Methods for preparation of heterobifunctional nitroxides: α,β-unsaturated ketones, β-ketoesters, cyano-nitro-derivatives

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The reactions of 1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-carboxaldehyde (**1**), 1-oxyl-2,2,5,5-tetramethyl-3-cyano-3-pyrroline (**2**), ethyl 1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-carbonyl carbonate (**3**), and *N*-(1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-carbonyl)imidazolide (**4**) are investigated with several nucleophiles (dialkylcadmium, diethyl sodio-malonate, magnesium enolate of ethyl hydrogen malonate, nitromethane-anion) to obtain α,β-unsaturated ketones, β-ketoesters, or conjugated reaction products. Aldehyde **1** is reacted with several methyl ketones (acetylmethylene-triphenylphosphorane, acetophenone, 2-acetylpyridine, 4-phenyl-3-butyn-2-one) to give spin labeled polyenones. The reaction of 4-(1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-yl)-2-buten-2-one (**24***a*) with ethylenediamine gives 7,14-bis-(1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-yl)-5,12-dimethyl-1,4,8,11-tetraazatetradeca-7,14-diene biradical (**25**).

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On a étudié les réactions de l'oxyl-1 tétraméthyl-2,2,5,5 pyrroline-3 carboxaldéhyde-3 (**1**), de l'oxyl-1 tétraméthyl-2,2,5,5 cyano-3 pyrroline-3 (**2**), de l'oxyl-1 tétraméthyl-2,2,5,5 pyrroline-3 carbonyl-3 carbonate d'éthyle (**3**) et de la *N*-(oxyl-1 tétraméthyl-2,2,5,5 pyrroline-3 carbonyl-3) imidazolide (**4**) avec plusieurs nucleophiles (dialkylcadmium sodio malonate de diéthyle, énolate magnesien du malonate double d'éthyle et d'hydrogène et anion du nitrométhane) en vue d'obtenir des cétones α,β non saturées, des β-cétoesters ou des produits de réactions conjuguées. L'aldéhyde **1** réagit avec plusieurs méthyl cétones (acétylméthylène-triphénylphosphorane, acétophénone, acétyl-2 pyridine, phényl-4 butyne-3 one-2) pour donner des polyénones avec des spins marqués. La réaction de la (oxyl-1 tétraméthyl-2,2,5,5 pyrroline-3 yl-3)-4 butène-2 one-2 (**24***a*) avec l'éthylènediamine donne le biradical, bis-(oxyl-1 tétraméthyl-2,2,5,5 pyrroline-3 yl-3)-7,14 diméthyl-5,12 tétraaza-1,4,8,11 tétradecadiène-7,14 (**25**).

[Traduit par le journal]

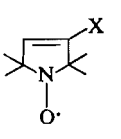
Introduction

There is a continuing interest in research of novel nitroxide free radicals having reactive functional groups by which they can be coupled to biologically important macromolecules for the study of the internal properties of the spin-labeled biomolecules by esr spectroscopy (1–3). We have published in the previous papers in this series the synthesis and reactions of 1-oxyl-2,2,6,6-tetramethylpiperidine-4-sulfonates with nucleophiles (4), synthesis and reactions of reactive derivatives of 1-oxyl-2,2,5,5-tetramethylpyrroline-3-carboxylic acid (5), nitroxide acylated aminoacids and peptides (6), synthesis of spin-label azidoformate (7), spin-labeled local anesthetics (8), synthesis and reactions with 1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-carboxaldehyde (9), preparation and reaction of a highly reactive allylic sulfonate (or halide) nitroxide (10), and synthesis and reaction of spin-label phosphinimines (11). Very recently as a part of the collaboration between J. F. W. Keana's and our laboratory a joint paper was prepared about the reactions of 3-substituted 1-oxyl-2,2,5,5-tetramethyl-3-pyrroline derivatives, carboxaldehyde, acid, and nitrile, with methyllithium (12). A characteristic of all of these reactions with methyllithium was that the diamagnetic *N*-methoxy derivative was also formed beside the *N*-hydroxy derivative which spontaneously oxidized in air to the corresponding paramagnetic nitroxide derivative.

In the present study we wish to report our attempts to prepare novel spin label nitroxides having two possible reaction centres for biomolecules.

Results and discussion

For starting compounds the 3-substituted derivatives of 1-oxyl-2,2,5,5-tetramethyl-3-pyrroline were chosen: the aldehyde (**1**), nitrile (**2**), acyl carbonate (**3**), and acyl imidazole (**4**).



1 X = CHO
2 X = CN
3 X = COOCOOEt
4 X = CO—N

The reactions of aldehyde **1** with alkyllithium to prepare the nitroxide secondary alcohol were accompanied by the formation of *N*-alkoxy alcohol (12). The reaction of aldehyde **1** with methylmagnesium iodide or ethylmagnesium bromide has resulted in essentially the same mixture of secondary alcohols which was oxidized to diamagnetic and paramagnetic ketones (**5**, **6** and **7**, **8**). The reaction

