Conjugate Addition with Organometallics and Nitration of Nitroxide (Aminoxyl) Free Radicals. Synthesis of Potentially Useful Cross-Linking Spin Label Reagents

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3-Methoxycarbonyl-2,2,5,5-tetramethyl-2,5-dihydropyrrol-1-oxyl (**1**) was converted with PhMgBr–CuI in a conjugate addition into 3-methoxycarbonyl-2,2,5,5-tetramethyl-4-phenylpyrrolidin-1-oxyl (**2**). Compound (**2**) was nitrated with concentrated H_2SO_4/HNO_3 to give the *para*-nitrophenyl derivative (**5**). Nitration of other phenyl substituted nitroxides was also investigated. The reaction in strong acids could be carried out in the presence of an acid-sensitive nitroxide free radical. An alkyl nitro compound has also been prepared: a conjugate addition of nitromethane to (**1**) gave 3-methoxycarbonyl-2,2,5,5-tetramethyl-4-nitromethylpyrrolidin-1-oxyl (**20**). Both (**2**) and (**20**) could be transformed by transfer hydrogenation to amino compounds and then in several steps to potentially useful cross-linking spin label reagents.

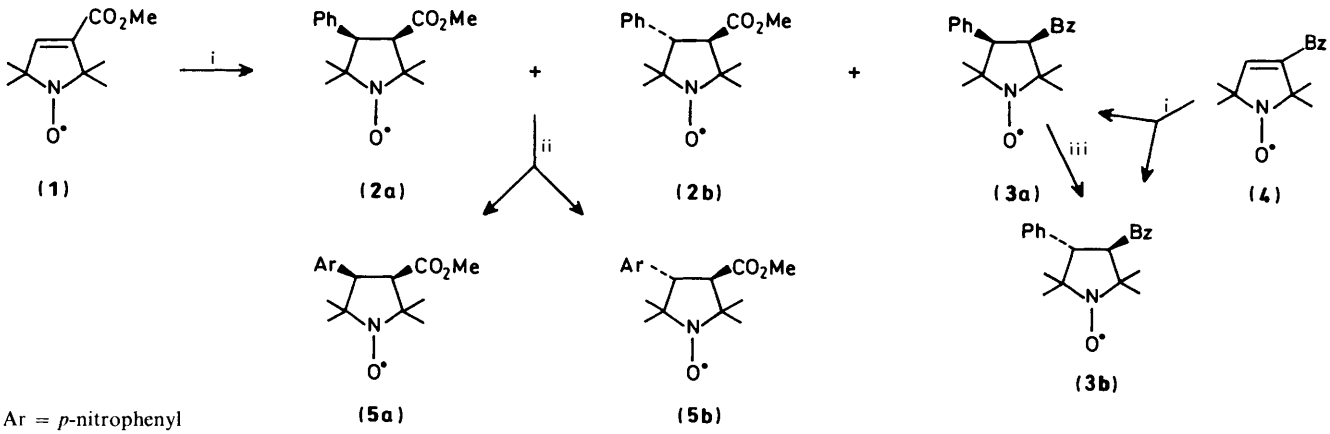
It is well known that chemically reactive groups containing nitroxide free radicals are useful reporter groups for spin labelling biomacromolecules.[1,2] Most of these reagents are one arm-reagents and, until recently, relatively few two-arm spin label reagents had been reported.[3–11] There is a need to produce such bifunctionalized spin label reagents having reactive groups (*e.g.* azido, maleimido, acylimidazole, *etc.*) analogues to the well known non-spin-label cross-linking reagents, in order to establish cross-links in biomacromolecules containing two functional groups so that their anisotropic motion can be studied by saturation e.s.r. spectroscopy (ST-e.s.r.).[12–14]

Since the main driving force has been in developing new spin label reagents to achieve better selectivity and less perturbation on the biological system, the chemistry of nitroxide compounds has rapidly expanded.[15,16] Utilization of nitroxides in medicine as contrast enhancers in n.m.r. imaging[17,18] has also given impetus to research in this area. It has generally been accepted that while the nitroxide entity is relatively inert to many chemical reactions, it is somewhat sensitive towards thiols, ascorbate, organometallic (Grignard reagents, alkyl-lithium) reagents and strong acids.

Our laboratory, which has a research programme for the development of new nitroxides, has shown that under carefully chosen reaction conditions and with suitably functionalized nitroxides even free thiol group-containing nitroxides[19] can be prepared and nitroxide acid derivatives can be used in Friedel-Crafts reactions with $AlCl_3$ as electrophilic reagent for the acylation of aromatics (*e.g.* benzene or toluene).[20]

Both Grignard reagents and alkyl-lithiums have also been successfully applied to the alkylation of 3-carboxy-2,5-dihydropyrrol-1-oxyl derivatives and 3-aldehydes.[5,6] In these reactions 1,2-addition takes place without any 1,4-conjugate addition so that the α,β-double bond remains intact.

The main objectives of the present work were to investigate various conjugate additions to the double bond of the unsaturated nitroxide ester (**1**) in order to build reactive functional groups into the 3,4-position of pyrrolidin-1-oxyl and thus prepare new compounds analogous to known cross-linking reagents. 3-Methoxycarbonyl-2,2,5,5-tetramethyl-4-phenylpyrrolidin-1-oxyl (**2**) could be obtained from conjugate addition of diphenylcopper(I)–magnesium bromide to the α,β-unsaturated ester (**1**). In the reaction, beside the *cis*- and *trans*-isomer of (**2**), a minor side-product 3-benzoyl-2,2,5,5-tetramethyl-4-phenylpyrrolidin-1-oxyl (**3a**) was also isolated, this arose as a result of a reaction at the ester group, probably as a result of PhMgBr in the reaction mixture. The reaction of 3-benzoyl-2,2,5,5-tetramethyl-2,5-dihydropyrrol-1-oxyl (**4**)

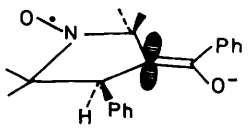


Ar = *p*-nitrophenyl

Scheme 1. *Reagents:* i, PhMgBr, CuI, Et_2O, then at work-up PbO_2/air; ii, H_2SO_4, HNO_3; iii, MeONa

with diphenylcopper(I)–magnesium bromide upon conjugate addition gave a mixture of stereoisomers of (**3**). The spontaneous reoxidation of labile *N*-hydroxy compounds formed in these reactions can be catalysed by PbO_2/air (Scheme 1).

In good agreement with the kinetic protonation of enolates,[21,22] as described in an early report on the protonation of the enolate obtained from Cu^{I}Cl catalysed 1,4-conjugate addition of PhMgBr to 1-benzoylcyclohexene,[21] the protonation of the enolate of (**3**) from the less hindered side resulted in the predominant formation of the less stable *cis*-isomer saturated ketone (**3a**) over the *trans*-isomer (**3b**). The *cis*-isomer (**3a**) could be converted into the *trans*-(**3b**) with sodium methoxide in methanol.



