Mononitroxides and Proximate Dinitroxides Derived by Oxidation of 2,2,4,4,5,5-Hexasubstituted Imidazolidines. A New Series of Nitroxide and Dinitroxide Spin Labels

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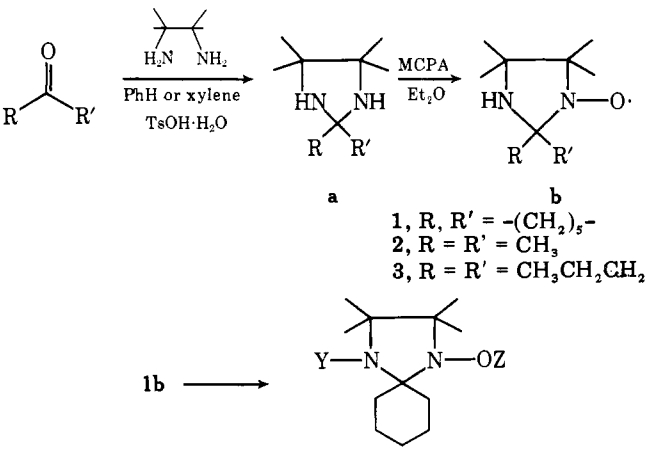
Abstract: A new series of mono- and dinitroxide spin labels derived by oxidation of 2,2,4,4,5,5-hexasubstituted imidazolidines is described. Condensation of 1 equiv of 2,3-diamino-2,3-dimethylbutane with a ketone (PhH, $TsOH\cdot H_2O$) led to the corresponding imidazolidine **a**. Substances **1a**, **2a**, **3a**, and **11** were thus prepared. Oxidation with ~1.5 equiv of *m*-chloroperoxybenzoic acid (MCPA) (ether) gave the corresponding mononitroxide **b**. Nitroxides **1b**, **2b**, **3b**, and **12** were thus prepared. Catalytic hydrogenation (Pd/C, THF) of **1b** gave **5** which, without isolation, was acetylated (AcCl, Et_3N, THF) to give **6**. Oxidation of **6** with MCPA (ether) gave nitroxide **7**. Hydrolysis (KOH, MeOH) gave **8**, mp 86–87 °C. Oxidation of **8** (*t*-BuOH–0.11 M KO-*t*-Bu, O_2) gave dinitroxide **9**, mp 70.5–71.5 °C. The structure of **9** was confirmed by x-ray analysis. The N---N distance is an unexceptional 2.22 (1) Å and the N–C–N angle is 98.5 (5)°. The ESR spectrum of **9** in an EPA glass (−196 °C) showed a dipolar splitting, $2D$ = 1606 G, a value about three times larger than splittings previously observed for a nonconjugated dinitroxide. Steroid dinitroxide **16** was similarly prepared and showed comparable spectra. The relative small steric size of these new dinitroxide labels, the ease of synthesis, and the enormous dipolar splitting exhibited by the labels should all combine to enhance their importance in theoretical studies as well as in spin labeling studies in which extreme sensitivity to motion is required.

Molecules of reasonably well defined geometry and containing two stable nitroxide free radical groupings have been of considerable interest in recent years both from the point of view of their use as dinitroxide spin labels[3,4] and as substrates for studying the interaction of unpaired spins.[5] We disclose herewith the synthesis and some properties of two members of a new series of rigid dinitroxides in which *the nitroxide groups are separated from each other by only one carbon atom*. Additionally, we disclose the synthesis of several intermediate imidazolidine mononitroxides which may have potential as new spin labels.[3]