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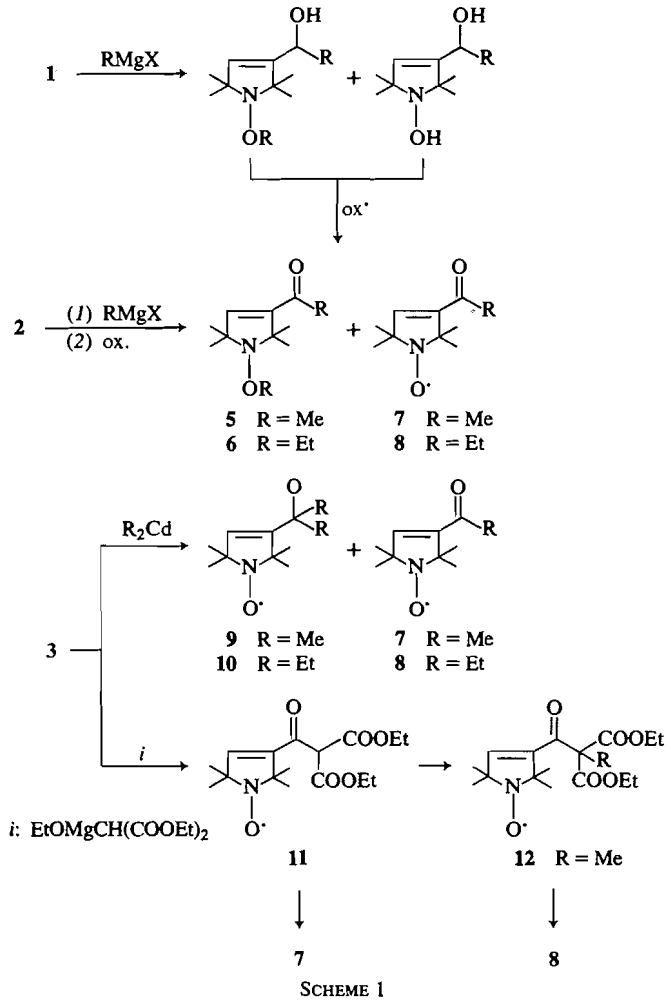
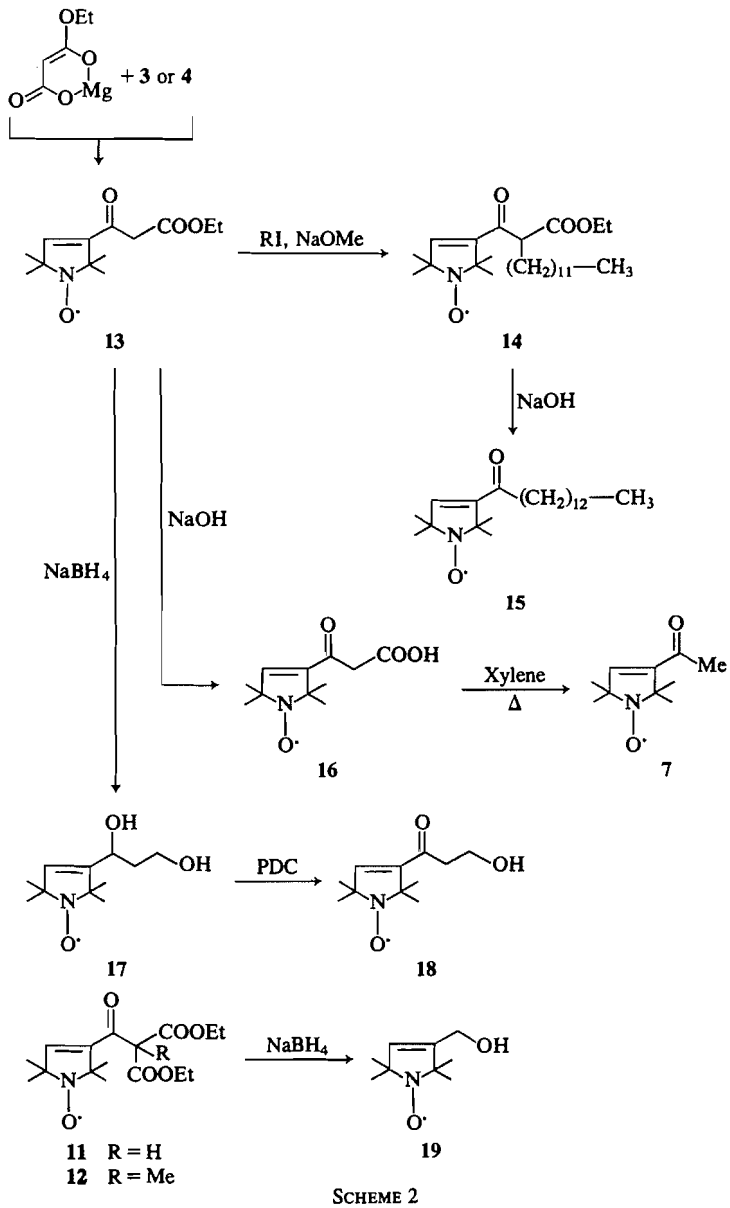


SCHEME 1

of nitrile **2** with ethylmagnesium iodide was even less promising because the dominating product after the oxidations was the diamagnetic ketone (**6**), while the paramagnetic ketone (**8**) was the minor product. Therefore it was necessary to find a reactive derivative of carboxylic acid which exhibits suitable reactivity toward milder organometallic reagents which are inert toward the nitroxide radical. The synthesis of the ketone directly from the reaction of acyl carbonate **3** with methylmagnesium iodide could not be carried out in a reasonable yield. However, the reaction of acyl carbonate **3** with *in situ* formed dialkylcadmium (dimethylcadmium or diethylcadmium) has resulted in a fair yield of the desired ketone (**7** or **8**) without any loss of the radical. In this reaction, as a minor product, the tertiary alcohol (**9** or **10**) was also formed, which could be separated by column chromatography.

Another approach was also successful in obtaining the ketones by an alternative route. The diethyl ethoxymagnesium malonate (13), either with acyl carbonate **3** or acyl imidazole **4**, could be converted in a good yield to the acyl malonate (**11**). The **11** could be alkylated with methyl iodide to **12**. The diethyl acyl malonates (**11** or **12**) could be hydrolysed with dilute acid via the *N*-hydroxy derivative to the corresponding ketone (**7** or **8**).