(**3**) - enolate

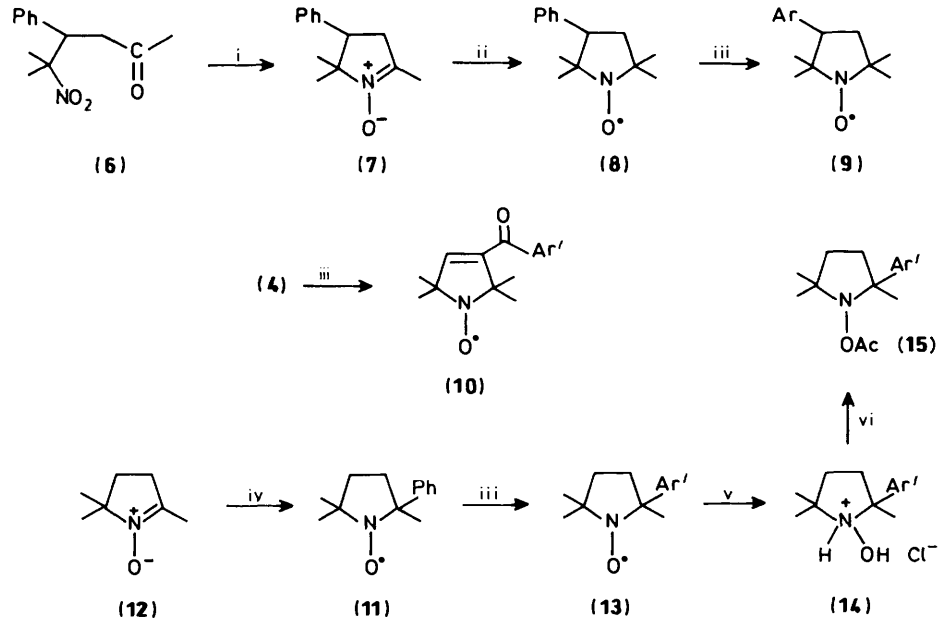
The n.m.r. spectra of paramagnetic species could be determined when the sample was examined in the presence of diphenylhydrazine as a reducing agent or converted into the diamagnetic *O*-acetyl derivative. The isomeric mixture (**2a,b**) was nitrated in concentrated H_2SO_4 at 0 °C with 67% HNO_3 to give the *para*-nitrophenyl derivatives (**5a,b**) (Scheme 1). The stereoisomers (**5a,b**) could be separated by chromatography. Because no special care was necessary during nitration and the work-up procedure the *N*-oxyl function was unaffected and the nitration seems to be generally applicable to other nitroxides. For a better understanding of the directing influence of substituents the nitration has been investigated with other phenyldihydropyrrole and pyrrolidine nitroxides. The synthesis of 2,2,2,5-tetramethyl-3-phenylpyrrolidin-1-oxyl (**8**) was achieved by the following steps (see Scheme 2): treating 4-phenylbut-3-en-2-one with 2-nitropropane to give the γ-nitroketone (**6**)[23] and then treatment of the latter with Zn–NH_4Cl to give the nitrone (**7**); the nitrone then reacted with MeMgI to give (**8**). Nitration of the compound (**8**) gave the *para*-nitro derivative (**9**). The 3-benzoyl-2,2,5,5-tetramethyl-2,5-dihydropyrrol-1-oxyl (**4**) prepared earlier was nitrated like acetophenone in the *meta*-position to afford (**10**). The 2,5,5-trimethyl-2-phenylpyrrolidin-1-oxyl (**11**) was obtained from the reaction of known 2,5,5-trimethyl-3,4-dihydro-2*H*-pyrrole 1-oxide (**12**)[24] with PhMgBr. Compound (**11**) was nitrated in the *meta*-position to give (**13**) owing to the influence of the protonated *N*-oxyl group of pyrrolidine.

Compound (**13**) was converted with ethanol–HCl into the labile diamagnetic *N*-hydroxy compound (**14**) which then further reacted with acetyl chloride to give the *O*-acetyl derivative (**15**) (Scheme 2).

The nitro group of (**5**) could be reduced by transfer hydrogenation with an ammonium formate palladium–charcoal catalysed reaction[25,26] to give the amino compound (**16**). The latter was diazotized to afford the azide (**17**) and this upon hydrolysis gave the acid (**18**); upon utilizing Staab's method[27] (**8**) could be converted with 1,1-carbonylbis-1*H*-imidazole (CDI) into the acylimidazole (**19**) (Scheme 3).

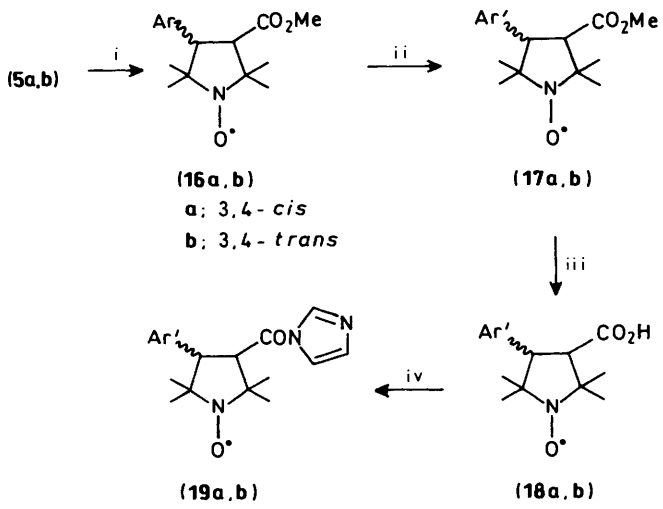
It has been demonstrated in earlier work that the tosylate salt of an acylimidazole has a greatly enhanced reactivity toward nucleophiles (*e.g.* azide) capable of reacting even in aqueous media.[28] Recently this type of reagent has been used for preparing retinoic esters.[29]

In a further approach to achieve a 3,4-bifunctionalized reagent, a nitromethyl substituent was introduced into the β-position of the unsaturated ester (**1**) to give 3-methoxycarbonyl-2,2,5,5-tetramethyl-4-nitromethylpyrrolidin-1-oxyl (**20**). In this reaction only the *trans*-isomer was formed. The reduction of a nitro group to an amine group (**21**) was achieved by transfer hydrogenation. Compound (**21**) on reaction with maleic anhydride and then with acetic anhydride–NaOAc gave the maleimido ester (**22**); the latter was then hydrolysed to the acid (**23**). The introduction of a photosensitive arm could be achieved when (**21**) was treated with 4-fluoro-3-nitrophenyl azide to afford the azidophenylamino ester (**24**) which was hydrolysed to the acid (**25**) (Scheme 4).



