester (**5a**) (1.52 g, 5 mmol) and anhydrous sodium acetate (1.64 g, 20 mmol) in dry CH_2Cl_2 (30 cm^3) at –10 °C was added N_2O_4 (340 μl, 5.5 mmol) over 1 min. The suspension was stirred for 30 min at –10 °C, then diluted with water (50 cm^3). The organic phase was separated, washed successively with 5% aq. Na_2CO_3 (4 × 20 cm^3), water (2 × 20 cm^3), and brine (20 cm^3), and then dried ($MgSO_4$). Removal of the solvent under reduced pressure gave *N*-(*N'*-acetyl-L-prolyl)-*N*-nitrosoglycine benzyl ester (**1a**) as a yellow–orange oil (1.62 g, 97%); λ_{max}(EtOH) 369 (ε 32 dm^3 mol^{-1} cm^{-1}), 386 (66), 402 (108), and 421 nm (107); $\nu_{max}(CCl_4)$ 1 750 (ester CO and nitrosamide CO), 1 660 (amide CO), and 1 510 cm^{-1} (NO); $\delta_H(CDCl_3)$ 2.20 (3 H, s, Ac), 1.7–2.6 (4 H, m, 3-H_2 and 4-H_2), 3.5–3.9 (2 H, m, 5-H_2), 4.60 (2 H, s, $CH_2CO_2CH_2Ph$), 5.10 (2 H, s, CH_2Ph), 5.6–5.8 (1 H, m, 2-H), and 7.30 (5 H, s, Ph).

The benzyl ester (**1a**) (667 mg, 2 mmol) in absolute EtOH (20 cm^3) containing 5% Pd–C (50 mg) was stirred at room temperature under 760 mmHg of hydrogen. When the uptake of hydrogen was complete (*ca.* 1 h), the catalyst was removed by filtration and the EtOH removed under reduced pressure and replaced by EtOAc (10 cm^3). On addition of light petroleum (35 cm^3, b.p. 40–60 °C) a yellow precipitate formed, which was recrystallised from EtOAc at 60 °C to give *N*-(*N'*-acetyl-L-prolyl)-*N*-nitrosoglycine (**1f**) as a yellow crystalline solid (210 mg, 43%), m.p. 110 °C (Found: C, 44.55; H, 5.3; N, 17.1. $C_9H_{13}N_3O_5$ requires C, 44.45; H, 5.39; N, 17.28%); λ_{max}-

(EtOH) 389 (ε 53 dm^3 mol^{-1} cm^{-1}), 403 (88), and 426 nm (91); ν_{max}(Nujol) 3 200–2 300 (CO_2H), 1 740 (nitrosamide CO), 1 720 (acid CO), 1 590 (amide CO), and 1 510 cm^{-1} (NO); δ_H[$(CD_3)_2CO$] 2.10 (3 H, s, Ac), 1.7–2.6 (4 H, m, 3-H_2 and 4-H_2), 3.4–3.8 (2 H, m, 5-H_2), 4.60 (2 H, s, CH_2CO_2H), and 5.5–5.9 (1 H, m, 2-H); *m/z* (FAB +ve ion) 244 (7%, MH^+), 180 (3), 171 (6, *M* − NO − CH_2CO), 158 (23), 140 [66, *M* − N(NO)CH_2CO_2H], 112 [77, *M* − CON(NO)CH_2CO_2H], 70 [100, *M* − Ac − CON(NO)CH_2CO_2H], and 43 (19, Ac).