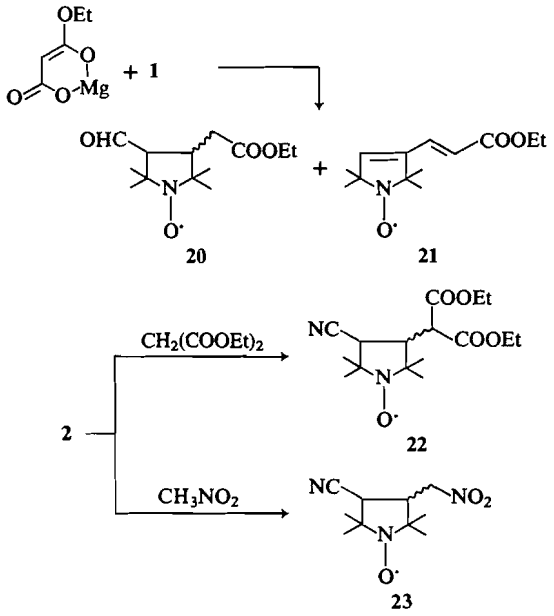
The reaction of the magnesium enolate of ethyl hydrogen malonate (14) either with acyl carbonate **3** or acyl imidazole **4** with concomitant decarboxylation gave the β-ketoester (**13**) in good yield. Utilization of the well-known reactivity of the β-ketoesters (15) may make this spin-label β-ketoester a very versatile building block in further synthesis starting from β-ketoesters. As an example of this type of reaction **13** was alkylated with dodecyl bromide to α-dodecyl-β-ketoester (**14**). The ester group could be eliminated by hydrolysis



SCHEME 2

with dilute sodium hydroxide to get a long chain alkyl α,β-unsaturated ketone (**15**). However, the preparation of α-unsubstituted β-ketoacid (**16**) was also possible when **13** was hydrolysed with dilute sodium hydroxide. The ketoacid (**16**) could be decarboxylated to **7** when refluxed in xylene. By the reduction of the β-ketoester (**13**) with sodium borohydride the dihydroxy compound **17** could be obtained in a fair yield. Diol **17** could be selectively oxidized at the allylic hydroxy group to a β′-hydroxy-α,β-unsaturated ketone (**18**). It is interesting to note that, in contrast, the reduction of another type of β-ketoester, the diethyl acyl malonates (**11**, **12**), has resulted in the elimination of the malonate and gives the hydroxymethylpyrroline derivative (**19**). By analogy to the recently described reaction of addition of ethyl monomagnesium enolate to the α,β-unsaturated ketones (16), the aldehyde **1** was reacted with ethyl monomagnesium enolate affording the conjugate adduct (**20**) in moderate yield. The 1,2-condensation reaction product diene ester (**21**), which can be separated by column chromatography, was also obtained from the reaction.

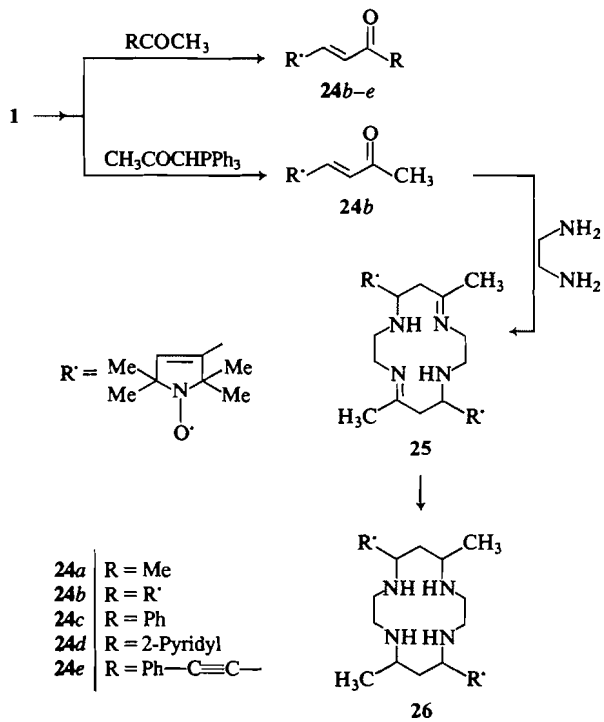
A conjugate addition reaction could be carried out on unsaturated nitrile (**2**) with diethyl malonate or with nitromethane (in the presence of base



SCHEME 3

catalysts sodium ethoxide or Triton B) to obtain cyanoalkylated diethyl malonate (**22**) and cyanoalkylated nitro spin label (**23**). These molecules may be very versatile synthons in further synthesis.

We also wished to prepare potentially more reactive, novel dienones suitable for nucleophilic addition of thiol or amino groups. While in the aldol type of condensation of unsaturated aldehyde **1** with various methyl ketones (acetophenone, 2-acetylpyridine, or acetylphenylacetylene) polyenones (**24***b–e*) could be obtained in reasonable yield, the methyl dienone (**24***a*) could be obtained only by a Wittig reaction with acetylmethylene-triphenylphosphorane. Utilizing the method for preparation of a 14-membered macrocyclic ligand (17, 18), a 14-membered macrocycle (**25**) can be prepared from **24***a* with ethylenediamine. By the reduction of the azomethine groups with sodium borohydride, the more stable saturated macrocycle (**26**) could be prepared, which may be a useful spin-labeled tetradentate ligand in complex formation reaction.