Ar = *p*-nitrophenyl, Ar′ = *m*-nitrophenyl

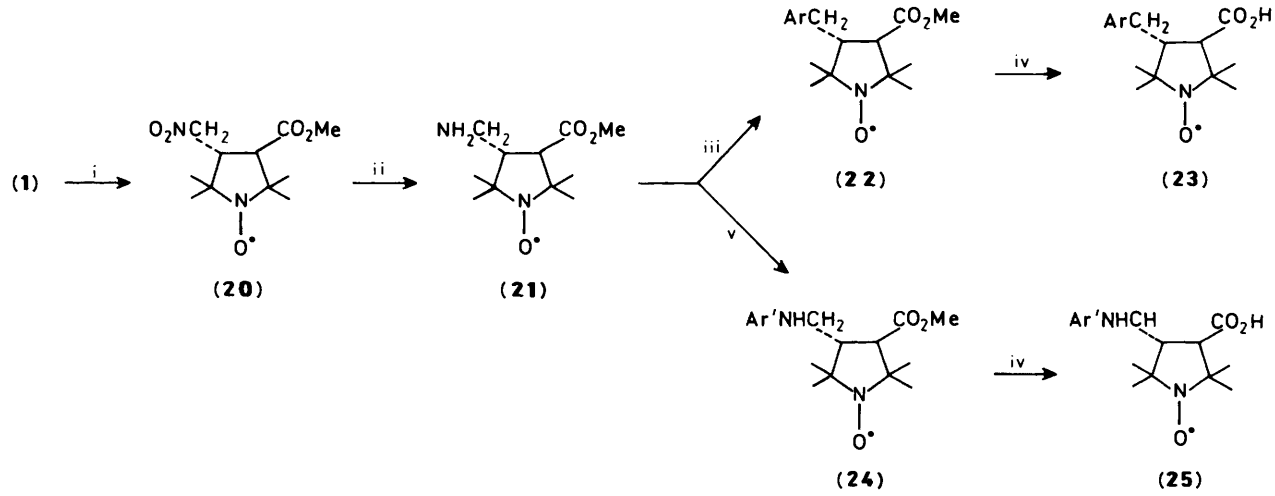
Scheme 2. *Reagents:* i, NH_4Cl, Zn; ii, MeMgI, Et_2O; iii, H_2SO_4, HNO_3; iv, PhMgBr, Et_2O; v, EtOH, HCl, Et_2O; vi, TEA, AcCl



Ar = *p*-aminophenyl, Ar′ = *p*-azidophenyl

Scheme 3. *Reagents:* i, HCO_2NH_4, Pd–C, MeOH; ii, H_2SO_4, $NaNO_2$, NaN_3; iii, NaOH, MeOH; iv, CDI, THF

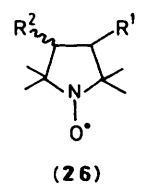
(**3a,b**), (**4a,b**), and (**20**) were run as KBr discs on a Bruker IFS-113v vacuum optic FT-spectrometer equipped with an Aspect 2000 computer. I.r. spectra of the other compounds were measured with Zeiss Specord 75 spectrophotometer, unless otherwise stated, all samples being either suspended in Nujol or dissolved in chloroform. The n.m.r. spectra were recorded in 5 or 10 mm tubes at room temperature on a Bruker WM-250 (^{1}H) or WP.80SY FT-spectrometer (^{13}C) controlled by an Aspect 2000 computer at 250.13 (^{1}H) or 20.14 MHz (^{13}C) in $CDCl_3$ solution using the deuterium signal of the solvent as the lock and TMS as internal reference. To get high resolution spectra of radicals PhNHNHPh additive was used for n.m.r. measurements. The most important measuring parameters were as follows: sweep width: 5 kHz; pulse width: 1 (^{1}H) or 3.5 (^{13}C) μs (*ca.* 20° or 30° flip angle); acquisition time: 1.64: number of scans: 16 or 2^{10}—2^{15}; computer memory: 16 K; Lorentzian exponential multiplication for signal-to-noise enhancement (line width 0.7 or 1.0 Hz) and complete proton noise decoupling (*ca.* 1.5 W) for ^{13}C measurements were applied. The assignments of ^{13}C n.m.r. lines in the spectra of compounds (**5a,b**) were proved by DEPT measurements. DEPT Experiments[30] were performed in a standard way[31] using only the $\theta = 135°$ pulse to separate CH/CH_3 and CH_2 lines phased 'up' and 'down', respectively. Typical acquisition data were: number



Ar = 2,5-dioxopyrrol-1-yl, Ar′ = 4-azido-2-nitrophenyl

Scheme 4. *Reagents:* i, $MeNO_2$, DBU; ii, HCO_2NH_4, Pd–C, MeOH; iii, maleic anhydride, Ac_2O, AcONa; iv, NaOH then H_2SO_4; v, 4-fluoro-3-nitrophenyl azide, TEA, EtOH

Overall, the methodology described here allows the utilization of organomagnesio-cuprate reagents, the nitration in concentrated H_2SO_4 with nitric acid, and transfer hydrogenation for the preparation of such 3,4-bifunctionalized nitroxides such as (**26**): these may establish a novel group of potentially useful spin-labelled cross-linkers.



(**26**)

Experimental

M.p.s were determined using a Büchi micromelting-point apparatus and are uncorrected. The i.r. spectra of compounds

of scans: 128—12 K, relaxation delay for protons 3 s, 90° pulse width: 10.8 and 22.8 μs for ^{13}C and ^{1}H, respectively. The estimated value *J*(C,H) resulted in a 3.7 ms delay for polarization.

Mass spectra were obtained using a MAT-SM-1 spectrometer. The e.s.r. spectra were obtained for 10^{-3} M chloroform solutions on a Zeiss ER-9 spectrometer. All the monoradicals exhibited three equidistant lines a_N = 14.3—14.8 G.

Flash column chromatography on silica gel was performed using Merck Kieselgel 60 (0.040—0.063 mm). Qualitative t.l.c. was carried out commercially prepared plates coated with Merck Kieselgel GF_{254}. Preparative t.l.c. was performed on plates (20 × 20 × 0.2 cm) coated with the same material.

3-Methoxycarbonyl-2,2,5,5-tetramethyl-4-phenylpyrrolidin-1-oxyl (**2**).—A suspension of CuI (19.04 g, 0.10 mol) was added, under nitrogen, to a stirred solution of phenylmagnesium bromide, freshly prepared from bromobenzene (51.84 g, 0.33

mol) and magnesium (7.29 g, 0.30 mol) in dry ether (120 ml) at −30 °C. The mixture was stirred at −30 °C for 30 min after which a solution of the unsaturated ester (**1**) (9.91 g, 0.05 mol) in dry ether (50 ml) was added slowly during 15 min. After being stirred at −30 °C for 3 h the reaction mixture was poured into ice-cooled saturated aqueous ammonium chloride. The ether phase was separated and the aqueous phase further extracted with ether (3 × 50 ml). The ethereal extracts were combined, dried, and evaporated to give a yellow oil which was taken up in $CHCl_3$ (100 ml). PbO_2 Catalyst (2 g) was added to the solution and oxygen was bubbled through it for 15 min. The deep orange solution was evaporated again to dryness and flash chromatographed on silica gel with hexane–ethyl acetate (6:1) as eluant. The first yellow band was a mixture of *cis*, *trans*-isomers of (**2**) (8.4 g, 61%), m.p. 115—116 °C (hexane) (Found: C, 69.3; H, 7.9; N, 4.9. $C_{16}H_{22}NO_3$ requires C, 79.5; H, 8.0; N, 5.0%); $\nu_{max.}$ 1 680 cm^{-1} (C=O); *m/z* 276 (M^+, 25%), 261 (5), 132 (100), and 117 (68).