N-(N′-*Acetyl*-L-*prolyl*)-N-*nitroso*-L-*alanine* (**1g**). From *N*-(*N*′-acetyl-L-prolyl)-L-alanine benzyl ester (**5b**) (159 mg, 0.5 mmol) as for (**5a**) above to give *N*-(*N*′-acetyl-L-prolyl)-*N*-nitroso-L-alanine benzyl ester (**1b**) as a yellow-orange oil (175 mg, 97%); λ_{max}(EtOH) 405 (ε 33 dm^3 mol^{-1} cm^{-1}) and 423 nm (30); ν_{max}(CCl_4) 1 750 (ester CO and nitrosamide CO), 1 660 (amide CO), and 1 510 cm^{-1} (NO); δ_H($CDCl_3$), 1.35 (3 H, d, *J* 7 Hz, CH*Me*), 2.01 (4 H, m, 3-H_2 and 4-H_2), 2.09 (3 H, s, Ac), 3.61 (2 H, m, 5-H_2), 5.04 (2 H, s, CO_2CH_2Ph), 5.19 (1 H, q, *J* 7 Hz, C*H*Me), 5.58 (1 H, m, 2-H), and 7.18 (5 H, m, Ph).

The benzyl ester group of (**1b**) (518 mg, 1.5 mmol) was removed by catalytic hydrogenolysis as for (**1a**) above. In this case, however, the product was precipitated from EtOAc by the addition of ether and recrystallised from EtOAc–ether to give *N*-(*N*′-acetyl-L-prolyl)-*N*-nitroso-L-alanine (**1g**) as a yellow solid (180 mg, 47%); m.p. 109 °C (Found: C, 46.55; H, 5.8; N, 15.65. $C_{10}H_{15}N_3O_5$ requires C, 46.69; H, 5.88; N, 16.33%); ν_{max}(Nujol) 3 300–2 200 (CO_2H), 1 750 (acid CO and nitrosamide CO), 1 600 (amide CO), and 1 510 cm^{-1} (NO); δ_H($CDCl_3$) 1.26 (3 H, d, *J* 7 Hz, CH*Me*), 1.80–2.35 (4 H, m, 3-H_2 and 4-H_2), 2.23 (3 H, s, Ac), 3.40–3.70 (2 H, m, 5-H_2), 4.52 (1 H, q, *J* 7 Hz, C*H*Me), and 5.29 (1 H, m, 2-H); *m/z* (FAB +ve ion) 259 (6%, MH^+), 185 (15, *M* − NO − CH_2CO), 158 (23), 140 [57, *M* − N(NO)CH_2CO_2H], 112 [64, *M* − CON(NO)CH_2CO_2H], 70 [100, *M* − Ac − CON(NO)CH_2CO_2H], and 43 (16, Ac).

N-(N′-*Acetyl*-L-*prolyl*)-N-*nitroso*-L-*phenylalanine* (**1h**). From *N*-(*N*′-acetyl-L-phenylalanine benzyl ester (**5c**) (395 mg, 1 mmol) as for (**5a**) above to give *N*-(*N*′-acetyl-L-prolyl)-*N*-nitroso-L-phenylalanine benzyl ester (**1c**) as a thick yellow oil (415 mg, 98%); λ_{max}(EtOH) 391 (ε 35 dm^3 mol^{-1} cm^{-1}), 406 (48), and 427 nm (47); ν_{max}(CCl_4) 1 745 (ester CO and nitrosamide CO), 1 655 (amide CO), 1 515 (NO), and 700 cm^{-1} (Ph); δ_H ($CDCl_3$) 1.33–2.17 (4 H, m, 5-H_2 and $CHCH_2Ph$), 5.02 (2 H, s, CO_2CH_2 Ph), 5.30–5.69 (2 H, m, 2-H and $CHCH_2Ph$), and 6.76–7.38 (10 H, m, Ph); *m/z* (FAB +ve ion) 140 [13%, *M* − N(NO)$CH(CH_2Ph)CO_2CH_2Ph$], 112 [38, *M* − CON(NO)$CH(CH_2Ph)CO_2CH_2Ph$], 91 (63, $C_7H_7^+$), 70 [97, *M* − Ac − CON(NO)$CH(CH_2Ph)CO_2CH_2Ph$], 52 (100), and 39 (32).