SCHEME 4

Experimental

Melting points were measured using a Boetius micro mp-determining instrument and were not corrected. The ir spectra were measured as a neat oil or in Nujol suspensions with a Zeiss Specord 75 type of instrument. The esr spectra were obtained from a 10^{-3} *M* solution using a Zeiss ER9 spectrometer. All the monoradicals exhibited three equidistant lines and the biradical showed five equidistant lines with n_N = 14.8–15.0 G. The ^1H nmr spectra were recorded on a Perkin–Elmer R12 instrument in $CDCl_3$, using tetramethylsilane as internal standard. The nmr spectra of radicals were taken in a 10% solution of diphenylhydrazine in $CDCl_3$ ($CDCl_3$–DPH). The mass spectra were taken in a Varian-MAT-SM-1 instrument. Solvents were reagent grade and dried prior to use. All organic extracts obtained during the work-up of the reaction mixture were dried over anhydrous magnesium sulfate and distilled off *in vacuo*. For column chromatography silica gel 60, for preparative tlc silica gel F(254 Merck-60 or Whatman PK6F), were used.

Reactions with organometallic reagents

(a). *Reaction of aldehyde 1 with alkylmagnesium iodides. Synthesis of 1-oxyl-2,2,5,5-tetramethyl-3-acetyl-3-pyrroline (7) and 1-oxyl-2,2,5,5-tetramethyl-3-ethanoyl-3-pyrroline (8)*

To a stirred solution of the Grignard reagent (prepared from magnesium turnings (0.36 g, 0.15 mol) and methyl iodide (2.1 g, 15 mmol) in dry ether (20 mL)) a solution of unsaturated aldehyde **1** (9) (1.68 g, 10 mmol) in dry ether (20 mL) was added at 0°C under nitrogen and stirred for 3 h. The reaction was quenched with saturated aqueous NH_4Cl (25 mL). The ether phase was separated, dried, and evaporated to dryness. A mixture of manganese(IV) dioxide (15 g) and lead(IV) dioxide (5 g) was added to the stirred solution of remaining pale yellow oil in carbon tetrachloride (50 mL) and refluxed for 6 h. The filtrate was subjected to column chromatography with carbon tetrachloride/ethyl acetate (10:1); two fractions were separated.

The first colorless fraction was the *N*-methoxy derivative **5**: 0.40 g (20%) as a colorless oil. The product was identical with the authentic (12) sample of **5**. The second yellow fraction was the main product **7** (from hexane), 1.01 g (55%), mp 71–72°C (lit. (12) mp 71–72°C).

The ethyl ketone **8** was prepared in the same way from aldehyde **1** (10 mmol) and ethylmagnesium bromide (15 mmol) to give the *N*-ethoxy derivative **6**, 0.70 g (31%) as a colorless oil. Infrared ν_{max}: 1670, 1620 cm^{-1}; ^1H nmr ($CDCl_3$) δ: 0.9–1.5 (m, 18H), 2.61 (q, 2H), 3.83 (q, 2H), 6.32 (s, 1H). *Anal.* calcd. for $C_{13}H_{23}NO_2$ (mol. wt. 225.3): C 69.30, H 10.29, N 6.22; found: C 69.33, H 10.35, N 6.18.

The second yellow band was the paramagnetic ethyl ketone **8**, yield 0.82 g (41%), mp 65–66°C; ir ν_{max}: 1670, 1620 cm^{-1}. *Anal.* calcd. for $C_{11}H_{18}NO_2$ (mol. wt. 196.3): C 67.32, H 9.24, N 7.14; found: C 67.45, H 8.93, N 7.16.

(b). *Reaction of nitrile (2) with ethylmagnesium bromide*

A solution of nitrile **2** (19) (1.65 g, 10 mmol) in dry ether was added to a freshly prepared solution of ethylmagnesium bromide (20 mmol) in ether under nitrogen at 0°C and stirred for 3 h, then the reaction was quenched with a saturated solution of ammonium chloride (5 mL) and the ether phase separated, dried, and evaporated to dryness. The pale oil was taken up in 5 *M* hydrochloric acid (20 mL) and refluxed for 2 h, then basified (to pH 10) and extracted with ether (3 × 10 mL). The ether solution was stirred with lead(IV) dioxide (3 g) for 3 h, then filtered. The filtrate was evaporated to dryness to give **6**, yield 1.58 g (70%), which was contaminated with only a minimal amount (less than 8%) of paramagnetic ketone **8**.

(c). *Reaction of acyl carbonate with dimethylcadmium: synthesis of 1-oxyl-2,2,5,5-tetramethyl-3-acetyl-3-pyrroline (7) and 2-(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolin-3-yl)-propan-2-ol (9)*

The dimethylcadmium (20) was freshly prepared as follows. Anhydrous cadmium chloride (3.6 g, 20 mmol) was added to a freshly prepared solution of Grignard reagent (magnesium turnings (0.96 g, 40 mmol) with methyl iodide (6.25 g, 44 mmol) in dry ether (40 mL)) under nitrogen. A solution of acyl carbonate **3** (5, 21) (5.12 g, 20 mmol) in dry ether (25 mL) was added to the stirred suspension of dimethylcadmium and the mixture was stirred overnight. The reaction was quenched with a saturated solution of NH_4Cl, the organic phase was dried and evaporated to dryness. The residual yellow oil was column chromatographed with carbon tetrachloride/ethyl acetate (1:1).

