atriene reported in Table I using a Perkin-Elmer 154 vapor fractometer with a $^1/_4$-in. packed column. Although the purity, as judged from analytical GLC, only increased from ca. 98 to 99%, the thermochemical results increased by about 5%, indicating some contamination by a component (probably a polymer) that was not detected by analytical GLC using a capillary column and a flame ionization detector. Old samples showed a distinct turbidity or tendency to precipitate from solutions in *n*-hexane, due, presumably, to polymer formation.

Completeness of reaction and absence of side reactions was indicated by a single hexane peak in the GLC trace of the reaction product. A competing polymerization reaction in the calorimeter would not have been detected by this method.

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Registry No. (*Z*)-1,3-Hexadiene, 14596-92-0; (*E*)-1,3-hexadiene, 20237-34-7; (*Z*)-1,4-hexadiene, 7318-67-4; (*E*)-1,4-hexadiene, 7319-00-8; 1,5-hexadiene, 592-42-7; (*Z,Z*)-2,4-hexadiene, 6108-61-8; (*E,Z*)-2,4-hexadiene, 5194-50-3; (*E,E*)-2,4-hexadiene, 5194-51-4; (*Z*)-1,3,5-hexatriene, 2612-46-6; (*E*)-1,3,5-hexatriene, 821-07-8.

Design of Modified Pyrroline *N*-Oxide Derivatives as Spin Traps Specific for Hydroxyl Radical

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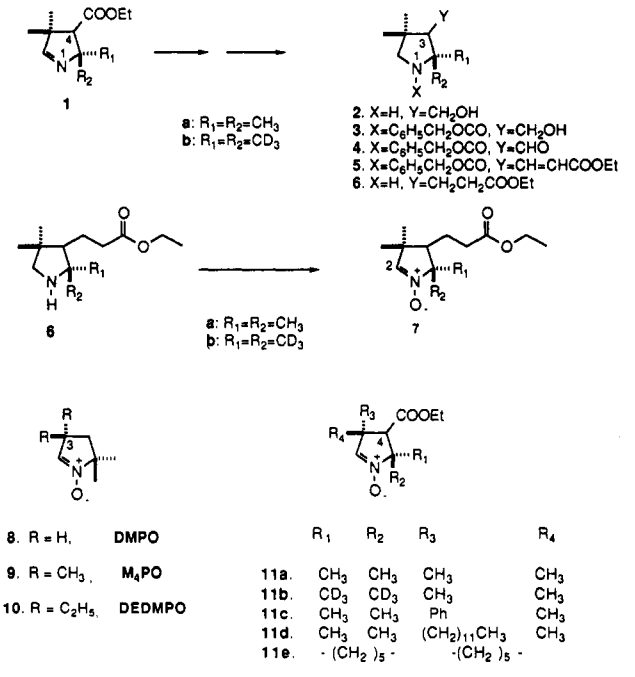
Nitrones, 4-[2-(ethoxycarbonyl)ethyl]-3,3,5,5-tetramethyl-1-pyrroline *N*-oxide (**7a**) and 4-[2-(ethoxycarbonyl)ethyl]-5,5-di([$^2H_3$]methyl)-3,3-dimethyl-1-pyrrolidine *N*-oxide (**7b**) have been synthesized. The ability of the nitrone (**7a**) to spin trap hydroxyl and superoxide radicals has been compared with nitrones **11a** and 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO, **8**). Nitrone **11a** bears a carbethoxy group at C-4, whereas nitrone **7a** has a spacer of two methylene units between the carbethoxy and the basic nitrone ring skeleton. Nitrone **11a** trapped both hydroxyl and superoxide radicals, while nitrone **7a** trapped only hydroxyl radical. The hydroxyl radical adducts of **7a** and **11a** were more resistant toward superoxide-mediated reduction than the hydroxyl radical adduct of DMPO (**8**).

In recent years reduced oxygen species, including superoxide and hydroxyl radical, have been proposed as mediators in a variety of cellular responses, such as phagocytosis, ischemia/reperfusion injury, aging, and cancer.[1] Despite numerous efforts to study the role of these free radicals in initiating tissue injury, the most difficult obstacle remains to be the unambiguous identification of these reactive species. Among the different methods for detecting superoxide and hydroxyl radicals in biological systems, electron spin resonance (ESR) spectroscopy combined with spin trapping offers the opportunity to simultaneously measure and characterize these oxygen-centered free radicals.[2-5] In this technique, transient free radicals are trapped by nitrone and nitroso compounds to give a persistent nitroxide spin trapped adduct that can be observed using a conventional ESR spectrometer.