It was possible to isolate a small amount of the *cis*-isomer (**2a**) in a repeated flash chromatography the mixture of the isomers using the same solvents as above, m.p. 111—112 °C (Found: C, 69.4; H, 7.9; N, 4.9. $C_{16}H_{22}NO_3$ requires C, 69.5; H, 8.0; N, 5.0%); $\nu_{max.}$ 1 680 cm^{-1} (C=O); δ_H 1.07, 1.28, 1.35, 1.36 (4 × 3 H, 4 s, 4-Me), 3.21, 3.40 (2 × 1 H, 2 d, *J* 8.6 Hz, 3- and 4-H), and 3.46 (3 H, s, OCH_3); δ_C 24.7 (2 overlapping lines), 25.4, 25.6 (CCH_3), 50.5 (OCH_3), 54.1, 55.0 (C-3 and -4), 63.9, 65.6 (C-2 and -5), 126.4 (C^pPh), 127.4, 129.9 ($C^{p,m}Ph$), 138.4 (C^sPh), and 171.8 (C=O).

The smaller yellow band proved to be cis-3-*benzoyl*-2,2,5,5-*tetramethyl*-4-*phenylpyrrolidin*-1-*oxyl* (**3a**) (0.8 g, 5%), m.p. 151—152 °C (Found: C, 78.1; H, 7.3; N, 4.45. $C_{21}H_{24}NO_2$ requires C, 78.2; H, 7.5; N, 4.35%); $\nu_{max.}$ 1 670 (C=O), 1 223 ($C_{Ar}C$=O), and 700 cm^{-1} (ArH); δ_H 1.12, 1.31, 1.35, 1.41 (4 × 3 H, 4 s, 4-Me), and 3.55 and 4.23 (2 × 1 H, 2 d, *J* 8.8 Hz, 3- and 4-H); δ_C 24.3, 26.6, 26.7 (CCH_3), 55.9 (C-3 and -4), 65.0, 66.1 (C-2 and -5), 126.4 [C-4′(Ph)], 127.6, 127.7, 128.2, 130.4 [C-2′ and -6′ (Ph + Bz)], 132.1 [C-4′(Bz)], 138.1, 139.9 [C-1′ (Ph + Bz)], and 200.4 (C=O); *m/z* 322 (M^+, 34%), 208 (30), 132 (100), and 105 (82).

Conjugate Addition of Diphenylcopper(I)–*Magnesium Bromide to* 3-*Benzoyl*-2,2,5,5-*tetramethyloxyl*-2,5-*dihydropyrrol*-1-*oxyl* (**4**).—A solution of the unsaturated ketone (**4**) (1.22 g, 5.0 mmol) was added dropwise to a stirred solution of freshly prepared diphenylcopper(I)–magnesium bromide [a tenth of that described above in the preparation of (**2**)] at −30 °C. After being stirred at −30 °C for 3 h the reaction mixture was worked-up as described in the preparation of (**2**). Flash chromatography of the product on silica gel with hexane–ethyl acetate (6:1) gave as the second, larger band the *cis*-isomer (**3a**) (0.97 g, 60%), m.p. 151—152 °C identical with the compound prepared above. The first, smaller band proved to be the *trans*-isomer (**3b**) (0.24 g, 15%), m.p. 193—194 °C (ether–hexane) (Found: C, 78.3; H, 7.5; N, 4.35. $C_{21}H_{24}NO_2$ requires C, 78.2; H, 7.5; N, 4.35%); $\nu_{max.}$ 1 660 (C=O), 1 220 ($C_{Ar}C$=O), 7.45, 695, and 680 cm^{-1} (ArH); δ_H 0.91, 1.00, 1.28, 1.41 (4 × 3 H, 4 s, 4-Me), and 3.78 and 4.41 (2 × 1 H, 2 d, *J* 12.1 Hz, 3- and 4-H); δ_C 20.7, 23.0, 26.6, 26.9 (CCH_3), 51.5, 54.6 (C-3 and -4), 63.6, 65.3 (C-2 and -5), 126.4 [C-4′ (Ph)], 127.7, 127.9, 128.2, 130.5 [C-2′ and -6′ (Ph + Bz)], the line at 130.5 p.p.m. is obscured by a signal of the $(PhNH)_2$ additive, 132.5 [C-4′ (Bz)], 137.9, 138.2 [C-1′ (Ph + Bz)], and 198.4 (C=O).

Isomerisation of (**3a**) *to* (**3b**).—A 30% solution of sodium methoxide (0.1 ml) in dry methanol was added to a solution of (**3a**) (322 mg, 1.0 mmol) in dry methanol (5 ml) and the mixture kept at 50 °C for 30 min. It was then diluted with water, extracted with ether (20 ml), and the extract dried ($MgSO_4$) and evaporated and the residual yellow solid crystallized from ether to give (**3b**) (305 mg, 95%), m.p. 193—194 °C, identical with the above minor product.