The first yellow band was the ketone (**7**), yield 2.21 g (60%), mp 71–72°C (from hexane). The ketone **7** was identical with an authentic sample of **7** obtained above or as it has been described earlier (12). The second yellow band was the tertiary alcohol **9**, yield 0.32 g, (8%), mp 127–129°C (from chloroform/hexane); ir ν_{max}: 3400–3200 cm^{-1}. *Anal.* calcd. for $C_{11}H_{20}NO_2$ (mol. wt. 198.3): C 66.63, H 10.17, N 7.06; found: C 66.79, N 9.98, N 6.96.

(d) *Reaction of acyl carbonate 3 with diethylcadmium: synthesis of 1-oxyl-2,2,5,5-tetramethyl-3-ethanoyl-3-pyrroline (8) and 3-(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolin-3-yl)pentan-3-ol (10)*

A solution of acyl carbonate **3** (2.56 g, 10 mmol) in dry ether (10 mL) was added to a solution of diethylcadmium (20) (10 mmol) (prepared from a solution of ethyl magnesium bromide (20 mmol) and dry cadmium chloride (20 mmol) in ether (20 mL)), reacted, and worked up as above for methylketone **7**. The first yellow band obtained from the silica gel column was ethyl ketone **8**, yield 1.00 g (51%), mp 65–66°C (from hexane); it was identical with **8** obtained from the Grignard method above. The second minor yellow band was the tertiary alcohol **10**, yield 0.30 g (13%), mp 117–119°C (from ether/hexane); ir ν_{max}: 3400–3200 cm^{-1}. *Anal.* calcd. for $C_{13}N_{24}NO_2$ (mol. wt. 226.3): C 68.98, H 10.69, H 6.19; found: C 69.04, H 10.76, H 6.26.

Diethyl (1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-carbonyl-malonate (11)

A solution of acyl carbonate **3** (2.56 g, 10.0 mmol) in dry ether was added slowly to a stirred solution of freshly prepared diethyl ethoxy magnesium malonate (13) (2.28 g, 10.0 mmol) in dry ether (30 mL) under nitrogen. The reaction mixture was kept at room temperature overnight, then saturated NH_4Cl solution (10 mL) was added. The ether phase was separated and washed with dilute (5%) sulphuric acid (20 mL), saturated $NaHCO_3$, water, dried, and evaporated to dryness. The remaining thick yellow oil was practically pure product **11**, yield 2.86 g (87.6%); ir ν_{max}: 1745, 1685, 1620 cm^{-1}; ^1H nmr ($CDCl_3$–DPH) δ: 1.1–1.5 (m, 18H), 4.2 (q, 4H), 4.90 (s, 1H), 6.36 (s, 1H). *Anal.* calcd. for $C_{16}H_{24}NO_6$ (mol. wt. 326.4): C 58.89, H 7.41, N 4.29; found: C 59.05, H 7.39, N 4.22.

1-Oxyl-2,2,5,5-tetramethyl-3-acetyl-3-pyrroline (7)

The acyl malonate (**11**) (3.26 g, 10.0 mmol) was dissolved in a mixture of acetic acid (6 mL), concentrated sulphuric acid (1 mL), and water (4 mL) and refluxed for 4 h. The cooled solution was extracted with ether (3 × 20 mL). The ether phase was worked up as above to give the pure ketone (**7**), yield 1.02 g (55%), mp 71–72°C. The product was identical with an authentic sample of **7**.

Reaction of 12 to ethyl ketone (8)

The reaction of alkylated acyl malonate (**12**) with acids under the same condition as above gave **8**, mp 65–66°C, yield 30%.

Diethyl 2-methyl-2-(1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-carbonyl)malonate (12)

A solution of sodium ethoxide (1 *M*) (10 mL) in ethanol was added under nitrogen to a stirred solution of acyl malonate **11** (3.26 g, 10.0 mmol) in dry ether (15 mL), followed by a solution of methyl iodide (2.13 g, 15.0 mmol) in dry THF (15 mL), and refluxed for 4 h. To the cooled solution saturated NaCl was added and the organic phase was separated, dried, and evaporated to dryness. The remaining yellow oil was purified on a silica gel column (solvent: hexane/ethyl acetate, 3:1). The first band was the unreacted **11** (0.90 g, 27%), the second band was **12** (1.03 g, 30%), mp 72–73°C (from hexane); ir ν_{max}: 1770, 1730, 1680, 1620 cm^{-1}. ^1H nmr ($CDCl_3$–DPH) δ: 1.0–1.5 (m, 18H), 1.7 (s, 3H), 4.2 (q, 4H), 6.3 (s, 1H). *Anal.* calcd. for $C_{17}H_{26}NO_6$ (mol. wt. 340.4): C 59.98, H 7.70, N 4.12; found: C 60.05, H 7.72, N 4.22.

Ethyl 2(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolin-3-yl)-2-oxopropionate (13)

To a freshly prepared and well stirred solution of magnesium monoethyl malonate (14) (2.20 g, 14 mmol) in dry tetrahydrofuran (20 mL) was added dropwise a solution of acyl carbonate **3** (2.56 g, 10.0 mmol) in dry tetrahydrofuran (20 mL) under nitrogen at room temperature and kept overnight. Saturated ammonium chloride solution was added to the reaction mixture and the organic phase separated and washed with dilute (5%) sulphuric acid, saturated sodium chloride, dried, and evaporated to dryness. The residue was a yellow oil contaminated with ethyl 1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-carboxylic acid, therefore it was column chromatographed with carbon tetrachloride. The fast moving band was the contaminating ester (identified by an authentic sample). The second band was the β-ketoester as a thick yellow oil after evaporating from the solvent. Yield 1.28 g (50%); ir ν_{max}: 1720, 1660, 1600 cm^{-1}. ^1H nmr ($CDCl_3$–DPH) δ: 1.0–1.5 (m, 15H), 3.5 (s, 2H), 4.1 (q, 2H), 6.4 (s, 1H). *Anal.* calcd. for $C_{13}H_{20}NO_4$ (mol. wt. 254.3): C 61.40, H 7.93, N 5.51; found: C 61.35, H 8.02, N 5.62. The ketoester **13** was prepared as above but acylimidazole (**4**) (22, 23), (2.34 g, 10.0 mmol) was used instead of **3**. Yield 1.85 g (73%).