Among several nitrones employed as spin traps, 5,5-dimethyl-1-pyrroline 1-oxide (DMPO, **8**) is most frequently used in biological systems to detect superoxide and hydroxyl radicals.[6-12] However, DMPO has several limitations.[4,5] To circumvent some of these problems, a family of 5,5-disubstituted, pyrroline 1-oxides have been prepared with enhanced sensitivity,[13] lipophilicity, and decreased susceptibility toward air oxidation.[14] More recently we have synthesized a spin trap, 3,3-diethyl-5,5-dimethylpyrroline 1-oxide (DEDMPO, **10**), which was found to be specific for hydroxyl radical.[15] It does not spin trap superoxide. However, the preparation of DEDMPO (**10**) is

Scheme I



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difficult with one step being potentially explosive. Furthermore, this nitrone is prone to air oxidation in the presence of metal ions.[16] It is clear that a new family of compounds is desired which lack the problems associated with DEDMPO, yet retain the selective nature of this nitrone.

Recently, a short and flexible synthesis of modified M_4PO derivatives (11) has been developed[17] (Scheme I). This not only allows the introduction of the different alkyl groups at C-3 and C-5 but also provides a carbethoxy group at the C-4 position. The introduction of a functional group at C-4 position is important since it can further be used to modify the structure.[18]

The spin traps 11 were effective as scavengers for many of the free radicals such as t-BuO•, •CH_2OH, •OH, and Ph•, and the adducts resulting from the trapping reaction had half-lives of several hours. Spin adducts obtained from the trapping of superoxide radical anion with spin traps 11 were short lived in comparison to DMPO. On the basis of our earlier kinetic studies on the spin trapping of superoxide with DMPO and 3,3,5,5-tetramethylpyrroline 1-oxide (M_4PO, 9), we believe that this is probably due to stereoelectronic interactions between the radical trapped and the COOEt group at C-4. In continuation of our program, we further decided to introduce a spacer between the basic ring skeleton and COOEt group of **11a** and examine its effects on the trapping of hydroxyl and superoxide radical.

In this paper, we report our successful synthesis of spin traps **7a** and **7b**. We also compare their selectivity toward superoxide and hydroxyl radicals generated from the model superoxide-generating system, xanthine/xanthine oxidase and ferrous salts. The incorporation of two deuteriomethyl groups in **7b** was planned to enhance its sensitivity toward superoxide and hydroxyl radical.[13] Of interest is the finding that spin trap **7a** reacts specifically with hydroxyl radical at near diffusion controlled rates and that the corresponding spin adduct is more stable than DMPO-OH in the presence of a superoxide flux.

Results and Discussion

Chemical Synthesis. Lithium aluminum hydride reduction of **1a** smoothly gave **2a** in 97% yield. The latter was treated with 1.1 equiv of benzyl chloroformate at −20 °C to room temperature to give **3a** in 95% yield. It was further subjected to pyridinium chlorochromate (PCC) oxidation at room temperature to give the corresponding aldehyde **4a** in 93%. When **4a** was subjected to standard Wittig reaction conditions by warming its benzene solution at 55–60 °C with (carbethoxymethylene)triphenylphosphorane, the *trans* unsaturated ester derivative **5a** was obtained in 76% yield. The ^1H NMR and ^{13}C NMR showed the characteristic signals for α,β-unsaturated ester derivative. The free amine **6a** was prepared by hydrogenation with 10% Pd over carbon and then oxidized to the corresponding nitrone **7a** using 30% H_2O_2 with Na_2WO_4 as a catalyst.[19] It was further purified over silica gel

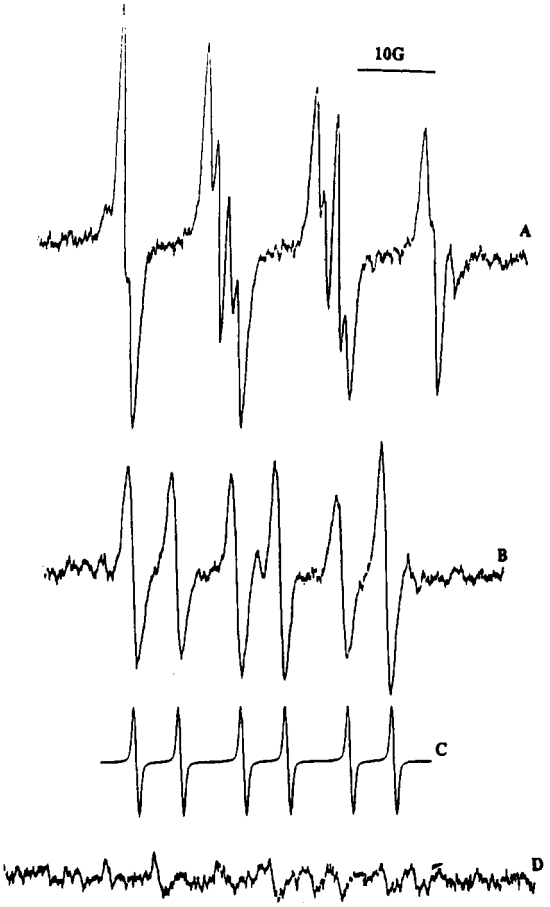

Figure 1. ESR spectra of superoxide spin adducts obtained as a consequence of the reaction of xanthine with xanthine oxidase in the presence of (A) 8, (B) **11a**, (C) computer simulation of B, (D) **7a**. Microwave power was 20 mW; modulation frequency was 100 KHz, with a modulation amplitude of 1 G; sweep time was 12.5 G/min, and the receiver gain was 2×10^4, with a response time of 1 s. Hyperfine splitting constants are listed in Table I.