Condensation of 1 equiv of 2,3-diamino-2,3-dimethylbutane with a ketone in refluxing benzene or xylene in the presence of *p*-$TsOH\cdot H_2O$[6] led to the corresponding imidazolidine **a**. Oxidation with 1.5 equiv of *m*-chloroperoxybenzoic acid (MCPA) in ether afforded the corresponding mononitroxide **b**. Several imidazolidines and mononitroxides prepared in this way are shown in Chart I. Because of the rigid attachment[7] of the nitroxide grouping to the parent molecule, this new series of nitroxide spin labels should show many of the advantages of doxyl nitroxides[6] in ESR spin labeling studies. The remaining unreacted amino function, moreover, offers a site for the attachment of a second grouping to the nitroxide moiety via an alkylation or acylation reaction.[8] Thus, reaction of **1b** with $Me_3O^+PF_6^-$ in CH_3CN at 0 °C for 1 h afforded the *N*-methyl derivative **4** in 70% yield.

Preliminary efforts toward further direct oxidation of the cyclohexanone derived mononitroxide **1b** with MCPA to dinitroxide **9** were not promising. Therefore, **1b** was reduced (H_2, 1 atm, Pd/C, THF) to *N*-hydroxy compound **5** which was immediately quenched, after removal of the catalyst, with 1.4 equiv of AcCl in the presence of excess Et_3N, producing crude acetate **6** (73%). Oxidation of **6** with 1.5 equiv of MCPA[6] in ether gave crude nitroxide **7** (80%). Hydrolysis of the *N*-acetoxy group was effected with 3.1 equiv of KOH in MeOH, producing crystalline *N*-hydroxy nitroxide **8** (56%). Nitroxide **8** was dissolved in *t*-BuOH–0.11 M KOtBu (1.6 equiv) and treated with O_2 gas at 25 °C for 3 min, affording crude orange crystals of dinitroxide **9** (84%). Two recrystallizations from pentane (−20 °C) afforded pure **9**, mp 70.5–71.5 °C.

Chart I



Treatment of a $CDCl_3$ solution of **9** with phenylhydrazine[9] led to an NMR spectrum of the corresponding di-*N*-hydroxy compound, identical with that obtained by phenylhydrazine reduction of precursor **8**. Catalytic reduction of **9** followed by a AcCl–Et_3N quench produced diacetate **10** (by NMR) (unstable to chromatography), which could also be prepared by a similar series of reactions applied to acetoxy nitroxide **7**. These results indicated that the starting imidazolidine ring was still present in dinitroxide **9**. The UV spectrum (*t*-BuOH) of **9** showed a maximum at 235 nm, with a molar extinction coefficient, 4420, about twice that of a mononitroxide.

Verification of the structure of dinitroxide **9** was obtained by x-ray analysis. An irregular-shaped crystal of **9** was sealed

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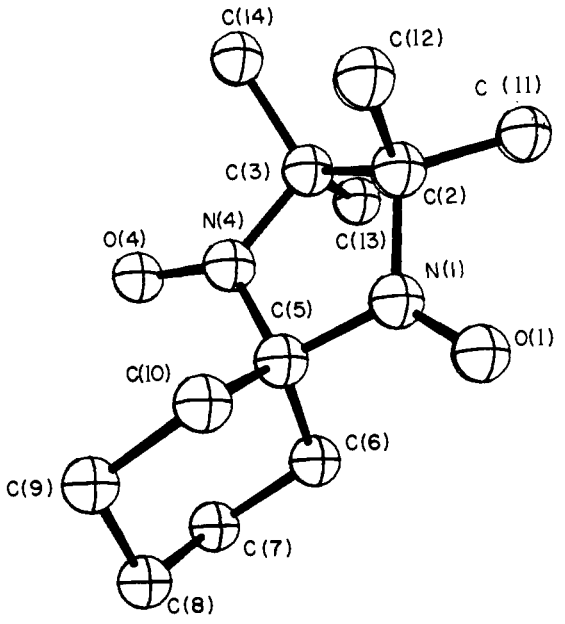


Figure 1. A computer-generated perspective drawing of dinitroxide **9**. Hydrogen atoms are omitted for clarity.