Ethyl 2-(1-oxyl-2,2,5,5-tetramethyl-3-pyrroline-3-carbonyl)tridecanoic acid (14)

Sodium methoxide (0.108 g, 2.00 mmol) was added under nitrogen to a solution of **13** (0.508 g, 2.00 mmol) and dodecylbromide (0.498 g, 2.0 mmol) in dry ethanol (10 mL) and refluxed for 8 h. The reaction mixture was diluted with water (20 mL) and extracted with ether (3 × 20 mL). The ether phase was dried and evaporated to dryness. The remaining yellow oil was purified from the unreacted starting compounds on preparative tlc (silica gel Whatman PK6F) with hexane/ethyl acetate (3:1) to give the

pure **14** as a thick yellow oil, yield 0.690 g (81%); ir ν_{max}: 1735, 1670, 1615 cm^{-1}. *Anal.* calcd. for $C_{25}H_{44}NO_4$ (mol. wt. 422.7): C 71.05, H 10.49, N 3.31; found: C 71.12, H 10.53, N 3.36.

*Hydrolysis of **14** to 1-oxyl-2,2,5,5-tetramethyl-3-*n*-tridecyl-3-pyrroline (**15**)*

Sodium hydroxide (10%) (3 mL) was added to a solution of **14** (211.3 mg, 0.5 mmol) in ethanol (3 mL) and allowed to stay at room temperature for two days. The reaction mixture was acidified (to pH 3) with sulfuric acid (5%) and extracted with chloroform (3 × 10 mL). The chloroform phase was evaporated to dryness. The crude oil was purified on preparative tlc with ethyl acetate/hexane (1:1) to give the pure ketone, 132 mg (75%); ir ν_{max}: 1675, 1610 cm^{-1}. *Anal.* calcd. for $C_{22}H_{40}NO_2$ (mol. wt. 350.6): C 75.37, H 11.50, N 4.00; found: C 75.31, H 11.43, N 4.12.

*2-(1-Oxyl-2,2,5,5-tetramethyl-3-pyrrolin-3-yl)-2-oxopropanoic acid (**16**)*

A solution of **13** (0.254 g, 1.00 mmol) in 2.5 *M* sodium hydroxide (2 mL) was kept at room temperature for 2 h, then extracted with chloroform (3 × 2 mL). The aqueous phase was acidified (pH 3) with dilute sulphuric acid (5%), saturated sodium chloride (10 mL) was added to the solution, and it was extracted again with chloroform (3 × 5 mL). The chloroform solution was dried and evaporated to dryness. The remaining solid was **16**, yield 183 mg (80%). The analytical sample was crystallized from ether/hexane, mp 107–108°C; ir ν_{max}: 1735, 1665, 1615 cm^{-1}. *Anal.* calcd. for $C_{11}H_{16}NO_4$ (mol. wt. 226.3): C 58.40, H 7.13, N 6.19; found: C 58.46, H 7.27, N 6.23.

*Decarboxylation of **16** to **7***

A solution of **16** (113 mg, 0.5 mmol) in xylene (5 mL) was boiled for 4 h and evaporated to dryness. The residue was the ketone **7**, yield 47 mg (51%), which was identical with an authentic sample of 7 obtained by an alternative method.

*Reduction of **13** to 1(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolin-3-yl)-1,3-dihydroxypropane (**17**)*

Sodium borohydride (756 mg, 20.0 mmol) was added to a solution of **13** (508 mg, 2.0 mmol) in dry ethanol (10 mL) and refluxed for 2 h. The reaction mixture was diluted with saturated sodium chloride solution and extracted with ether (3 × 10 mL). The ether phase was dried and evaporated to dryness. The residue was a thick oil, yield 342 mg (80%), and showed one spot on tlc; ir ν_{max}: 3600–3100 cm^{-1}, and no ester bond was present; ^1H nmr, δ: 1.0–1.3 (m, 12H) 1.5–2.0 (m, 2H), 3.2–3.6 (m, 2H, 2HO), 3.6–3.9 (m, 2H), 4.0–4.4 (m, 1H, CH); ms, *m/e*: M^+ 214 (56), 151 (29), 139 (72), 135 (100).

*Oxidation of diol **17** with pyridinium dichromate (ref. 24) to β-hydroxyketone (**18**)*

The diol (**17**) (428 mg, 2 mmol) was dissolved in methylene chloride (15 mL) and pyridinium dichromate (PDC) (2.78 g, 7.4 mmol) was added to the solution and stirred for 2 h. Then ether (20 mL) was added to the black suspension, which was stirred for one further hour and filtered. The filtrate was evaporated to dryness. The residue was subjected to preparative tlc (Whatman PK6F) with hexane/ethyl acetate (1:3); the intensive yellow band was the β-hydroxy ketone **18**, 300 mg (70%), mp 96–98°C; ir ν_{max}: 3600–3300, 1660, 1610 cm^{-1}; ms, *m/e*: M^+ 212 (50), 182 (100), 167 (45), 138 (55). *Anal.* calcd. for $C_{11}H_{18}NO_3$ (mol. wt. 212.3): C 62.24, H 8.55, N 6.60; found: C 62.12, H 8.40, N 6.49.