Table I

	7a	8	11a
A_N ($O_2^{\bullet-}$), G		14.1	13.5
A_H ($O_2^{\bullet-}$), G		11.2	5.6
A_H ($O_2^{\bullet-}$), G		1.2	
A_N ($OH^\bullet$), G	14.5	14.9	14.5
A_H ($OH^\bullet$), G	6.6	14.9	10.2
k,[a] M^{-1} s^{-1}	4.3×10^9	3.4×10^9 [b]	3.1×10^9

[a] Second order rate constant for the reaction of hydroxyl radical with each nitrone. Values represent a mean of at least three determinations. [b] Taken from ref 22.

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to give the pure nitrone **7a**, in 85% yield, which was free of any paramagnetic impurities. Similarly, the whole series was repeated with 5,5-bis(deuteriomethyl)-3,3-dimethyl-4-carbethoxypyrroline (**1b**) for the synthesis of required spin trap **7b**, in which two CD_3 groups have been incorporated at C-5 position.

Spin Trapping Studies. Superoxide was spin trapped by using nitrones **7a**, 8, and **11a** and a superoxide generating system, consisting of xanthine in the presence of xanthine oxidase at pH 7.4. The rate of superoxide production, as measured by the superoxide dismutase-inhibitable reduction of cytochrome c, was 10 μM/min. Nitrone **11a** reacts with superoxide to give an ESR spectrum with hyperfine splitting constants A_N = 13.5 G, A_H = 5.60 G. When the spin trap **7a** was used in place of **11a** we were unable to spin trap superoxide at the gain used (Figure 1). We believe that this is due to increased carbon length at the fourth position of the pyrroline ring. These findings parallel our earlier observation that as the bulkiness on the pyrroline ring increased, the ability to spin trap superoxide decreased.[15]

The production of the nitrone–superoxide spin adduct was completely inhibited by the addition of superoxide dismutase (30 units/mL), confirming that the observed ESR spectra resulted from the reaction of superoxide with the nitrones. Hyperfine splitting constants for superoxide spin trapped adducts are summarized in Table I.

Spin trapping of hydroxyl radical was conducted by the addition of ferrous ammonium sulfate (0.1 mM) to hydrogen peroxide (0.3%) according to the Fenton reaction.[20] All spin traps reacted efficiently with hydroxyl radical to give ESR spectra shown in Figure 2. Identical spectra were also obtained when hydroxyl radical was generated by photolysis of hydrogen peroxide and by the Haber–Weiss reaction.[20] The addition of catalase (300 units/mL) confirmed that the ESR spectrum resulted from the spin trapping of hydroxyl radical derived from hydrogen peroxide. The hyperfine splitting constants for hydroxyl radical spin trapped adducts obtained from computer simulation are presented in Table I.

Rate constants for the reaction of various spin traps with hydroxyl radical are given in Table I. In earlier reports, equations were developed to determine the rate constant for the reaction of hydroxyl radical with DMPO.[21,22] Because of this, we will only summarize our results. Rate constants for spin trapping of hydroxyl radical were determined by competitive kinetics where the known rate constant for reaction of hydroxyl radical with DMPO was chosen as the standard. Hydroxyl radical was generated by UV photolysis of hydrogen peroxide (0.3%) at pH 7.4. Data were analyzed according to the equations developed by Marriot et al.[21] The results showed that all nitrones tested react with hydroxyl radical at near diffusion controlled rates. In contrast to the spin trapping of superoxide, the increase in bulkiness of nitrones **7a** and **11a** did not prevent their reactions with the highly reactive hydroxyl radical.

One of the limitations of DMPO is the rapid decay of its hydroxyl radical spin adduct in the presence of a flux of superoxide.[9] Since the increase in bulkiness of nitrones **7a** and **11a** appears to slow down their reaction with superoxide, we thought that their corresponding hydroxyl radical spin adducts would be more resistant toward superoxide mediated reduction than DMPO-OH. To test this hypothesis, the stability of the hydroxyl radical spin adducts of **7a**, **11a**, and DMPO (8) in the presence of a flux of superoxide were compared.