The benzyl ester group was removed from (**1c**) (850 mg, 2 mmol) by catalytic hydrogenolysis as for (**1a**) above. *N*-(*N*′-Acetyl-L-prolyl)-*N*-nitroso-L-phenylalanine (**1h**) was isolated as a yellow solid following recrystallization from EtOAc–light petroleum (225 mg, 34%); m.p. 177 °C; ν_{max}(Nujol) 3 500–2 300 (CO_2H), 1 735 (acid CO and nitrosamide CO), 1 610 (amide CO), 1 520 (NO), and 705 cm^{-1} (Ph); δ_H($CDCl_3$) 1.52–2.34 (4 H, m, 3-H_2 and 4-H_2), 2.20 (3 H, s, Ac), 2.90–3.78 (4 H, m, CH_2Ph and 5-H_2), 4.37–4.83 (1 H, m, $CHCO_2H$), 5.31–5.78 (1 H, m, 2-H), 6.74–7.44 (5 H, m, Ph), and 920 (1 H, br s, CO_2H).

N-(N′-*Acetyl*-L-*prolyl*)-N-*nitrosoglycine ethyl ester* (**1d**). From *N*-(*N*′-acetyl-L-prolyl)-*N*-glycine ethyl ester (**5d**) as for (**5a**) above to give *N*-(*N*′-acetyl-L-prolyl)-*N*-nitrosoglycine ethyl ester (**1d**) as a thick yellow oil (70 mg, 98%); ν_{max}(CCl_4) 1 740 (ester CO and nitrosamide CO), 1 650 (amide CO), and 1 510 cm^{-1} (NO); δ_H($CDCl_3$), 1.20 (3 H, t, *J* 7 Hz, $CO_2CH_2CH_3$), 2.01 (3 H, s, Ac), 1.69–2.38 (4 H, m, 3-H_2 and 4-H_2), 3.42–3.83 (2 H, m, 5-H_2), 4.07 (2 H, q, *J* 7 Hz, $CO_2CH_2CH_3$), 4.40 (2 H, s, CH_2CO_2Et), and 5.4–5.78 (1 H, m, 2-H).

N-(N′-*Acetyl*-L-*prolyl*)-N-*nitroso*-L-*alanine ethyl ester* (**1e**). From *N*-(*N*′-acetyl-L-prolyl)-L-alanine ethyl ester (**5e**) as for (**5a**) above to give *N*-(*N*′-acetyl-L-prolyl)-*N*-nitroso-L-alanine ethyl ester (**1e**) as a thick, yellow oil (68 mg, 98%); λ_{max}(EtOH) 394 (ε 44 dm^3 mol^{-1} cm^{-1}), 407 (70), and 428 nm (72); ν_{max}(CCl_4) 1 745 (ester CO and nitrosamide CO), 1 660 (amide CO), and 1 510 cm^{-1} (NO); δ_H($CDCl_3$) 1.18 (3 H, t, *J* 7 Hz, $CO_2CH_2CH_3$), 1.32 (3 H, d, *J* 7 Hz, CH*Me*), 2.13 (3 H, s Ac), 1.9–2.5 (4 H, m, 3-H_2 and 4-H_2), 3.72 (2 H, m, 5-H_2), 4.10 (2 H, q, *J* 7 Hz, $CO_2CH_2CH_3$), 5.19 (1 H, q, *J* 7 Hz, C*H*Me), and 5.70 (1 H, m, 2-H); *m/z* (FAB +ve ion) 286 (0.3%, MH^+), 158 (17), 140 [34, *M* − N(NO)CHMeCO_2Et], 112 [75, *M* − CON(NO)-CHMeCO_2Et], and 70 [100, *M* − Ac − CON(NO)CHMe-CO_2Et].

N-*Phthalimidoacetyl*-N-*nitrosoglycine Esters* (**2a–b**).—These were also prepared by the aprotic nitrosation (using N_2O_4) of the corresponding ester, which was obtained by coupling phthalimidoacetic acid with glycine ester in the presence of DCC.