3-*Methoxycarbonyl*-2,2,5,5-*tetramethyl*-4-(4-*nitrophenyl*)-*pyrrolidin*-1-*oxyl* (**5a,b**).—To a stirred mixture of (**2a,b**) (8.29 g, 0.03 mol) dissolved in concentrated H_2SO_4 (20 ml) at −5 °C was added dropwise a mixture of concentrated H_2SO_4 (24 ml) and 67% HNO_3 (12 ml), the temperature being kept <0 °C. The deep red reaction mixture became at first colourless and then turned yellow. It was stirred at 0—5 °C for 30 min and then poured onto vigorously stirred crushed ice (*ca.* 300 g) and neutralized with 40% NaOH. The resulting yellow solid product was filtered off, washed with water, and dried (8.0 g, 83%). T.l.c. chromatography [ethyl acetate–hexane (2:4)] showed only two spots for *cis*,*trans*-isomers. These were separable by chromatography on a silica gel column with ethyl acetate–hexane (1:4) as the eluant. The second yellow band was isolated and proved to be the *para*-nitrated *cis*-isomer (**5a**), m.p. 127—128 °C (Found: C, 59.8; H, 6.6; N, 8.5. $C_{16}H_{21}N_2O_5$ requires C, 59.8; H, 6.6; N, 8.7%); $\nu_{max.}$ 1 728 (C=O), 1 524, 1 352 (NO_2), 1 205 ($C_{Ar}C$=O), and 860 cm^{-1} (ArH); δ_H 0.91, 1.25, 1.28, 1.36 (4 × 3 H, s, 4-Me), 3.32, 3.37 (2 × 1 H, 2 d, *J* 8.7 Hz, 3- and 4-H), 3.43 (3 H, s, OCH_3), and 8.04 (2 H, ~d, *J* 8.7 Hz, 3′- and 5′-H_{Ar}); δ_C 24.1, 24.5, 25.4, 26.6 (CCH_3), 50.9 (OCH_3), 54.3, 54.5 (C-3 and -4), 63.7, 65.9 (C-2 and -5), 122.6 [C-3′ and -4′ (Ar)], 131.2 [C-2′ and -6′ (Ar)], 146.8, 147.3 [C-1′ and -4′(Ar)], and 171.1 (C=O); *m/z* 321 (M^+, 38%), 307 (16), 216 (5), 177 (100), and 115 (35). The first yellow band was isolated and proved to the *para*-nitro *trans* isomer (**5b**), m.p. 169—170 °C (Found: C, 60.0; H, 6.4; N, 8.45. $C_{16}H_{21}N_2O_5$ requires C, 59.8; H, 6.6; N, 8.7%); $\nu_{max.}$ 1 734 (C=O), 1 520, 1 350 (NO_2), 1 196 ($C_{Ar}CO$), 862, and 851 cm^{-1} (split band-pair, ArH); δ_H 0.75, 1.11, 1.18, 1.43 (4 × 3 H, 4 s, 4-Me), 3.24, 3.51 (2 × 1 H, *J* 12.5 Hz, 3- and 4-H), 3.75 (3 H, s, OCH_3), 7.30 (2 H, ~d, *J* ≃ 9 Hz, 2′- and 6′-H_{Ar}), and 8.10 (2 H, d, 3′- and 5′-H_{Ar}); δ_C 20.8, 22.8, 26.8, 27.1 (CCH_3), 51.9 (OCH_3), 51.6, 54.7 (C-3 and -4), 63.8, 65.6 (C-2 and -5), 123.3 [C-3′ and -5′ (Ar)], 129.2 [C-2′ and -6′ (Ar)], 146.2, 147.1 [C-1′ and -4′ (Ar)], and 171.7 (C=O); *m/z* 321 (M^+, 75%), 307 (8), 216 (8), 177 (100), and 115 (34).

2,5,5-*Trimethyl*-4-*phenyl*-3,4-*dihydropyrrole* 1-*Oxide* (**7**).—To a stirred solution of the nitro ketone (**6**) (14.15 g, 0.06 mol) [obtained from 4-phenylbut-3-en-2-one and 2-nitropropane by a literature procedure [23]] in tetrahydrofuran (THF) was added a solution of NH_4Cl (3.21 g, 0.06 mol) in water (10 ml); the mixture was cooled (~5 °C) and zinc powder (15.59 g, 0.24 mol) was added at such a rate that the reaction temperature did not arise over 10 °C. The mixture was filtered, the filtrate evaporated to dryness, and the residue taken up in ether (100 ml). The solution was then washed with water and brine, dried ($MgSO_4$), and evaporated. The residue was distilled *in vacuo* (b.p. 140—144 °C/1.2 mmHg) to give the nitrone (**7**), m.p. 69—70 °C (ether–hexane) (Found: C, 76.95; H, 8.3; N, 6.9. $C_{13}H_{17}NO$ requires C, 76.8; H, 8.4; N, 6.9%); δ_H 0.96, 1.50, 2.12 (3 × 3 H, s, 3-Me), 2.6—3.6 (3 H, m, $CHCH_2$), and 7.1—7.4 (5 h, m, HAr).

2,2,5,5-*Tetramethyl*-3-*phenylpyrrolidin*-1-*oxyl* (**8**).—To a stirred solution of MeMgI [freshly prepared from MeI (11.35 g, 0.08 mol) and magnesium (1.92 g, 0.08 mol) in dry ether (100 ml)] was added slowly under a nitrogen atmosphere a solution of the nitrone (**7**) (8.2 g, 0.04 mol) in dry ether (30 ml); the mixture was stirred at room temperature for 6 h and then quenched with saturated aqueous NH_4Cl (40 ml). The ether phase was separated, washed with saturated brine, and evaporated to dryness. The residue was taken up in $CHCl_3$ (100 ml) and the solution well stirred over anhydrous $MgSO_4$ in the presence of PbO_2 (2 g) for 1 h; during this time the colour of

solution turned to orange-yellow. The filtrate was evaporated and the residue column chromatographed on silica gel [hexane–ethyl acetate (9:11)] to give the orange-yellow crystalline nitroxide (**8**) (6.54 g, 75%), m.p. 85—86 °C (Found: C, 76.95; H, 9.0; N, 6.45. $C_{14}H_{20}NO$ requires C, 77.0; H, 9.2; N, 6.4%); δ_H 0.82, 1.21, 1.29, 1.34 (4 × 3 H, 4 s, 4-Me), 1.82 (1 H, dd, ^2J 12.5 Hz, ^3J 6.9 Hz, 4-H *cis*), 2.22 (1 H, t, ^3J 13 Hz, 4-H *trans*), and 3.02 (1 H, dd, 3-H); δ_C 17.9, 26.3, 26.4, 28.3 (CCH_3), 40.7 (C-4), 48.6 (C-3), 60.8 (C-5), 66.9 (C-2), 127.2, 128.0 ($C^{o,m}$Ph), 125.9 (C^pPh), and 138.9 (C^sPh).

2,2,5,5-*Tetramethyl*-3-(4-*nitrophenyl*)*pyrrolidin*-1-*oxyl* (**9**).—A solution of compound (**8**) (2.18 g, 0.01 mol) in concentrated H_2SO_4 (10 ml) was nitrated with a mixture of concentrated H_2SO_4–67% HNO_3 [3:2 (v/v); 5 ml] and worked up as described in the preparation of compound (**5**). The yellow crystalline *para*-nitro compound (**8**) (1.71 g, 65%), m.p. 142—143 °C (Found: C, 63.9; H, 7.1; N, 10.6. $C_{14}H_{19}N_2O_3$ requires C, 63.85; H, 7.25; N, 10.6%); $\nu_{max.}$ 1 540 and 1 360 cm^{-1} (NO_2); δ_H 0.77, 1.19, 1.26, 1.33 (4 × 3 H, 4 s, 4-Me), 1.88 (1 H, dd, ^2J 12.5, ^3J 7.1 Hz, 4-H *cis*), 2.18 (1 H, t, ^3J 12.5 Hz, 4-H *trans*), 3.11 (1 H, dd), 7.34 (2 H, d, J 8.8 Hz, 2′- HAr 6′-HAr), and 8.12 (2 H, 3′- and HAr 5′-HAr); δ_C 18.7, 26.7, 26.8, and 2.83 (CCH_3), 41.0 (C-4), 49.3 (C-3), 61.4 (C-5), 67.2 (C-2), 122.9 [C-3′ and -5′ (Ar)], 129.5 [C-2′ and -6′ (Ar)], 146.7, 147.5 [C-1′ and -4′ (Ar)]; *m/z* 262 (M^+, 76%), 264 ($M^+ + 1$, 11%), 248 (8), 177 (100), and 130 (28).