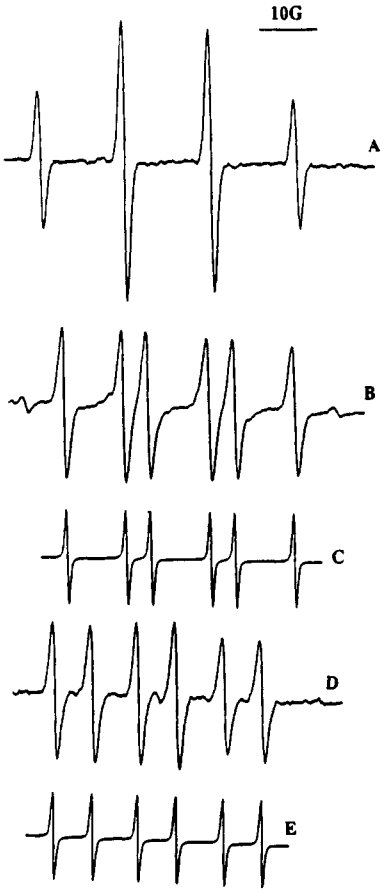

Figure 2. ESR spectra of hydroxyl radical spin adducts obtained as a result of the reaction of hydrogen peroxide (0.3%) with iron–DPTA, in the presence of (A) 8, (B) **11a**, (C) computer simulation of B, (D) **7a**, (E) computer simulation of D. Instrument settings were the same as described in Figure 1, except that the receiver gain was 5×10^3. Hyperfine splitting constants are listed in Table I.

The hydroxyl radical spin adducts of the three nitrones were generated by UV photolysis in the presence of hydrogen peroxide. The studies were conducted as shown in Figure 3. In the absence of superoxide, the signal intensity of the hydroxyl radical spin adducts of the three nitrones did not diminish over a period of at least 8 min. In the presence of 1 μM/min superoxide, the hydroxyl radical spin adducts of the three nitrones decayed slowly over the period of 8 min. Not surprisingly, when the rate of superoxide generation was increased to 5 μM/min, the rate of the hydroxyl radical spin adduct decomposition was more rapid. In the case of DMPO, the decomposition rate was more than those of **7a** and **11a**. Even when the rate of superoxide generation was increased to 10 μM/min, the rate of reduction of the hydroxyl radical spin adducts of **7a** and **11a** was still slow compared to DMPO-OH. In fact, the rate of decomposition of DMPO-OH was so rapid that no ESR signal was recorded by the time the scan started. It was estimated that the rates of reduction of the hydroxyl radical spin adducts of nitrones **7a** and **11a**, in the presence of 5 and 10 μM/min superoxide, were one-fourth and half that of DMPO-OH, respectively. Thus increased steric hindrance at the C-4 position of the nitrone makes the hydroxyl radical spin adduct more resistant to superoxide mediated reduction.

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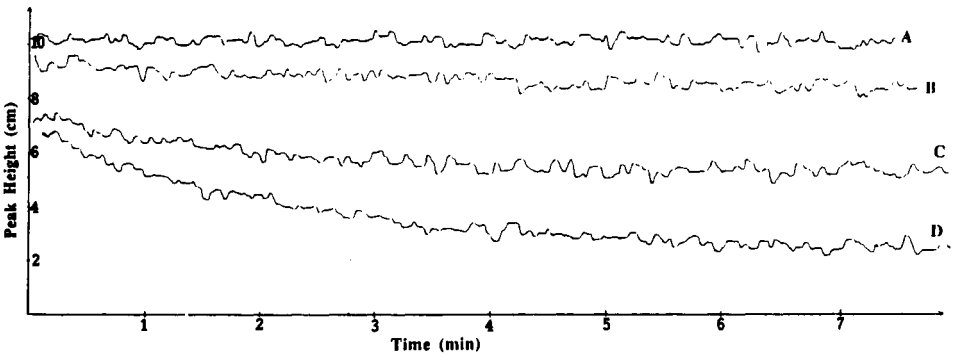

Figure 3. Effect of superoxide on the stability of hydroxyl radical spin adduct of **7a**. The magnetic field was set at the top of the first low-field peak of the ESR signal. Time zero is the time at which recording began, usually between 45 and 60 s after the addition of xanthine oxidase. Tracing A represents the peak height of the hydroxyl radical spin adduct of **7a** in the absence of superoxide. Tracing B, C, and D were generated in the presence of superoxide at 1 μM/min, 5 μM/min, and 10 μM/min. Instrument settings were the same as described in Figure 1, except that the receiver gain was 8×10^4.

In summary, new nitrone spin traps were synthesized (**7a** and **7b**). **7a** reacts with hydroxyl radical effectively to form the corresponding nitroxide which is more stable than DMPO-OH in the presence of a flux of superoxide. The spin trapping of hydroxyl and superoxide radicals with **7b** is in progress. Use of these spin traps may expand our ability to detect and facilitate our understanding of the role of hydroxyl radical in biological systems.