*Reduction of diethyl acyl malonates (**11** or **12**) with sodium borohydride to 1-oxyl-2,2,5,5-tetramethyl-3-hydroxymethyl-3-pyrroline (**19**)*

Sodium borohydride (378.3 mg, 10.0 mmol) was added to a solution of **11** (326.4 mg, 1.0 mmol) in dry ethanol (20 mL) and refluxed for 3 h. The reaction mixture was concentrated *in vacuo*, then the residue was taken up with saturated sodium chloride solution (10 mL) and extracted with chloroform (3 × 10 mL). The chloroform phase was dried and evaporated to dryness. The solid yellow residue was recrystallized from chloroform/hexane to give **19**, yield 140 mg (82%), mp 75–77°C. The **19** was also prepared from **12** (340.4 mg, 1.0 mmol) under the same condition as above, yield 133 mg (78%), mp 75–77°C; ir ν_{max}: 3500–3100 cm^{-1}. The above samples of **19** were identical with the authentic sample of **19** (9).

*Ethyl (1-oxyl-4-formyl-2,2,5,5-tetramethylpyrrolidin-3-yl)-acetate (**20**)*

A freshly prepared solution of magnesium monoethyl malonate (14) (2.20 g, 14 mmol) in dry DMF (10 mL) was added under a nitrogen atmosphere to a stirred solution of 1-oxyl-2,2,6,6-tetramethyl-3-pyrroline-3-carboxaldehyde (**1**) (1.68 g, 10 mmol). The reaction mixture was kept at 40°C for three days, then acetic acid (1.6 mL) was added and kept at 80°C for two further days to achieve the decarboxylation. The solution was diluted with water (about 50 mL) and extracted with ether (3 × 20 mL). The ether phase was washed with saturated sodium hydrogen carbonate, saturated sodium chloride, dried, and evaporated to dryness. The remaining yellow oil (1 g) showed 3 spots on tlc (hexane/ethyl acetate (1:1)). The crude oil was subjected to column chromatography with carbon tetrachloride/ether (10:1). The second band proved to be **20**, yield 0.80 g (31%) (yellow oil); ir ν_{max}: 1730, 1680 cm^{-1}; ms, *m/e*: M^+ 256 (100), $M^+ + 1$ 257(20), 226(27), 211(25), 143(91), 140(28), 111(32), 110(63). *Anal.* calcd. for $C_{13}H_{22}NO_4$ (mol. wt. 256.3): C 60.91, H 8.65, N 5.47; found: C 60.83, H 8.54, N 5.59. The first band was proved to be the unreacted **1** by ir. The third band isolated as yellow crystals was the diene ester, ethyl 3-(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolin-3-yl)-2-propenoate (**21**), yield 0.90 g (38%), mp 80–81°C (from hexane), identical with the authentic sample.

*Preparation of authentic **21** by Wittig reaction*

A solution of **1** (168 mg, 1.00 mmol) and (ethoxycarbonyl methylene)triphenylphosphorane (348 mg, 1 mmol) in dry THF was refluxed for 3 h, then evaporated to dryness. The solid residue was dissolved in hot petroleum ether (bp 40–70°C) (3 × 10 mL), then evaporated to a minimal volume and cooled. The yellow crystals were filtered off to give **21**, yield 210 mg (88%), mp 80–81°C (from hexane); ir ν_{max}: 1685, 1620, 1590 cm^{-1}. *Anal.* calcd. for $C_{13}H_{20}NO_3$ (mol. wt. 238.3): C 65.52, H 8.46, N 5.88; found: C 65.46, H 8.46, N 5.99.

*Diethyl 2(1-oxyl-4-cyano-2,2,5,5-tetramethylpyrrolidin-3-yl)-malonate (**22**)*

A solution of unsaturated nitrile (**2**) (1.65 g, 10.0 mmol) was added to a solution of diethyl malonate (2.40 g, 15.0 mmol) and sodium ethoxide (1 *M*) (3 mL, 3 mmol) in dry ethanol (10 mL) and refluxed for 16 h. The reaction mixture was diluted with a solution of saturated sodium chloride (20 mL) and extracted with ether (3 × 10 mL). The organic phase was dried and evaporated to dryness. The residual yellow oil (3 g) was subjected to column chromatography on silica gel with chloroform/petroleum ether (bp 40–70°C) (9:11). The first yellow band was the unreacted nitrile (**2**), 0.50 g (30%). The second wide band was a mixture of the two stereoisomers of **22**, yield 1.64 g (50%), mp 86–88°C (from hexane); ir ν_{max}: 2235, 1750, 1710 cm^{-1}; ^1H nmr δ: 0.5–1.5 (m, 18H), 2.3–2.5 (m, 2H), 3.3–3.5 (m, 1H), 3.9–4.4 (m, 4H). *Anal.* calcd. for $C_{16}H_{25}N_2O_5$ (mol. wt. 325.4): C 59.06, H 7.74, N 8.61; found: C 58.95, H 7.73, N 8.77.