2,2,5,5-*Tetramethyl*-3-(3-*nitrobenzoyl*)-2,5-*dihydropyrrol*-1-*oxyl* (**10**).—A solution of 3-benzoyl-2,2,5,5-tetramethyl-2,5-dihydropyrrol-1-oxyl (**4**)[20] (2.44 g, 0.01 mol) in H_2SO_4 (10 ml) was nitrated with a mixture of concentrated H_2SO_4–67% HNO_3 [3:22 (v/v), 5 ml] and worked up as described for compound (**8**): the product (2.0 g, 69%) had m.p. 135—136 °C (Found: C, 62.3; H, 6.0; N, 9.7. $C_{15}H_{17}N_2O_4$ requires C, 62.3; H, 5.9; N, 9.65%); $\nu_{max.}$ 1 660 (C=O), 1 610 (C=C), 1 530, and 1 340 cm^{-1} (NO_2); δ_H 1.34, 1.49 (2 × 6 H, 2 s 4-Me), 6.20 (1 H, s 4-H), ≃7.58 (1 H, t, 5′-HAr), ≃7.96 (1 H, t, 4′-HAr), 8.35 (1 H, dd, 6′-HAr), and 8.50 (1 H, t, J ≃2 Hz, 2′-HAr); δ_C 24.5, 24.9 (CCH_3), 68.4, 70.9 (C-2 and -5), 123.6 [C-2′ (Ar)], 126.2 [C-4′ (Ar)], 129.4 [C-5′ (Ar)], 134.2 [C-6′ (Ar)], 140.0, 142.6 [C-4 and -1′ (Ar)], 148.0, 149.3 [C-3 and C-3′ (Ar)], and 190.6 (CO); *m/z* 289 (M^+, 21%), 259 (67), 244 (55), 150 (100), and 109 (76).

2,5,5-*Trimethyl*-2-*phenylpyrrolidin*-1-*oxyl* (**11**).—2,2,5-Trimethyl-3,4-dihydropyrrole 1-oxide (**12**) (7.63 g, 0.06 mol) was treated with phenylmagnesium bromide [freshly prepared from bromobenzene (18.84 g, 0.12 mol) and magnesium (2.88 g, 0.12 mol) in dry benzene (200 ml)] and worked up as described in the preparation of compound (**8**) to give after column chromatography [CCl_4–ether (10:0)] a thick red oil (**11**) (6.35 g, 51%) (Found: C, 76.1; H, 9.0; N, 6.7. $C_{13}H_{18}NO$ requires C, 76.4; H, 8.9; N, 6.8%); $\nu_{max.}$ 1 600 cm^{-1} [C=C (Ar)].

2,5,5-*Trimethyl*-2-(3-*nitrophenyl*)*pyrrolidin*-1-*oxyl* (**13**).—A mixture of concentrated H_2SO_4–67% HNO_3 (3:2; 5 ml) was added dropwise to a stirred solution of the nitroxide (**11**) (2.04 g, 0.01 mol) in concentrated H_2SO_4 (10 ml) while the temperature was kept below −5 °C; the mixture was then stirred for an additional 30 min and worked up as described for compound (**5**). The crude product was purified by flash chromatography on a column of silica gel [hexane–ethyl acetate (4:1)] to give the *m*-nitrophenyl compound (**13**) as a thick red oil (1.5 g, 60%) (Found: C, 62.8; H, 6.95; N, 11.1. $C_{13}H_{17}N_2O_3$ requires C, 62.6; H, 6.9; N, 11.2%); *m/z* 249 (M^+, 30%), 219 (10), and 163 (100); $\nu_{max.}$ 1 520 and 1 350 cm^{-1} (NO_2).

1-*Hydroxy*-2,5,5-*trimethyl*-2-(3-*nitrophenyl*)*pyrrolidine Hydrochloride* (**14**).—A solution of the nitroxide (**13**) (498 mg, 2.0 mmol) in ethanol saturated with HCl (5 ml) was left overnight; ether was then added to induce crystallisation of the hydrochloride salt (**14**) (420 mg, 73%), m.p. 131—136 °C (Found: C, 54.3; H, 6.6; Cl, 12.2; N, 9.5. $C_{13}H_{19}ClN_2O_3$ requires C, 54.5; H, 6.7; Cl, 12.4; N, 9.8%).

1-*Acetoxy*-2,5,5-*trimethyl*-2-(3-*nitrophenyl*)*pyrrolidine* (**15**).—Acetyl chloride (157 mg, 2.0 mmol) was added to a stirred mixture of the hydrochloride salt (**14**) (573.5 mg, 2.0 mmol) and triethylamine (202 mg, 2.0 mmol) in dry chloroform (20 ml). After 30 min the mixture was diluted with water and the organic phase separated and washed with 3% H_2SO_4 and water, dried, and evaporated to give the *N*-acetoxy derivative (**15**) as a colourless oil (502.8 mg, 85%); subsequently this crystallised, m.p. 66—68 °C (Found: C, 61.4; H, 6.9; N, 9.5. $C_{15}H_{20}N_2O_4$ requires C, 61.6; H, 6.9; N, 9.6%); $\nu_{max.}$ 1 760 (C=O), 1 520, and 1 340 cm^{-1} (NO_2); δ_H 1.26, 1.27, and 1.40 (3 × 3 H, 3 s, 3-Me), 1.7—2.2 (4 H, m, 3- and 4-H), 2.01 (3 H, s, Ac), 7.47 (1 H, t, J 8.0 Hz, 5′-HAr), 8.05 (1 H, dd, 6′-HAr), 8.22 (1 H, dd, 4′-HAr), and 8.44 (1 H, dd, J ≃ 2 Hz, 2′-HAr); *m/z* 292 (M^+, 10%), 277 (15), 250 (80), and 235 (100).