Experimental Section

General Comments. Diethylenetriaminepentaacetic acid (DTPA), xanthine, xanthine oxidase, superoxide dismutase, and catalase were obtained from Sigma Chemical Co. (St. Louis, MO). Chelex-100 was purchased from Bio-Rad (Richmond, CA). All other reagents were used as received from commercial suppliers unless otherwise stated. All dried solvent was distilled according to Perrin et al.[23] All buffers used for quenching organic reactions were 50 mM sodium phosphate buffer, pH 7.4. All buffers in ESR experiments were passed through a Chelex-100 ion exchange column before use to remove metal ion impurities. All spin trapping experiments were conducted in an air-saturated 50 mM sodium phosphate buffer, pH 7.4, containing 1 mM DTPA.

^{13}C proton decoupled NMR and ^1H NMR spectra were obtained in $CDCl_3$. The chemical shifts of $CDCl_3$ at 77.0 and 7.27 ppm were used as internal standard for ^{13}C and ^1H spectra, respectively. J values are in hertz. GC–MS were obtained with a capillary (cross-linked methyl silicone gum, 12-m × 0.2-mm × 0.33-mm film thickness) column. Flash chromatography was performed according to Still et al.[24] Analytical TLC utilized Merck 60F-254, 250-mm precoated silica gel plates. All reactions were routinely run under argon atmosphere. $MgSO_4$ was used as drying agent for organic solutions.

The spin trap DMPO (**8**) was synthesized according to the procedure of Bonnett et al.[25] and freshly distilled prior to use. The spin trap **11a** was synthesized according to the procedure of Dehnel et al.[17] and was recrystallized from pentane. Computer simulation of ESR spectra was obtained according to the algorithm described by Oehler and Janzen.[26]

3-(Hydroxymethyl)-2,2,4,4-tetramethyl-1-pyrrolidine (2a). To a mixture of lithium aluminum hydride (0.8 g, 20.0 mmol) in ether (10 mL) was added dropwise a solution of 4-carbethoxy-3,3,5,5-tetramethyl-1-pyrroline[17] (**1a**; 4.95 g, 25.12 mmol) in ether (100 mL) over a period of 3 h. After the addition was completed, the mixture was refluxed for 1 h. It was then quenched by careful dropwise addition of 1/1 solution of 10% NaOH and 95% ethanol. The organic layer was separated, dried, and evaporated to dryness. Crystallization in 95% ethanol give 3.8 g of **2a** (97%) as a white crystal: mp 91–92 °C; ^1H NMR δ 3.54, 3.51 (AB, J = 5.6, 2 H, H-3′), 2.55 (AB, J = 1.5, 2 H, H-5), 1.45 (t, J = 7.4, 1 H, H-3), 1.48, 1.01, 1.00, and 0.85 (4 s, 12 H, 4 CH_3); ^{13}C NMR δ 61.4, 60.0, 59.6, 42.0, 31.8, 29.5, 23.6, and 22.3; MS 157 (M^+, 6), 142 (76), 124 (31), 112 (10), 96 (18), 81 (5), 72 (6), 71 (100), 70 (22), 58 (11), 55 (20), 43 (13), 42 (19), and 41 (27); HRMS $C_9H_{19}ON$ calcd 157.1462, found 157.1486.

2,2-Di([$^2H_3$]methyl)-4,4-dimethyl-3-(hydroxymethyl)-1-pyrrolidine (2b): yield 96%; MS 164 (M + 1, 1), 163 (M^+, 6), 146 (19), 145 (67), 144 (13), 127 (18), 115 (8), 99 (12), 78 (14), 77 (100), 76 (48), 75 (15), 59 (7), 55 (9), 45 (11), 41 (12), 31 (9), and 29 (6); HRMS $C_9H_{13}D_6ON$ calcd 163.1840, found 163.1855.

***N*-(Benzyloxycarbonyl)-3-(hydroxymethyl)-2,2,4,4-tetramethylpyrrolidine (3a).** To a mixture of **2a** (4.7 g, 30.0 mmol) and K_2CO_3 (4.8 g, 35.0 mmol) in acetonitrile (100 mL) was added a solution of benzyl chloroformate (4.7 mL, 33 mmol) in acetonitrile (15 mL). After stirring at −20 °C for 2.5 h, the mixture was brought to 25 °C, quenched with a phosphate buffer solution (20 mL), and extracted with dichloromethane (200 mL). The combined organic phase was dried and evaporated. The oily residue was purified by flash chromatography over silica gel and eluted with 1:3 ethyl acetate–hexane to give 8.1 g of **3a** (92%) as a white solid: mp 62–63 °C; R_f 0.64 (EtOAc–hexane, 1:1); ^1H NMR δ 7.41 (s, 5 H, Ar-H), 5.15 (s, 2 H, OCH_2Ph), 3.81 (AB, J = 7.1, 2 H, H-3′), 3.46, 3.16 (AB, J = 10 each, 2 H, H-5), 2.62 (bs, 1 H, OH), 1.96 (t, J = 7.3, 1 H, H-3), 1.57, 1.52, 1.15 and 1.03 (4 s, 12 H, 4 CH_3); ^{13}C NMR δ 154.2, 66.9, 66.0, 60.2, 60.0, 37.5, 30.2, 29.9, 27.4, 22.0, and 21.0; MS 292 (M + 1, 0.6), 276 (44.3), 232 (69), 214 (2), 184 (2), 107 (28), 92 (21), 91 (100), 70 (10), 65 (18), 55 (14), 41 (13), and 29 (3); HRMS $C_{17}H_{25}O_3N$ calcd 291.1827, found 291.1829.