N-*Phthalimidoacetylglycine ethyl ester* (**3a**). To a solution of phthalimidoacetic acid (2.05 g, 10 mmol) in dry THF (30 cm^3) was added a solution of DCC (2.27 g, 11 mmol) also in dry THF (10 cm^3) followed by a solution of glycine ethyl ester (1.03 g, 10 mmol) in dry THF (10 cm^3). A white precipitate formed immediately, and the suspension was stirred at room temperature under N_2 for 4 h. Glacial HOAc (1 cm^3) was then added, and the solvent was removed under reduced pressure and replaced by CH_2Cl_2 (50 cm^3). The precipitated dicyclohexylurea was removed by filtration, and the filtrate was washed with water (6 × 10 cm^3) and dried ($MgSO_4$). Solvent removal under reduced pressure left a white solid which was recrystallised from EtOH to give *N*-phthalimodoacetylglycine ethyl ester (**3a**) (2.75 g, 95%); m.p. 192 °C (Found: C, 58.0; H, 4.85; N, 9.55. $C_{14}H_{14}N_2O_5$ requires C, 57.93; H, 4.86; N, 9.65%); ν_{max}(Nujol) 3 290 (NH), 1 725 (imide CO and ester CO), 1 715 (imide CO), and 1 645 cm^{-1} (amide CO); δ_H($CDCl_3$) 1.24 (3 H, t, *J* 7 Hz, $CO_2CH_2CH_3$), 4.01 and 4.07 (2 H, s, $NHCH_2CO_2Et$), 4.35 (2 H, q, *J* 7 Hz, $CO_2CH_2CH_3$), and 7.7–8.0 (5 H, m, Ph and CONH); *m/z* (electron impact) 290 (13%, M^+), 217 (15, *M* − CO_2Et), 188 (27, *M* − $NHCH_2CO_2Et$), and 161 (100).

N-*Phthalimidoacetyl*-N-*nitrosoglycine ethyl ester* (**2a**). To a mixture of *N*-phthalimidoacetylglycine ethyl ester (**3a**) (870 mg, 3 mmol) and anhydrous NaOAc (0.98 g, 12 mmol) in dry CH_2Cl_2 (25 cm^3) at −10 °C was added liquid N_2O_4 (220 μl, 3.5 mmol) over 1 min. The suspension was stirred for 30 min at −10 °C, then diluted with water (40 cm^3). The organic phase was separated, washed successively with 5% aq. $NaHCO_3$ (3 × 20 cm^3), water (2 × 20 cm^3), and brine (20 cm^3), and then dried ($MgSO_4$). Solvent removal under reduced pressure left a yellow solid, which was recrystallised from ether to give *N*-phthalimidoacetyl-*N*-nitrosoglycine ethyl ester (**2a**) as yellow crystals (805 mg, 84%); m.p. 117–118 °C (Found: C, 52.55; H, 4.0; N, 13.1. $C_{14}H_{13}N_3O_6$ requires C, 52.67; H, 4.10; N, 13.16%); λ_{max}(Et_2O) 404 (ε 100 dm^3 mol^{-1} cm^{-1}) and 423 nm (110); ν_{max}(Nujol) 1 765 (imide CO), 1 730 (ester CO, nitrosamide CO, and imide CO), and 1 500 cm^{-1} (NO); δ_H($CDCl_3$) 1.23 (3 H, t, *J* 7 Hz, CO_2CH_2Me), 4.15 (2 H, q, *J* 7 Hz, CO_2CH_2Me), 4.49 (2 H, s, CH_2CO_2Et), 5.33 (2 H, s, $CH_2CON(NO)$], and 7.78 (4 H, m, ArH).

N-*Phthalimidoacetylglycine benzyl ester* (**3b**). As for (**3a**) using glycine benzyl ester (1.65 g, 10 mmol). After recrystallization from EtOH, *N*-phthalimidoacetylglycine benzyl ester (**3b**) was obtained as a white solid (2.54 g, 72%); m.p. 158 °C (Found: C, 64.9; H, 4.55; N, 7.95. $C_{19}H_{16}N_5O_5$ requires C, 64.77; H, 4.58; N, 7.95%); ν_{max}(Nujol) 3 320 (NH), 1 730 (ester CO and imide CO), 1 675 (amide CO), 1 570 (amide II), and 760, 720 and 705 cm^{-1} (Ph); δ_H($CDCl_3$) 3.98 and 4.06 (2 H, s,

$CH_2CO_2CH_2Ph$), 4.31 (2 H, s, CH_2CONH), 5.11 (2 H, s, CO_2CH_2Ph), 7.26 (5 H, s, Ph), and 7.61–7.82 (5 H, m, ArH and CONH).