4-(4-*Aminophenyl*)-3-*methoxycarbonyl*-2,2,5,5-*tetramethylpyrrolidin*-1-*oxyl* (**16**).—10% Pd/C (100 mg) and then anhydrous ammonium formate (1.26 g, 20 mmol) were added to a stirred solution of the nitro compound (**5a**) or (**5b**) (642.7 mg, 2.0 mmol) in dry methanol (10 ml) under argon. The reaction mixture was warmed to *ca.* 40 °C and the stirring was maintained for 30 min; the catalyst was then filtered off and the filtrate was diluted with saturated brine and extracted with chloroform (3 × 10 ml). The combined extracts were dried (Na_2SO_4) and oxidized (PbO_2; 100 mg) with stirring and aeration for 30 min at room temperature; the mixture was then evaporated to dryness and the residue crystallized from ether–hexane to give the appropriate *cis*-amino compound (**16a**) (478 mg, 82%), m.p. 186—187 °C (Found: C, 65.9; H, 8.0; N, 9.8. $C_{16}H_{23}N_2O_3$ requires C, 66.0; H, 8.0; N, 9.6%); $\nu_{max.}$ 3 320, 3 230 (NH_2), and 1 722 cm^{-1} (C=O). The *trans*-isomer (**16b**) (420 mg, 72%) had m.p. 157—158 °C (Found: C, 65.9; H, 8.0; N, 9.7. $C_{16}H_{23}N_2O_3$ requires C, 66.0; H, 8.0; N, 9.6%); $\nu_{max.}$ 3 320, 3 230 (NH_2), and 1 720 cm^{-1} (C=O).

4-(4-*Azidophenyl*)-3-*methoxycarbonyl*-2,2,5,5-*tetramethylpyrrolidin*-1-*oxyl* (**17**).—Sodium nitrite (82.8 mg, 1.2 mmol) was added to a cold (0 °C) solution of the amino compound (**16a**) or (**16b**) (291.4 mg, 1.0 mmol) in dilute (3%) sulphuric acid (10 ml), the temperature of the reaction being kept in the range 0—5 °C. After 30 min urea (20 mg) was added to destroy the excess of nitrous acid and this was followed by sodium azide (130 mg, 2.0 mmol); after a further 30 min the reaction mixture was extracted with ether and the extract washed with brine, dried, and evaporated to dryness. The residual yellow thick oil was analytically pure *cis*- and *trans*-azide (**17a,b**). The *cis*-isomer (**17a**) (225 mg, 71%) had m.p. 87—88 °C (Found: C, 60.5; H, 6.4; N, 17.55. $C_{16}H_{21}N_4O_3$ requires C, 60.55; N, 6.7; N, 17.65%); $\nu_{max.}$ 2 020, 1 980 (N_3), and 1 720 cm^{-1} (C=O). The *trans*-isomer of (**17b**) (245 mg, 77%) had m.p. 77—78 °C (Found: C, 60.4; H, 6.7; N, 17.4. $C_{16}H_{21}N_4O_3$ requires C, 60.55; H, 6.7; N, 17.65%); $\nu_{max.}$ 2 110, 2 090 (N_3), and 1 720 cm^{-1} (C=O).

4-(4-*Azidophenyl*)-3-*carboxy*-2,2,5,5-*tetramethylpyrrolidin*-1-*oxyl* (**18**).—The azide ester (**17**) (636 mg, 2.0 mmol) was dissolved in aqueous methanol (60%; 10 ml) containing NaOH (2 g) and the solution stirred at room temperature for 2 h; it was then extracted with ether (10 ml) to remove any unhydrolysed ester. The aqueous phase was acidified with 3% H_2SO_4 and extracted with ether (50 ml), and the extract was washed, dried,

and evaporated to dryness. The solid residue was crystallized from ether–hexane to give the pure acid (**18**). The *cis*-isomer (**18a**) had m.p. 164—165 °C (Found: C, 59.3; H, 6.4; N, 18.3. $C_{15}H_{19}N_4O_3$ requires C, 59.4; H, 6.3; N, 18.5%); $\nu_{max.}$ 2 115 (N_3), 1 725, and 1 710 cm^{-1} (C=O). The *trans*-isomer (**18b**) (520 mg, 86%) had m.p. 180—181 °C (Found: C, 59.2; H, 6.4; N, 18.4. $C_{15}H_{19}N_4O_3$ requires C, 59.4; H, 6.3; N, 18.5%); $\nu_{max.}$ 2 115 (N_3), 1 725, and 1 710 cm^{-1} (C=O).

4-(4-*Azidophenyl*)-2,2,5,5-*tetramethyl*-3-*imidazol*-1-*yl-carbonylpyrrolidin*-1-*oxyl* (**19**).—1,1-Carbonylbis-1*H*-imidazole (195 mg, 1.2 mmol) was added at room temperature to a solution of acid (**18a**) or (**18b**) (303 mg, 1.0 mmol) in dry tetrahydrofuran (100 ml). After 1 h the mixture was diluted with saturated brine and extracted with ether (3 × 10 ml). The ether phase was washed with 5% aqueous sodium hydrogen carbonate and saturated brine, dried, and evaporated to dryness. The residue was crystallized from ether–hexane to give compound (**19**). Compound *cis*-(**19a**) (300 mg, 85%) had m.p. 132—143 °C (Found: C, 61.4; H, 6.0; N, 23.9. $C_{18}H_{21}N_6O_2$ requires C, 61.2; H, 6.0; N, 23.8%); $\nu_{max.}$ 2 100 (N_3) and 1 730 cm^{-1} (C=O). Compound *trans*-(**19b**) (270 mg, 76%) had m.p. 166—167 °C (Found: C, 61.3; H, 5.9; N, 23.7. $C_{18}H_{21}N_6O_2$ requires C, 61.2; H, 6.0; N, 23.8%); $\nu_{max.}$ 2 100 (N_3) and 1 730 cm^{-1} (C=O); *m/z* 354 (M^+ + 1, 73%), 325 (16), 310 (10), 175 (31), and 158 (47).

Tosylate Salt of (**19b**).—Anhydrous toluene-*p*-sulphonic acid (95 mg, 0.6 mmol) was added to a solution of the base (**19b**) (177 mg, 0.5 mmol) in dry acetone (5 ml); dilution of the mixture with ether then precipitated the crystalline title salt. The latter was filtered off (210 mg, 80%) and had m.p. 130—131 °C (Found: C, 57.0; H, 5.3; N, 16.2; S, 6.3. $C_{25}H_{28}N_6O_5S$ requires C, 57.2; H, 5.4; N, 16.0; S, 6.1%); $\nu_{max.}$ 2 100 (N_3), 1 695, and 1 580 cm^{-1} (CON=).

trans-3-*Methoxycarbonyl*-2,2,5,5-*tetramethyl*-4-*nitromethyl-pyrrolidin*-1-*oxyl* (**20**).—1,5-Diazabicyclo[5.4.0]undec-7-ene (DBU) (4.56 g, 0.03 mmol) was added to a solution of the unsaturated ester (**1**) (5.95 g, 0.03 mol) and nitromethane (3.66 g, 0.06 mol) in dry acetonitrile (30 ml). The dark pink solution was kept at 50 °C for 8 h and then diluted with chloroform (60 ml); the organic phase was successively washed with 3% sulphuric acid and saturated brine, dried, and evaporated to dryness. The yellow crystalline residue was recrystallized from chloroform–hexane to give the nitro ester (**20**) (6.38 g, 82%), m.p. 118—119 °C (Found: C, 50.8; H, 7.5; N, 10.75. $C_{11}H_{19}N_2O_5$ requires C, 50.95; H, 7.4; N, 10.8%); $\nu_{max.}$ 1 738 (C=O), 1 555, 1 391, and 1 371 cm^{-1} (NO_2); δ_H 0.94, 1.00, 1.16, 1.28 (4 × 3 H, 4 s, 4-Me), 2.56 (1 H, d, *J* 11.4 Hz, 3-H), 2.99 (1 H, dt, 4-H), 3.65 (3 H, s, OCH_3), and 4.16 and 4.32 (2 × 1 H, 2 dd, ^2J 12.1 Hz, ^3J 7.2 Hz, CH_2); δ_C 19.5, 21.9, 26.1, 26.7 (CCH_3), 43.9 (C-4), 51.5 (C-3), 53.8 (OCH_3), 63.5, 63.6 (C-2 and -5), 75.8 (CH_2), and 171.2 (C=O).