***N*-(Benzyloxycarbonyl)-3-(hydroxymethyl)-2,2-di([$^2H_3$]methyl)-4,4-dimethylpyrrolidine (3b):** yield 91%; R_f 0.38 (EtOAc–hexane, 1:2); MS 297 (M^+, 1), 279 (16), 280 (6), 278 (6), 235 (30), 92 (8), 91 (100), 77 (2), 76 (3), 75 (2), 65 (9), 63 (2), 58 (2), 51 (2), 44 (3), and 41 (4); HRMS $C_{17}H_{19}D_6O_3N$ calcd 297.2206, found 297.2210.

***N*-(Benzyloxycarbonyl)-3-formyl-2,2,4,4-tetramethylpyrrolidine (4a).** To a solution of pyridinium chlorochromate (Aldrich) (3.2 g, 15.0 mmol) in dichloromethane (50 mL) was added dropwise at 25 °C a solution of **3a** (2.9 g, 10.0 mmol) in dichloromethane (50 mL). After stirring at 25 °C for 3 h, it was diluted with ether (200 mL) and was passed over fluorosil (10 g). The solution was washed with brine, dried, and evaporated. The resulting solid residue was purified by flash chromatography over silica gel and eluted with 1:7 ethyl acetate–hexane to give 2.7 g of **4a** (93%) as a white oil: R_f 0.62 (EtOAc–hexane, 1:3); ^1H NMR δ 9.86 (s, 1 H, CHO), 7.38 (bs, 5 H, Ar-H), 5.14 (s, 2 H, OCH_2Ph), 3.52, 3.2 (AB, J = 10.8 each, 2 H, H-5), 2.40 (bs, 1 H, H-3), 1.69, 1.59, 1.31, and 1.30 (4 s, 12 H, 4 CH_3); MS 289 (M^+, 2), 274 (14), 205 (10), 92 (8), 91 (100), 65 (9), 55 (6), 51 (2), 43 (2), 42 (2), and 41 (6); HRMS $C_{17}H_{23}O_3N$ calcd 289.1672, found 289.1680.

***N*-(Benzyloxycarbonyl)-3-formyl-2,2-di([$^2H_3$]methyl)-4,4-dimethylpyrrolidine (4b):** yield 91%; R_f 0.54 (EtOAc–hexane, 1:2); ^1H NMR δ 7.43 (bs, 5 H, Ar-H), 5.18 (s, 2 H, OCH_2Ph), 3.56, 3.21 (AB, J = 10.8 each, 2 H, H-5), 2.76 (d, J = 6.5, 1 H, H-3), 1.26 and 1.25 (2 s, 6 H, 2 CH_3); ^{13}C NMR δ 175.6,

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155.0, 67.2, 66.3, 63.7, 60.2, 38.2, 22.3, and 20.5; MS 295 (M^+, 1), 278 (3), 277 (10), 233 (13), 232 (3), 206 (2), 205 (9), 92 (8), 91 (100), 65 (8), 58 (2), 55 (2), 44 (2), and 41 (2); HRMS $C_{17}H_{17}D_6O_3N$ calcd 295.2049, found 295.2050.

N-(Benzyloxycarbonyl)-3-[1,2-didehydro-2-(ethoxycarbonyl)ethyl]-2,2,4,4-tetramethylpyrrolidine (5a). A solution of **4a** (2.9 g, 10.0 mmol) and (carbethoxymethylene)triphenylphosphorane (Aldrich) (5.2 g, 15.0 mmol) in benzene (50 mL) was stirred at 60 °C for 1 h and was diluted with an additional 50 mL of benzene. It was then washed with aqueous saturated ammonium chloride solution (20 mL), dried, and evaporated. The resulting solid residue was purified by flash chromatography over silica gel and eluted with 1:10 ethyl acetate–hexane to give 2.74 g of **5a** (76%) as white solid: mp 79–80 °C; R_f 0.7 (EtOAc–hexane, 1:3); ^{1}H NMR δ 7.39 (bs, 5 H, Ar-H), 7.05 (dd, J = 10.7 and 15.4, 1 H, H-3′), 5.94 (dd, J = 2.9 and 15.4, 1 H, H-3′), 5.15 (s, 2 H, OCH_2Ph), 4.26 (q, J = 7.2, 2 H, OCH_2CH_3), 3.57, 3.21 (AB, J = 10.8 each, 2 H, H-5), 2.35 (dd, J = 2.9 and 10.7, 1 H, H-3), 1.25 (t, J = 7.1, 3 H, OCH_2CH_3), 1.51, 1.44, 1.06, and 1.01 (4 s, 12 H, 4 CH_3); ^{13}C NMR δ 165.5, 143.5, 143.2, 125.3, 66.0, 63.4, 60.3, 60.0, 39.6, 27.7, 27.0, 26.5, 26.5, 22.4, 22.2, and 14.1; MS 360 (M + 1, 2), 359 (M^+, 7), 300 (9), 224 (19), 168 (4), 109 (4), 92 (13), 91 (100), 81 (5), 71 (51), 65 (9), 55 (6), 41 (5), and 29 (4); HRMS $C_{21}H_{29}O_4N$ calcd 359.2088, found 359.2077.