in a Lindemann capillary. Preliminary photographs revealed no symmetry other than an inversion center. Precise lattice constants of $a = 6.537\ (3)$, $b = 14.328\ (7)$, $c = 14.724\ (7)$ Å, $\alpha = 105.98\ (4)$, $\beta = 81.52\ (4)$, and $\gamma = 96.19\ (4)$ ° were obtained by least-squares fitting of 15 high-angle reflections. A measured density of 1.31 g/cm^3 indicated four molecules of composition $C_{12}H_{22}N_2O_2$ per unit cell. A total of 2948 unique reflections with $2\theta \leq 114°$ were surveyed using graphite monochromated Ca Kα (1.541 78 Å) radiation and an ω-scan technique. After correction for Lorentz, background, and polarization effects, 1751 (59%) reflections were judged observed ($F_o \geq 3\sigma(F_o)$). The intensity data were converted to normalized structure factors and sign determination was undertaken in the space group P_1.[10] Sign determination proceeded uneventfully and most atoms of both molecules in the asymmetric unit were visible in the E synthesis. Hydrogen atoms were located in subsequent difference syntheses. Full-matrix, least-squares refinements with anisotropic nonhydrogen atoms and isotropic hydrogen atoms have converged to an unweighted residual of 0.078 for the observed data.[11] See the paragraph on supplementary material for further crystallographic details.

Both molecules in the asymmetric unit have essentially the same conformation. A perspective drawing of one is presented in Figure 1. All bonds distances and angles generally agree well with accepted values[12] and numbers in the following discussion refer to average values for the two molecules in the asymmetric unit. The N–O bond is a relatively short 1.258 (8) Å. This is slightly shorter than the N–O bonds in the closely related nitroxides, potassium 2,2,5,5-tetramethyl-3-carboxypyrroline-1-oxyl[13] (1.277 (8), 1.263 (13) Å), 2,2,5,5-tetramethylpyrrolidin-3-one-1-oxyl azine[14] (1.266 (7) Å), and doxylcyclohexane[15] (1.259 (4) Å). All these model substances have a nitroxide in a five-membered ring flanked by fully substituted carbons. They also have planar nitroxide groups which may represent averaged structures given the proclivity of nitroxides to undergo modest distortions from planarity. In dinitroxide **9**, however, the nitroxide groups show an average distortion from planarity of 12 (3)°. The C–N–C angle is 114.9 (10)° and the N–O bond length is 1.478 (12) Å, in excellent agreement with related nitroxides.[12-15]

The most important structural feature of **9** is the proximate nitroxide groups. Interestingly, the N---N distance is an unexceptional 2.22 (1) Å and the N–C–N angle is 98.5 (5)°. The C(2)–C(3) bond is a surprisingly short 1.513 (6) Å, however.

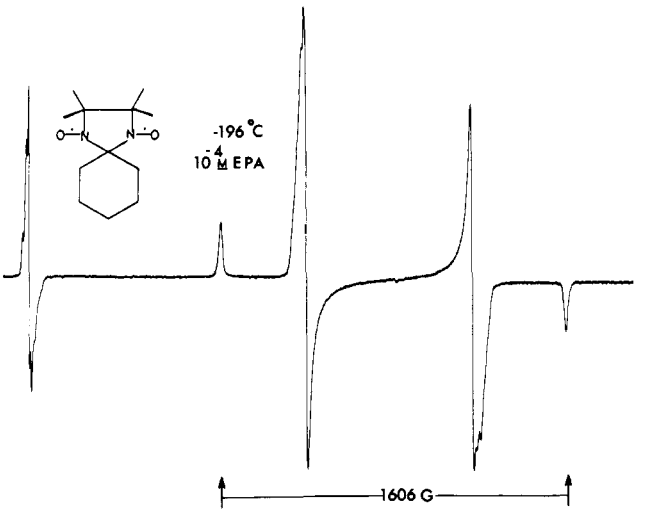
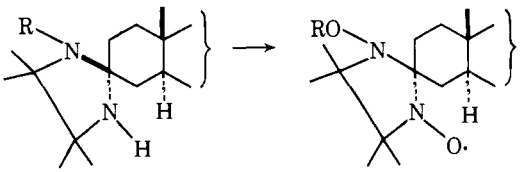


Figure 2. EPA glass spectrum (−196 °C) of dinitroxide **9**.