4-*Aminomethyl*-3-*methoxycarbonyl*-2,2,5,5-*tetramethyl-pyrrolidin*-1-*oxyl* (**21**).—10% Pd/C (0.5) followed by anhydrous ammonium formate (5.0 g, 0.08 mol) in one portion were added to a stirred solution of the nitro ester (**20**) (2.59 g, 0.01 mol) in dry methanol (200 ml) under argon. The reaction mixture was stirred at 40 °C for 1 h after which the catalyst was filtered off and the filtrate diluted with saturated brine and extracted with chloroform (3 × 20 ml); the dried extract was then evaporated to dryness. The residual pale yellow oil was dissolved again in chloroform (20 ml) and treated with PbO_2 to oxidize the 1-hydroxy compound to the yellow 1-oxyl radical (**21**). Crude (**21**) was chromatographed on silica gel column with chloroform–methanol (9:1) as eluant and the slowest moving yellow band yielded as a thick, deep yellow oil the amino ester (**21**) (1.1 g, 48%), m.p. 127—128 °C (Found: C, 57.5; H, 9.3; N, 12.1. $C_{11}H_{21}N_2O_3$ requires C, 57.6; H, 9.2; N, 12.2%); $\nu_{max.}$ 3 400—2 300 (NH_2) and 1 730 cm^{-1} (C=O).

trans-4-*Maleimidomethyl*-3-*methoxycarbonyl*-2,2,5,5-*tetra-methylpyrrolidin*-1-*oxyl* (**22**).—A solution of maleic anhydride (147 mg, 1.5 mmol) in dry ether (10 ml) was added to a stirred solution of the amino ester (**21**) (344 mg, 1.5 mmol) in dry ether (10 ml) and the mixture was stirred at room temperature for 30 min; it was then evaporated to dryness. The residue was dissolved in freshly distilled acetic anhydride (7 ml) and anhydrous sodium acetate (60 mg) was added to the solution which was then heated at 90 °C for 3 h. The dark solution was evaporated to dryness under reduced pressure and the residue was taken up in dry benzene. The benzene solution was evaporated to dryness and the residue was purified by p.l.c. [hexane–ethyl acetate (1:1)]. The fast moving yellow band was identified as (**22**) (241 mg, 52%), m.p. 145—148 °C (chloroform–hexane) (Found: C, 58.2; H, 7.0; N, 9.2. $C_{15}H_{21}N_2O_5$ requires C, 58.2; H, 6.8; N, 9.05%); *m/z* 309 (M^+, 30%), 295 (15), 204 (8), 165 (100), and 130 (15); $\nu_{max.}$ 1 760, 1 730, and 1 720 cm^{-1} (C=O); *m/z* 310 (M^+ + 1, 10%), 309 (M^+, 30%), 165 (100), and 110 (60).

trans-4-*Maleimidomethyl*-2,2,5,5-*tetramethylpyrrolidin*-1-*oxyl* (**23**).—The methyl ester (**22**) (155 mg, 0.5 mmol) was dissolved in aqueous methanol (50%; 3 ml) containing NaOH (20 mg, 0.5 mmol) and the mixture was stirred at room temperature for 30 min; it was then diluted with saturated brine, acidified with 3% H_2SO_4, extracted with chloroform (30 ml), and the extract dried and evaporated to dryness. The residual crude acid was purified by p.l.c. [chloroform–methanol (9:11)] to give the acid (**23**) (86 mg, 58%), m.p. 139—140 °C (Found: C, 56.8; H, 6.5; N, 9.4. $C_{14}H_{19}N_2O_5$ requires C, 57.0; H, 6.5; N, 9.5%); $\nu_{max.}$ 1 760, 1 730, and 1 725 cm^{-1} (C=O).

trans-4-[N-(4-*Azido*-2-*nitrophenyl*)*aminomethyl*]-3-*methoxy-carbonyl*-2,2,5,5-*tetramethylpyrrolidin*-1-*oxyl* (**24**).—4-Fluoro-3-nitrophenyl azide (182 mg, 1.0 mmol) was added to a solution of the amino ester (**21**) (229 mg, 1.0 mmol) and triethylamine (111 mg, 1.1 mmol) in ethanol (5 ml) was added and the mixture was stirred in the dark at 50 °C for 6 h; it was then diluted with saturated brine and extracted with chloroform (3 × 10 ml). The combined extracts were dried and evaporated to dryness and the red residue was purified by p.l.c. in the dark with hexane–ethyl acetate (2:1) as eluant to give pure title compound (**24**) as red crystals (294 mg, 75%), m.p. 120—122 °C (chloroform–hexane) (Found: C, 52.1; H, 5.9; N, 21.5. $C_{17}H_{23}N_6O_5$ requires C, 52.2; H, 5.9; N, 21.5%); *m/z* 392 (M^+ + 1, 32%), 348 (34), 302 (15), 192 (45), 184 (20), and 166 (100); $\nu_{max.}$ 3 335 (NH), 2 095 (N_3), 1 720 (C=O), and 1 515 cm^{-1} (NO_2).

trans-4-[N-(4-*Azido*-2-*nitrophenyl*)*aminomethyl*]-3-*carboxy*-2,2,5,5-*tetramethylpyrrolidin*-1-*oxyl* (**25**).—The ester (**24**) (377 mg, 1.0 mmol) was added to a warm (40 °C) stirred solution of NaOH (40 mg, 1.0 mmol) in aqueous methanol (50%, 5 ml). After 1 h the mixture was diluted with saturated brine and extracted with chloroform (10 ml) to remove any non-hydrolysed ester. The aqueous phase was acidified with 3% H_2SO_4, extracted again with chloroform (30 ml), and the extract dried and evaporated to dryness. The red solid residue was crystallized from THF–ether to give the pure red acid (**25**) (287 mg, 76%), m.p. > 250 °C (Found: C, 50.9; H, 5.7; N, 22.1. $C_{16}H_{21}N_6O_5$ requires C, 50.9; H, 5.6; N, 22.3%); $\nu_{max.}$ 3 100—2 800 (NH), 2 105 (N_3), 1 718 (C=O), and 1 525 cm^{-1} (NO_2).

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