N-(Benzyloxycarbonyl)-3-[1,2-didehydro-2-(ethoxycarbonyl)ethyl]-2,2-di([$^2H_3$]methyl)-4,4-dimethylpyrrolidine (5b): yield 85%; R_f 0.59 (EtOAc–hexane, 1:3); MS 366 (M + 1, 2), 365 (8), 347 (3), 303 (10), 279 (7), 278 (30), 277 (68), 230 (19), 201 (12), 199 (16), 183 (11), 167 (11), 152 (9), 149 (26), 128 (4), 113 (4), 109 (5), 108 (21), 107 (16), 105 (7), 92 (26), 91 (100), 79 (14), 77 (29), 76 (47), 75 (20), 71 (9), 65 (24), 57 (13), 55 (12), 43 (12), 41 (13), and 29 (11); HRMS $C_{21}H_{23}D_6O_4N$ calcd 365.2467, found 365.2490.

3-[2-(Ethoxycarbonyl)ethyl]-2,2,4,4-tetramethyl-1-pyrrolidine (6a). A solution of **5a** (3.6 g, 10.0 mmol) in absolute ethanol (30 mL) and palladium on charcoal (10%, 200 mg) was hydrogenated under atmospheric pressure for 10 h. The mixture was filtered over fluorosil (5 g), and the solvent was evaporated to give 2.1 g of **6a** (95%) as a white oil: ^{1}H NMR δ 4.16 (q, J = 7.1, 2 H, OCH_2CH_3), 2.80 (bs, 2 H, H-5), 2.37 (t, J = 7.8, 2 H, H-3″), 1.66 (m, 3 H, H-3 and H-3′), 1.28 (t, J = 7.1, 3 H, OCH_2CH_3), 1.21, 1.09, 1.06, and 0.97 (4 s, 12 H, 4 CH_3); ^{13}C NMR δ 173.4, 61.9, 60.2, 59.4, 57.7, 42.5, 33.7, 31.3, 29.3, 23.7, 21.8, and 14.3; MS 227 (M^+, 2), 212 (22), 182 (4), 140 (9), 125 (3), 124 (24), 112 (3), 111 (5), 96 (5), 81 (4), 72 (8), 71 (100), 70 (12), 69 (7), 58 (7), 56 (6), 55 (12), 43 (5), 42 (7), and 41 (15); HRMS $C_{13}H_{25}O_2N$ calcd 227.1879, found 227.1872.

3-[2-(Ethoxycarbonyl)ethyl]-2,2-di([$^2H_3$]methyl)-4,4-dimethyl-1-pyrrolidine (6b): yield 92%; MS 233 (M^+, 2), 231 (2), 216 (4), 215 (16), 213 (6), 186 (5), 146 (4), 127 (13), 99 (3), 78 (15), 77 (100), 76 (47), 75 (13), 64 (6), 55 (9), 45 (8), 44 (5), and 41 (8).

4-[2-(Ethoxycarbonyl)ethyl]-3,3,5,5-tetramethyl-1-pyrroline N-Oxide (7a). To a stirred solution of **6a** (1.13 g, 5.0 mmol) and $Na_2WO_4 \cdot 2H_2O$ (0.82 g, 20.00 mmol) in methanol (25 mL) was added dropwise hydrogen peroxide 30% (2 mL) at 0 °C. After the mixture was at 0 °C for 4 h, the solvent was evaporated to dryness. The solid residue was taken up with dichloromethane (50 mL), washed with brine (10 mL), dried, and evaporated to dryness. The residue was purified by flash chromatography over silica gel and eluted with 20:1 dichloromethane–methanol to give 1.02 g of **7a** (85%) as a yellow oil which solidified at 0 °C. This material was of sufficient purity for spin trap experiments: R_f 0.61 (CH_2Cl_2–MeOH, 9:1); ^{1}H NMR δ 6.48 (s, 1 H, H-2), 3.99 (q, J = 7.1, 2 H, OCH_2CH_3), 2.25 (t, J = 7.7, 2 H, H-4″), 1.70 (m, 3 H, H4 and H4′), 1.10 (t, J = 7.1, 3 H, OCH_2CH_3), 1.27, 1.18, 1.05, and 0.96 (4 s, 12 H, 4 CH_3); ^{13}C NMR δ 171.9, 139.9, 76.1, 60.0, 52.6, 40.5, 32.4, 27.8, 26.9, 21.1, 20.9, 20.5, and 13.7; MS 242 (M + 1, 7), 241 (29), 227 (16), 226 (92), 196 (23), 180 (26), 141 (23), 140 (90), 138 (76), 126 (23), 123 (18), 109 (19), 95 (28), 85 (77), 81 (36), 69 (50), 67 (27), 58 (32), 55 (100), 41 (74), 29 (61), and 27 (26); HRMS $C_{13}H_{23}O_3N$ calcd 241.1671, found 241.1687. Anal. Calcd for $C_{13}H_{23}O_3N \cdot 0.1H_2O$: C, 64.21; H, 9.55; N, 5.76. Found: C, 64.04; H, 9.69; N, 5.76.