The five-membered ring is not planar and shows a symmetric distortion with the nitrogens above and below the best least-squares plane by 0.11 Å and the $C(CH_3)_2$ carbons oppositely disposed by 0.16 Å. The spiro carbon is on the best plane of the ring. The average torsional angles are C(5)–N(1) 11°, N(1)–C(2) 26°, C(2)–C(3) 27°, C(3)–N(4) 24°, and N(4)–C(5) 9°. The five-membered ring is essentially orthogonal (85°) to the best plane through C(6), C(7), C(9), and C(10) of the cyclohexane ring. The average C–H bond is 1.0 Å, although there are substantial deviations from this value. There are no abnormally short intermolecular contacts.

A 10^{-4} M solution of **9** in $CHCl_3$ gave no observable ESR signal owing to the anisotropy in the electron–electron interaction. At 0.1 M concentration in $CHCl_3$, a single intense exchange narrowed line with a peak to peak width of 100 G was observed. Figure 2 shows the ESR spectrum of a 10^{-4} M solution of **9** in an EPA glass at −196 °C. The separation between the two outermost lines, $2D$, was 1606 G, giving a dipolar splitting, D = 803 G. This value is about *three times larger than splittings previously observed for a nonconjugated dinitroxide*. It is clear from the spectrum that the other dipolar splitting parameter, $E \ll D$, is probably of the order of a_N or less. A half-field ($\Delta m = 2$) transition was observed at 1563 G.

We have also prepared the dinitroxide derivative **16** of 5α-cholestan-3-one by a route analogous to **1b** → **9**. Thus, MCPA oxidation of imidazolidine **11** gave nitroxide **12**.[16] Nitroxide **12** was then converted into *N*-hydroxy nitroxide **15**



11, R = H
12, R = O·
13, R = OAc

14, R = Ac
15, R = H
16, R = ·

via intermediates **13** and **14**. Oxidation of **15** with *t*-BuOH–KO-*t*-Bu–O_2 gave crystalline dinitroxide **16** in 85% yield. The EPA rigid glass ESR spectrum of **16** was very similar in appearance to that shown in Figure 2. For **16**, $2D$ = 1606 G.

The relatively small steric size of these new dinitroxide ketone spin labels (cf. ref 4), the ease with which they may be attached at the site of a ketone group, and the enormous dipolar

splitting exhibited by the labels should all combine to enhance their importance in theoretical studies as well as in spin labeling studies in which extreme sensitivity to motion is required. Finally, these new labels offer the possibility of "synthesizing" in situ a mononitroxide spin label through chemical *reduction* of one of the two nitroxide functions.

Experimental Section

Melting points were determined with a Hoover melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Beckman IR5A infrared spectrophotometer. Ultraviolet (UV) spectra were determined on a Cary 15. The nuclear magnetic resonance (NMR) spectra were obtained with a Varian XL-100 spectrometer using Me_4Si as an internal standard. X-Band electron spin resonance spectra were recorded with a Varian E-9 spectrometer which was calibrated with a Varian F-8 nuclear flux meter and a Hewlett-Packard 5383A frequency counter. Elemental analyses and mass spectra were determined at the University of Oregon by Dr. R. Wielesek.