*(1-Oxyl-2,2,5,5-tetramethyl-4-cyanopyrrolidin-3-yl) nitromethane (**23**)*

A solution of unsaturated nitrile (**2**) (1.65 g, 10 mmol) and nitromethane (0.91 g, 15 mmol) in dry THF (20 mL) was refluxed

in the presence of Triton B catalyst (0.2 mL) for 3 days, then worked up as above for the reaction of **22**. The products were separated on a silica gel column; the first band was unreacted nitrile (**2**) (0.70 g, 42%) and the second one the two nonseparated stereoisomers of **23** (0.70 g, 31%). The stereoisomers were separated on a second column with carbon tetrachloride/ether (6:4); R_f I: 0.50, R_f II: 0.65. Isomer I: 0.50 g (17.6%), mp 69–70°C; ir ν_{max}: 2230, 1550 cm^{-1}. *Anal.* calcd. for $C_{10}H_{16}N_3O_3$ (mol. wt. 226.3): C 53.08, H 7.13, N 18.57; found: C 52.98, H 6.96, N 18.43. Isomer II: 0.30 g (13.4%), mp 55–56°C; ir ν_{max}: 2230, 1540 cm^{-1}. *Anal.* calcd. for $C_{10}H_{16}N_3O_3$ (mol. wt. 226.3): C 53.08, H 7.13, N 18.57; found: C 53.17, H 7.20, N 18.40.

4-(1-Oxyl-2,2,5,5-tetramethyl-3-pyrrolin-3-yl)-3-buten-2-on (24a)

A solution of (acetylmethylene)triphenylphosphorane (3.18 g, 10.0 mmol) and unsaturated aldehyde **1** (1.68 g, 10.0 mmol) in dry tetrahydrofuran (30 mL) was refluxed for 16 h, then evaporated to dryness. The remaining semisolid was dissolved in light petroleum ether (bp 40–70°C) (50 mL), then evaporated again. The orange oil was subjected to column chromatography with chloroform/ether (1:1) to give an orange oil, yield 1.31 g (63%); ir ν_{max}: 1670, 1620, 1590 cm^{-1}; ^1H nmr ($CDCl_3$–DPH) δ: 1.28 (s, 6H), 1.38 (s, 6H) 2.24 (s, 3H), 5.95 (s, 1H), 6.32 (d, 1H, HC=C, J = 16.8 Hz). *Anal.* calcd. for $C_{12}H_{18}NO_2$ (mol. wt. 208.3): C 69.20, H 8.71, N 6.73; found: C 69.19, H 8.69, N 6.71.

General procedure for Claisen condensation of 1 with methyl ketones

The equivalent amount (0.5 mmol) of **1** and a methyl ketone were dissolved in aqueous methanol (50%) (5 mL) and sodium hydroxide (3 drops of 10% solution) was added to the reaction mixture and kept at room temperature overnight, then diluted with water (5 × 10 mL) to make the crystallization more complete. The crystals were filtered and the analytical samples were crystallized from ether/hexane.

Compound **24b**: yield 75%, mp 170–171°C; ir ν_{max}: 1650, 1595, 1578 cm^{-1}. *Anal.* calcd. for $C_{19}H_{28}N_2O_3$ (mol. wt. 332.4): C 68.65, H 8.49, N 8.43; found: C 68.74, H 8.42, N 8.37.

Compound **24c**: yield 68%, mp 106–107°C; ir ν_{max}: 1660, 1620, 1590 cm^{-1}. *Anal.* calcd. for $C_{17}H_{20}NO_2$ (mol. wt. 270.4): C 75.53, H 7.46, N 5.18; found: C 75.44, H 7.36, N 5.10.

Compound **24d**: this derivative was isolated after extraction of the reaction mixture with ether, then chromatographed on preparative tlc with ethyl acetate/hexane (1:1). Yield: 50%, mp 100–101°C; ir ν_{max}: 1660, 1595, 1570 cm^{-1}. *Anal.* calcd. for $C_{16}H_{19}N_2O_2$ (mol. wt. 271.3): C 70.83, H 7.06, N 10.32; found: C 70.82, H 6.98, N 10.46.

Compound **24e**: yield 74%, mp 111–112°C; ir ν_{max}: 2205, 1610, 1575 cm^{-1}. *Anal.* calcd. for $C_{19}H_{20}NO_2$ (mol. wt. 294.4): C 77.52, H 6.85, N 4.76; found: C 77.44, H 7.00, N 4.96.

7,14-Bis-(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolin-3-yl)-5,12-dimethyl-1,4,8,11-tetraazatetradeca-7,11-diene (25)

Ethylene diamine (0.72 g, 12.0 mmol) and anhydrous K_2CO_3 (1 g) were added to a solution of dienone (**24a**) (2.08 g, 10.0 mmol) in cyclohexane/ether (2:1) (30 mL) and refluxed for 6 h then filtered. The filtrate was evaporated to dryness at 0°C *in vacuo*. The residue was crystallized from ether to give macrocyclic diene **25**, yield 3.75 g (75%), mp 139–140°C; ir ν_{max}: 3270, 1660, 1570 cm^{-1}. *Anal.* calcd. for $C_{28}H_{48}N_6O_2$ (mol. wt. 500.7): C 67.16, H 9.67, N 16.78; found: C 67.08, H 9.76, N 16.80.

7,14-Bis-(1-oxyl-2,2,5,5-tetramethyl-3-pyrrolin-3-yl)-5,12-dimethyl-1,4,8,11-tetraazatetradecane (26)

The diene (**25**) (500 mg, 1 mmol) was added to a suspension of sodium borohydride (76 mg, 2.0 mmol) in dry ethanol (5 mL) and refluxed for 2 h, then diluted with water and extracted with chloroform (3 × 10 mL). The chloroform phase was dried and evaporated to dryness. The solid residue was recrystallized from chloroform/hexane to give **26**, yield 362 mg (72%), mp 200°C; ir ν_{max}: 3240 cm^{-1}. *Anal.* calcd. for $C_{28}H_{52}N_6O_2$ (mol. wt. 504.8): C 66.63, H 10.38, N 16.65; found: C 66.70, H 10.22, N 16.55.

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