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New 2-Substituted Pyrroline-*N*-oxides: An EPR Solvent Study of the Radical Spin Adducts

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Ten substituted 5,5-dimethyl-1-pyrroline-*N*-oxides as well as the parent nitrene spin trap (DMPO) were prepared: 5,5-dimethyl-1-pyrroline-*N*-oxide, 2,5,5-trimethyl-1-pyrroline-*N*-oxide, 2-*tert*-butyl-5,5-dimethyl-1-pyrroline-*N*-oxide, 2-phenyl-5,5-dimethyl-1-pyrroline-*N*-oxide, 2-d_5-phenyl-5,5-dimethyl-1-pyrroline-*N*-oxide, 2-phenyl-5,5-dimethyl-1-pyrroline-*N*-oxide-*nitronyl*-^{13}C, 2-(4-fluorophenyl)-5,5-dimethyl-1-pyrroline-*N*-oxide, 2-(4-chlorophenyl)-5,5-dimethyl-1-pyrroline-N-oxide, 2-(4-*tert*-butylphenyl)-5,5-dimethyl-1-pyrroline-*N*-oxide, 2-(4-methylphenyl-5,5-dimethyl-1-pyrroline-*N*-oxide and 2-(2- methylphenyl)-5,5-dimethyl-1-pyrroline-*N*-oxide. Analytical (i.e. EPR-grade) samples of these novel cyclic nitrones were obtained and characterized by (among other methods) ^{1}H NMR spectroscopy. Reduction of DMPO and these various 2-substituted cyclic nitrones gave the corresponding cyclic *N*,*N*-dialkylhydroxylamines, whose structures and conformations were also analyzed by ^{1}H NMR spectroscopy. Air oxidation of these cyclic *N*,*N*-dialkylhydroxylamines provided access to the EPR spectra of the hydrogen, methyl, *tert*-butyl, phenyl, d_5-phenyl, *nitronyl*-^{13}C-phenyl, 4-fluorophenyl, 4-chlorophenyl, 4-*tert*-butylphenyl, 4-methylphenyl and 2-methylphenyl cyclic aminoxyl (pyrrolidine *N*-oxyl nitroxide) radical spin adducts of DMPO. The ^{14}N, ^{13}C (where applicable) and ^{1}H hyperfine splitting constants of these aminoxyl adducts in ten solvents of widely different polarities (e.g., hexane to water) were measured and the solvent effect on these parameters was evaluated. It was found that for the various 2-substituted DMPO-type spin adducts both the nitrogen and β-hydrogen EPR hyperfine splittings correlated linearly ($r^2 \geqslant 0.90$) with typical solvent polarity parameters such as $E_{T(30)}$. The correlation between the nitrogen and β-hydrogen hyperfine splitting constants were even more linear ($r^2 \geqslant 0.97$).

KEY WORDS electron paramagnetic resonance NMR ^{1}H, ^{14}N and ^{13}C hyperfine splittings Spin traps Spin adducts Nitrones (1-pyrroline-*N*-oxides)

INTRODUCTION

It was 1973 when 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) was introduced as a spin trap.[1,2] Many DMPO analogs[3–14] have since been synthesized. Some of the objectives with these modified spin traps were to increase the spin trap solubility in a lipid environment, to decrease the nitrone susceptibility toward air oxidation and to study spin trap stereochemistry. Most of the DMPO-type spin traps reported have various substituents on the 3-, 4- or 5-positions of the DMPO ring. However, no systematic study is available on the effect of replacing the hydrogen atom in the 2-position with a variety of radical inert groups (i.e. without abstractable β-Hs). We have initiated a program of synthesis of 2-substituted DMPO derivatives for evaluation as better spin traps for biological systems. Although this approach produces spin adducts without the β-H hyperfine splitting constant information normally needed for assignment of spectra, promising spin trapping results would prompt the synthesis of *nitronyl*-^{13}C-DMPOs which could provide the EPR parameter needed for diagnostic purposes.[15] The advantage expected from 2-substituted DMPO spin traps is that the spin traps might be less susceptible to air oxidation and the spin adducts would be less vulnerable to oxidative degradation or disproportionation. Along with DMPO (**1a**), we have prepared ten 2-substituted DMPOs: 2,5,5-trimethyl-1-pyrroline-*N*-oxide (**1b**), 2-*tert*-butyl-5,5-dimethyl-1-pyrroline-*N*-oxide (**1c**), 2-phenyl-5,5-dimethyl-1-pyrroline-*N*-oxide (**1d**), 2-d_5-phenyl-5,5-dimethyl-1-pyrroline-*N*-oxide (**1e**), 2-phenyl-5,5-dimethyl-1-pyrroline-*N*-oxide-*nitronyl*-^{13}C (**1f**), 2-(4-fluorophenyl)-5,5-dimethyl-1-pyrroline-*N*-oxide (**1g**), 2-(4-chlorophenyl)-5,5-dimethyl-1-pyrroline-*N*-oxide (**1h**), 2-(4-*tert*-butylphenyl)-5,5-dimethyl-1-pyrroline-*N*-oxide (**1i**), 2-(4-methylphenyl)-5,5-dimethyl-1-pyrroline-