2,2-Pentamethylene-4,4,5,5-tetramethylimidazolidine (1a). A 100-mL flask was fitted with a Dean-Stark water separator containing anhydrous K_2CO_3 and then was charged with 60 mL of benzene, 4.43 g (38.2 mmol) of 2,3-diamino-2,3-dimethylbutane, 3.74 g (38.1 mmol) of cyclohexanone, and 15 mg of toluenesulfonic acid monohydrate. After a 48-h reflux period fractional distillation afforded imidazolidine **1a** as a colorless oil: bp 122 °C (18 mm) (4.49 g, 60%); NMR ($CDCl_3$) δ 1.10 (s, 12 H), 1.56 (bm, 10 H), 1.87 (bs, 2 H, NH). Anal. Calcd for $C_{12}H_{24}N_2$: C, 73.41; H, 12.32; N, 14.27. Found: C, 73.80; H, 12.48; N, 14.32.

2,2,4,4,5,5-Hexamethylimidazolidine (2a). The imidazolidine was prepared following the above described general procedure except that 25 equiv of acetone was used. Fractional distillation afforded imidazolidine **2a** as a colorless oil, bp 60 °C (12 mm), which slowly crystallized as the monohydrate upon exposure to the atmosphere (76%, mp 41 °C): NMR ($CDCl_3$) δ 1.10 (s, 12 H), 1.35 (s), 1.73 (bs, 2 H). Anal. Calcd for $C_9H_{22}N_2O$: C, 62.06; H, 12.64; N, 16.09. Found: C, 61.71; H, 12.75; N, 16.12.

2,2-Dipropyl-4,4,5,5-tetramethylimidazolidine (3a). This substance was prepared following the above described general procedure except that 15 equiv of 4-heptanone was used. Fractional distillation afforded imidazolidine **3a**: bp 110 °C (30 mm) (32%); NMR δ 1.10 (s, 12 H), 0.90–1.45 (bm), 2.15 (bs); mass spectrum m/e 169 ($M - CH_2CH_2CH_3$).

2,2-Pentamethylene-4,4,5,5-tetramethylimidazolidine-1-oxyl (1b). To a stirred solution of **1a** (4.49 g, 22.9 mmol) in 40 mL of ether at 0 °C was added dropwise overnight a solution of MCPA (7.00 g, 40.7 mmol) dissolved in ether (70 mL). The yellow solution was then washed thrice with 10% Na_2CO_3, dried (K_2CO_3), and then evaporated. Chromatography of the resulting solid over silica gel (elution with $CHCl_3$) afforded a yellow solid, mp 96–115 °C (1.64 g). Recrystallization from hexane afforded analytically pure nitroxide **1b**: mp 121–123 °C; ESR (4:1 MeOH–$CHCl_3$) three lines (a_N = 13.8 G); UV max (EtOH) 237 nm (ϵ 2700), vis max 450 nm (ϵ 6.5); m/e 211 (M^+); NMR ($CDCl_3$) (after phenylhydrazine reduction[9]) δ 1.10 (s, 6 H), 1.66 (s, 6 H), 1.66 (bs, 10 H). Anal. Calcd for $C_{12}H_{23}N_2O$: C, 68.25; H, 10.90. Found: C, 68.19; H, 10.96.

2,2,4,4,5,5-Hexamethylimidazolidine-1-oxyl (2b). To a 0 °C stirred solution of 300 mg (1.92 mmol) of **2a** in 1.5 mL of ether was added 585 mg (3.39 mmol) of MCPA dissolved in 10 mL of ether dropwise overnight. The yellow mixture was then washed thrice with 10% Na_2CO_3, dried (K_2CO_3) (crude yield of **2b** by UV spectroscopy was 36%), and then concentrated by distilling the ether through a Vigreux column. The nitroxide was isolated by preparative vapor phase chromatography. Recrystallization from pentane afforded the analytical specimen, mp 55 °C. The isolated yield was much lower than the crude yield owing to the high volatility of **2b**. Anal. Calcd for $C_9H_{19}N_2O$: C, 63.12; H, 11.18; N, 16.36. Found: C, 63.26; H, 11.23; N, 16.25.