N-*Phthalimidoacetyl*-N-*nitrosoglycine benzyl ester* (**2b**). As for (**2a**) from the benzyl ester (**3b**) (96 mg, 300 μmol). After recrystallization from MeOH, *N*-phthalimidoacetyl-*N*-nitrosoglycine benzyl ester (**2b**) was obtained as a yellow solid (97 mg, 92%); m.p. 119 °C (Found: C, 59.2; H, 4.2; N, 11.35. $C_{19}H_{15}N_3O_6$ requires C, 59.84; H, 3.97; N, 11.02%); $\lambda_{max}(Et_2O)$ 386 (ε 67 dm^3 mol^{-1} cm^{-1}), 403 (110), and 422 nm (116); ν_{max}(Nujol) 1 735 (ester CO, nitrosamide CO, and imide CO), 1 520 (NO), and 720 cm^{-1} (Ph); $\delta_H(CDCl_3)$ 4.47 (2 H, s, $CH_2CO_2CH_2Ph$), 5.03 (2 H, s, $CH_2CON(NO)$], 5.28 (2 H, s, CO_2CH_2Ph), 7.25 (5 H, s, Ph), and 7.63–7.89 (4 H, m, ArH).

N-(N′-*Triphenylmethylglycyl*)*glycine Benzyl Ester* (**4**). A suspension of *N*-glycylglycine (3.30 g, 25 mmol) and toluene-4-sulphonic acid monohydrate (4.95 g, 26 mmol) in a mixture of benzyl alcohol (25 cm^3) and benzene (30 cm^3) was heated at 60 °C to form a yellow solution. The solution was heated under reflux with a Dean and Stark trap until azeotropic removal of water was complete (*ca.* 12 h). The resulting solution was allowed to reach ambient temperature, diluted with CH_2Cl_2 (75 cm^3), and the resulting white precipitate was collected and air dried, then suspended in CH_2Cl_2 (30 cm^3) and treated with Et_3N (2.5 g, 25 mmol), to form a clear solution. A solution of chlorotriphenylmethane (6.8 g, 25 mmol) in dry CH_2Cl_2 (10 cm^3) was then added over 10 min and the resulting solution allowed to stand under argon for 20 h at ambient temperature. The solution was then washed with water (3 × 30 cm^3) and dried (Na_2SO_4). Solvent removal under reduced pressure followed by recrystallization from EtOH gave *N*-(*N*′-triphenylmethylglycyl)glycine benzyl ester (**4**) as a white solid (5.8 g, 50%); m.p. 152–153 °C (Found: C, 77.6; H, 6.05; N, 6.0. $C_{30}H_{28}N_2O_3$ requires C, 77.56; H, 6.07; N, 6.03%); ν_{max}(Nujol) 3 320 (NH), 1 740 (ester CO), 1 650 (amide I), and 750, 720, 710 and 700 cm^{-1} (Ph); $\delta_H(CDCl_3)$ 2.20 (1 H, br s, D_2O exch., Ph_3CNH), 2.98 (2 H, s, $NHCH_2CONH$), 4.06 and 4.15 (2 H, s, $CH_2CO_2CH_2Ph$), 5.19 (2 H, s, CO_2CH_2Ph), and 7.1–7.4 (21 H, m, 4 × Ph and CONH); *m*/*z* (electron impact) 464 (3%, M^+), 387 (30, M − Ph), 243 (100, Ph_3C^+), 165 (23, Ph_3C^+ − PhH), and 91 (25, $C_7H_7^+$).

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