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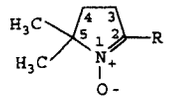
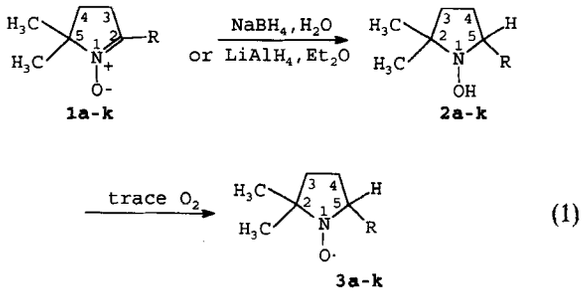
N-oxide (**1j**) and 2-(2-methylphenyl)-5,5-dimethyl-1-pyrroline-*N*-oxide **1k**):



1a-k

R = H (**1a**), CH_3 (**1b**), $C(CH_3)_3$ (**1c**), C_6H_5 (**1d**), C_6D_5 (**1e**), C_6H_5(*nitronyl*-^{13}C) (**1f**), 4-F-C_6H_4 (**1g**), 4-ClC_6H_4 (**1h**), 4-$C(CH_3)_3C_6H_4$ (**1i**), 4-$CH_3C_6H_4$ (**1j**), 2-$CH_3C_6H_4$ (**1k**).

In addition to superoxide/hydroperoxyl and hydroxyl radical adducts of DMPO, C-centered radical adducts of DMPO are the common spin trapping products in biological milieu when DMPO is used as a spin trap.[16,17] It is difficult to determine exactly what kind of C-centered radical is trapped. One of the reasons is that EPR data for many C-centered radical adducts of DMPO in an aqueous solvent have not been well established because of the difficulty of generating the spin adducts in aqueous media. Most C-centered radical adducts can easily be generated in benzene,[1] but direct generation in water is more difficult. In this paper, an indirect method is described to generate hydrogen (**3a**), methyl (**3b**), *tert*-butyl (**3c**), phenyl (**3d**), d_5-phenyl (**3e**), phenyl(*nitronyl*-^{13}C) (**3f**), 4-fluorophenyl (**3g**), 4-chlorophenyl (**3h**), 4-*tert*-butylphenyl (**3i**), 4-methylphenyl (**3j**) and 2-methylphenyl (**3k**) radical adducts of DMPO as illustrated in Eqn (1).



1a-k **2a-k** **3a-k** (1)

We initially tried the reduction of nitrones **1a–e** with sodium borohydride and found that the reduction was not complete when the 2-position is substituted. Subsequently, lithium aluminum hydride was used to reduce readily nitrones **1a–k** to their corresponding cyclic *N*,*N*-dialkylhydroxylamines (**2a–k**).

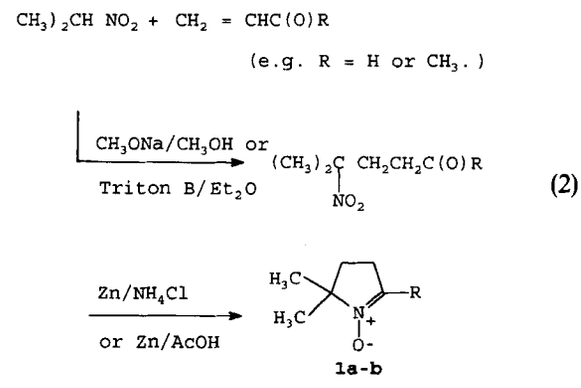
Although spin trapping with DMPO in a biological system is usually conducted in aqueous solution, extraction with organic solvents is commonly used to concentrate spin adducts. Nitrogen and hydrogen hyperfine splitting constants (^{14}N hfsc or a^N; ^{1}H hfsc, a_β^H) of DMPO adducts are strongly dependent on the kind of solvent used.[18] In this respect, it is of importance to measure a^N and a_β^H values in various solvents. Further, it would be most useful if correlations of solvent with a^N, solvent with a_β^H and a^N with a_β^H were available for each spin adduct so that the hyperfine values in any solvent could be predicted.