2,2-Dipropyl-4,4,5,5-tetramethylimidazolidine-1-oxyl (3b). To a stirred solution of 70 mg (0.33 mmol) of **3a** in 1.0 mL of ether at 0 °C was added 100 mg (0.583 mmol) of MCPA dissolved in 7 mL of ether dropwise overnight. The yellow solution was then washed thrice with 10% Na_2CO_3, dried (K_2CO_3), and then evaporated. Preparative silica gel thick layer chromatography of the resulting oil afforded solid nitroxide **3b** (8 mg, 10%), mp 60–61 °C, mass spectrum m/e 227.212 (calcd, 227.212).

2,2-Pentamethylene-1,4,4,5,5-pentamethylimidazolidine-3-oxyl (4). To a stirred solution of 154 mg (0.735 mmol) of **1b** dissolved in 5 mL of acetonitrile at 0 °C was added 274 mg (133 mmol) of trimethyloxonium hexafluorophosphate portionwise over a 15-min period. After a 1-h stir at 0 °C the solution was diluted with 20 mL of CH_2Cl_2, washed thrice with chilled 10% Na_2CO_3, dried (K_2CO_3), and then evaporated. Chromatography of the resulting oil over silica gel afforded 116 mg (70%) of nitroxide **4** as an oil: NMR ($CDCl_3$) (after phenylhydrazine reduction[9]) δ 1.02 (s, 6 H), 1.17 (s, 6 H), 1.70 (bs, 10 H), 2.29 (s, 3 H); ESR ($CHCl_3$) three lines, a_N = 15.0 G; mass spectrum m/e 225.196 (calcd, 225.197).

***N*-Acetoxy-2,2-pentamethylene-4,4,5,5-tetramethylimidazolidine (6).** To a 25 °C stirred mixture of 7 mg of 10% Pd/C and 3.0 mL of THF under an H_2 atmosphere was added dropwise a solution of 328 mg (1.55 mmol) of **1b** in 2.5 mL of THF. Stirring was continued until H_2 uptake ceased (10 min). The mixture was filtered. The filtrate (colorless) was cooled to 0 °C and treated with 263 mg (2.60 mmol) of triethylamine dissolved in 0.3 mL of THF followed immediately by the addition of 177 mg (2.25 mmol) of acetyl chloride dissolved in 0.3 mL of THF. After a 1-h stir, the mixture was filtered and the filtrate was evaporated. The residual oil was treated with hexane and this mixture was filtered. Evaporation of the filtrate afforded crude acetate **6** as a pale yellow, hydrolytically unstable oil (288 mg, 73%): IR 1775 cm^{-1} (N–OAc); NMR ($CDCl_3$) δ 1.06 (s, 6 H), 1.16 (s, 6 H), 1.63 (bs, 10 H), 2.12 (s, 3 H); mass spectrum m/e 254.198 (calcd, 254.199).

1-Acetoxy-2,2-pentamethylene-4,4,5,5-tetramethylimidazolidine-3-oxyl (7). The crude nitroxide was prepared in a manner similar to the preparation of **1b**. From 996 mg of **6** there was obtained 930 mg of crude **7**. Crude **7** was purified by silica gel chromatography (elution with $CHCl_3$), producing 838 mg (80%) of **7** as an orange oil: IR 1775 cm^{-1} (N–OAc); NMR ($CDCl_3$) (after phenylhydrazine reduction[9]) δ 1.10 (s, 6 H), 1.16 (s, 6 H), 1.40–1.85 (b), 2.12 (s, 3 H).