4-[2-(Ethoxycarbonyl)ethyl]-5,5-di([$^2H_3$]methyl)-3,3-dimethyl-1-pyrrolidine N-oxide (7b): yield: 84%; R_f 0.58 (CH_2Cl_2–MeOH, 9:1); ^{1}H NMR δ 6.66 (s, 1 H, H-2), 4.03 (q, J = 7.1, 2 H, OCH_2CH_3), 2.43 (t, J = 7.7, 2 H, H-4″), 1.80 (m, 3 H, H4 and H4′), 1.24 (t, J = 7.1, OCH_2CH_3), 1.19, and 1.11 (2 s, 6 H, 2 CH_3); ^{13}C NMR δ 172.3, 140.6, 77.0, 60.4, 52.9, 40.9, 32.7, 28.1, 21.4, 20.8, and 14.0; MS 248 (M + 1, 8), 247 (M^+, 31), 232 (26), 231 (14), 230 (30), 229 (88), 228 (19), 202 (25), 186 (19), 147 (27), 146 (95), 145 (45), 144 (56), 143 (26), 142 (16), 141 (39), 132 (20), 131 (12), 130 (14), 129 (20), 128 (15), and 127 (11); HRMS $C_{13}H_{17}D_6O_3N$ calcd 247.2049, found 247.2042. Analysis by MS indicated that **7b** consisted of 85% of $^2H_6$.

Spin Trapping of Superoxide. The superoxide generating system consisted of xanthine oxidase and xanthine (400 μM). The rate of superoxide production was 10 μM/min as determined by following the superoxide dismutase-inhibitable reduction of cytochrome c at 550 nm,[27] using a molar absorptivity of 21 mM^{-1} cm^{-1}. The reaction was initiated by the addition of xanthine oxidase to a solution of xanthine and the various spin traps (0.1 M) to a final volume of 0.5 mL. Reaction mixtures were transfered to a flat quartz ESR cell, fitted into the cavity of an ESR spectrometer, and spectra were recorded at 25 °C. No free radical could be spin trapped in the presence of SOD (30 units/mL) or if any of the components of the above reaction were not present.

Spin Trapping of Hydroxyl Radical. The spin trapping of hydroxyl radical was performed by the addition of ferrous sulfate (0.1 mM) to a solution of hydrogen peroxide (0.3%) containing the various spin traps (0.1 M). Reaction mixtures were transferred to a flat quartz ESR cell, fitted into the cavity of an ESR spectrometer, and spectra were recorded at 25 °C.

Reduction of Hydroxyl Radical Spin Adducts. The hydroxyl radical spin adducts were generated by irradiating an ESR flat cell containing a 0.5-mL solution of the various spin traps (10 mM), hydrogen peroxide (0.3%) and placing the cell 7 cm from the UV light source (Ultra-violet Product, Inc., San Gabriel, CA, Model no. SCT1) for 1 min. To 0.1 mL of the irradiated reaction mixture were added to a final volume of 0.25 mL. A solution of xanthine (400 μM) and varying amounts of xanthine oxidase to generate the desired flux of superoxide. Reaction mixtures were transferred to a flat quartz ESR cell, fitted into the cavity of an ESR spectrometer, and spectra were recorded at 25 °C.

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Registry No. **1a**, 113086-38-7; **1b**, 139461-59-9; **2a**, 139461-48-6; **2b**, 139461-54-4; **3a**, 139461-49-7; **3b**, 139461-55-5; **4a**, 139461-50-0; **4b**, 139493-27-9; **5a**, 139461-51-1; **5b**, 139461-56-6; **6a**, 139461-52-2; **6b**, 139461-57-7; **7a**, 139461-53-3; **7b**, 139461-58-8; **8**, 3317-61-1; superoxide, 11062-77-4; hydroxyl radical, 3352-57-6.

Supplementary Material Available: ^{1}H and ^{13}C NMR spectra of compounds **2a**, **3a**, **4b**, **5a**, **6a**, **7a**, and **7b** and ^{1}H NMR spectrum of **4a** (15 pages). Ordering information is given on any current masthead page.

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