1-Hydroxy-2,2-pentamethylene-4,4,5,5-tetramethylimidazolidine-3-oxyl (8). To a stirred solution of 385 mg (1.43 mmol) of acetate **7** in 15 mL of methanol at 0 °C was added dropwise a solution of 250 mg of KOH dissolved in 10 mL of methanol. After 1.5 h at 25 °C, the volatiles were removed at reduced pressure. The residue was leached with ether. The ether was then washed with water (four times), dried ($MgSO_4$), and evaporated, giving nitroxide **8** as yellow crystals (242 mg, 75%). Recrystallization from hexane afforded 182 mg (56%) of pure nitroxide: mp 86–87 °C; UV max (EtOH) 245 nm (ϵ 2430); mass spectrum m/e 227.174 (calcd, 227.176); ESR ($CHCl_3$) three lines (a_N = 15.5 G); NMR ($CDCl_3$) (after phenylhydrazine reduction[9]) δ 1.10 (s, 12 H), 1.76 (b, 10 H). Anal. Calcd for $C_{12}H_{23}N_2O_2$: C, 63.40; H, 10.20; N, 12.32. Found: C, 63.23; H, 10.20; N, 12.30.

2,2-Pentamethylene-4,4,5,5-tetramethylimidazolidine-1,3-dioxyl (9). To a solution of 115 mg (0.51 mmol) of **8** in 5 mL of *tert*-butyl alcohol at 25 °C was added 2.0 mL of 0.4 M potassium *tert*-butoxide in *tert*-butyl alcohol. Oxygen gas was then bubbled through this solution for 3 min, giving an orange mixture. The solvent was removed at reduced pressure and the residue was treated with several fresh portions of hexane. The combined hexane extracts were evaporated and the residue once again was leached with hexane. Centrifugation followed by evaporation of the supernatant solution gave 97 mg (84%) of orange crystals. Low-temperature recrystallization from pentane gave pure dinitroxide as orange needles: mp 70.5–71.5 °C; UV max (*t*-BuOH) 235 nm (ϵ 4020); mass spectrum m/e 226.167 (calcd, 226.168); ESR (EPA glass at −196 °C) $2D$ = 1606 G, Δm = 2 transition at 1563 G. An x-ray crystallographic analysis confirmed the assigned structure (see text).

1,3-Diacetoxy-2,2-pentamethylene-4,4,5,5-tetramethylimidazolidine (10). Using a procedure analogous to the preparation of **6**, both acetoxy nitroxide **7** and dinitroxide **9** gave diacetate **10** (by NMR) (unstable to chromatography): NMR ($CDCl_3$) δ 1.18 (bs, 12 H), 1.35–1.96 (bm, 10 H), 2.13 (s, 6 H).

4′,4′,5′,5′-Tetramethylspiro[5α-cholestane-3,2′-imidazolidine] (11). Crude imidazolidine **11** was prepared in a manner similar to the preparation of **1a**. From 1.47 g (3.78 mmol) of 5α-cholestan-3-one and 440 mg (3.79 mmol) of 2,3-dimethyl-2,3-diaminobutane there was obtained 1.26 g of white solid (68%). Recrystallization from methanol gave **11** as white needles, mp 145–147 °C. Anal. Calcd for $C_{33}H_{60}N_2$: C, 81.75; H, 12.47; N, 5.78. Found: C, 81.58; H, 12.79; N, 5.36.

4′,4′,5′,5′-Tetramethylspiro[5α-cholestane-3,2′-imidazolidine]-1β-yloxy 12. The crude nitroxide **12** (55%) was prepared in a manner similar to the preparation of **1b**. Pure **12**, free of 5α-cholestan-3-one formed during the MCPA oxidation, was obtained by repeated recrystallizations from hexane, mp 160–163 °C, ESR see note 16. Anal. Calcd for $C_{33}H_{59}N_2O$: C, 79.30; H, 11.90; N, 5.60. Found: C, 78.97; H, 12.01; N, 5.53.

1′β-Acetoxy-4′,4′,5′,5′-Tetramethylspiro[5α-cholestane-3,2′-imidazolidine] (13). Crude acetate **13** was prepared in a manner similar to the preparation of **6**. From 179 mg (0.357 mmol) of nitroxide **12** there was obtained 170 mg (88%) of crude **13**. This was used for the next step without further purification.

1′β-Acetoxy-4′,4′,5′,5′-Tetramethylspiro[5α-cholestane-3,2′-imidazolidin]-3′α-yloxy (14) and 1′β-Hydroxy-4′,4′,5′,5′-tetramethylspiro[5α-cholestane-3,2′-imidazolidin]-3′α-yloxy (15). The oxidation of 170 mg (0.312 mmol) of acetate **13** was effected in a manner similar to the preparation of **1b**. Chromatography of the crude product over silica gel (elution with $CHCl_3$) afforded **14** (96 mg, 55%) as an orange foam. Hydrolysis of **14** in the usual manner afforded **15** (63 mg, 72%). Analytically pure **15** was obtained after two recrystallizations from hexane, mp 159.5–160.5 °C. Anal. Calcd for $C_{33}H_{59}N_2O_2$: C, 76.84; H, 11.53; N, 5.43. Found: C, 76.64; H, 11.25; N, 5.26.

4′,4′,5′,5′-Tetramethylspiro[5α-cholestane-3,2′-imidazolidin]-1′β,3′α-ylidioxy (16). Crude dinitroxide **16** was prepared in a manner similar to the preparation of **9**, From 21 mg of **15**, there was obtained 17 mg of crude **16** (84%). Analytically pure **16** was obtained after recrystallization from pentane (13 mg, 43%), mp 135 °C. Anal. Calcd for $C_{33}H_{58}N_2O_2$: C, 76.99; H, 11.35; N, 5.44. Found: C, 76.74; H, 11.51; N, 5.33. ESR, see text; UV max (*t*-BuOH) 236 nm (ε 4215).

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Supplementary Material Available: Fractional coordinates, bond distances, bond angles, and observed and calculated structure factors (15 pages). Ordering information is given on any current masthead page.

References and Notes

(1) Alfred P. Sloan Foundation Fellow; NIH Research Career Development Award Recipient.
(2) Camille and Henry Dreyfus Teacher–Scholar Grant Awardee and Alfred P. Sloan Foundation Fellow.
(3) For a review see G. R. Luckhurst in "Spin Labeling. Theory and Applications", L. J. Berliner, Ed., Academic Press, New York, N.Y., 1976, Chapter 4.
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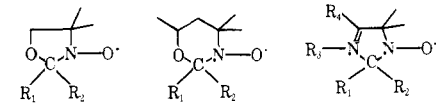
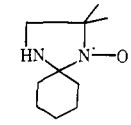
(7) At least three other methods are available for the rigid attachment of a nitroxide moiety to another molecule at the site of a ketone grouping: the synthesis of doxyl nitroxides i,[6] tetrahydrooxazine nitroxides ii (A. Rassat and P. Rey, *Tetrahedron*, **30**, 3315 (1974)), and Δ^3-imidazoline-1-oxyl nitroxides iii (T. K. Sevast'yanova and L. B. Volodarskii, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 2339 (1972); *Chem. Abstr.*, **78**, 58313f (1973)).



i ii iii

(8) Because the amino function in nitroxides **b** is quite hindered toward further reactions, we have recently synthesized nitroxide iv and are currently investigating its reactions. J. F. W. Keana and S. Magnuson, unpublished work.



iv

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(16) The ESR spectrum ($CHCl_3$) of **12** showed the usual three-line spectrum (a_N = 15.0 G). However, upon deoxygenation of the sample and with a sweep width of 10 G, each line showed additional splitting due to neighboring protons characteristic of the 3β-nitroxide epimer of 3-doxylcholestane.[17,18] These data, taken together with the observation that *N*-hydroxy nitroxide **15** did not show this additional splitting, permits the assignment of the β configuration for the nitroxide group in **12** and the α configuration for the nitroxide group in **15**. The ESR lines in both **12** and **15** were noticeably broader than those observed with the corresponding doxyl nitroxides.[17] This is likely due to an additional coupling to the neighboring ^{14}N nucleus in amino nitroxides **12** and **15**.
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