There have been a number of studies dealing with relationships between solvent and a^N value for some alkyl and alicyclic aminoxyls[19] and for some stable aminoxyls.[20] Also, correlations between solvent and a^N and a_β^H values for spin adducts from *C*-phenyl-*N*-*tert*-butyl nitrone (PBN)[18] and DMPO,[18,21] and the ratio of a^N to a_β^H for spin adducts derived from DMPO[22] have been reported. Current interest in this area prompted us to examine the EPR spectra of the ten 2-substituted DMPO adducts (**3b–k**) in ten common solvents.

RESULTS AND DISCUSSION

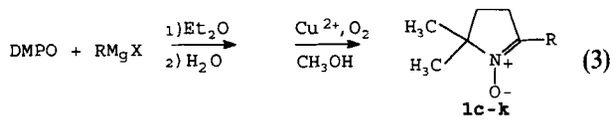
The various DMPO-type cyclic nitrones (1a–k)

Two simple methods are available for the preparation of DMPO-type nitrone spin traps. The first is the Michael addition between 2-nitropropane and the α,β-unsaturated ketone which produces the nitro-ketone intermediate, which is reduced with zinc–ammonium chloride [23] or zinc–acetic acid[9] to generate the desired nitrone [Eqn (2)]. DMPO (**1a**) and 2,5,5-M_3PO (**1b**)[7] were synthesized by this method.



1a-b (2)

The second is the addition of Grignard reagents to DMPO followed by Cu^{2+}-catalyzed air oxidation [Eqn (3)].[23,24] This is a convenient method with a fair yield for the *tert*-butyl derivative (**1c**) and excellent yields for the aryl-substituted nitrones (**1d–k**). The ^{1}H NMR data for the various cyclic nitrones (**1a–k**) are collected in Table 1.



1c-k (3)

It should be noted that synthetic chemists publishing nitrone preparations are normally not faced with the problem of making very pure (EPR-grade) compounds. We have found that more effort is needed to purify nitrones for analytical spin trapping purposes than is usually required. For example, one recrystallization from hexane and subsequent sublimation of **1d** provided a sample which gave an EPR spectrum from its 0.02 mol l^{-1} benzene solution. This spectrum consisted of a triplet due to an aminoxyl impurity. Chromatography on silica gel with CH_2Cl_2–CH_3OH (95:5, v/v) was performed but the product still had an aminoxyl impurity.

Table 1. ^{1}H NMR data for the various cyclic nitrones (1-pyrroline-*N*-oxides) (1a–k)[a,b]

Position	1b	1c	1d	1e	1f[c]
3	2.63 (*m*, 2H, CH_2)	2.61 (t, 2H, CH_2), J = 7.5 Hz	2.98 (t, 2H, CH_2), J = 7.5 Hz	3.05 (t, 2H, CH_2), J = 7.5 Hz	3.04 (q, 2H, CH_2), $J(^1H–^1H) = J(^1C–^{13}H)$ = 7.4 Hz
4	2.02 (m, 2H, CH_2)	1.93 (t, 2H, CH_2), J = 7.4 Hz	2.06 (t, 2H, CH_2), J = 7.4 Hz	2.12 (t, 2H, CH_2), J = 7.4 Hz	2.12 (td, 2H, CH_2), $J(^1H–^1H)$ = 7.4 Hz, $J(^1H–^{13}C)$ = 2.6 Hz
5	1.40 (s, 6H, $2CH_3$)	1.36 (s, 6H, $2CH_3$)	1.44 (s, 6H, $2CH_3$)	1.50 (s, 6H, $2CH_3$)	1.50 (s, 6H, $2CH_3$)
R	2.02 (m, 3H, CH_3)	1.30 (s, 9H, *tert*-butyl)	7.39 (m, 3H, Ar-H), 8.34 (m, 2H, Ar-H)	—	7.24–7.44 (m, 3H, *O*- and *P*-Ar-H), 8.36–8.40 (m, 2H, *m*-Ar-H)

Position	1g	1h	1i	1j	1k
3	3.03 (t, 2H, CH_2), J = 7.4 Hz	3.02 (t, 2H, CH_2), J = 7.5 Hz	3.02 (t, 2H, CH_2), J = 7.4 Hz	3.02 (t, 2H, CH_2), J = 7.4 Hz	2.92 (t, 2H, CH_2), J = 7.4 Hz
4	2.13 (*t*, 2H, CH_2), J = 7.5 Hz	2.13 (t, 2H, CH_2), J = 7.4 Hz	2.10 (t, 2H, CH_2), J = 7.5	2.10 (t, 2H, CH_2), J = 7.5 Hz	2.17 (t, 2H, CH_2), J = 7.4 Hz
5	1.50 (s, 6H, $2CH_3$)	1.49 (s, 6H, $2CH_3$)	1.48 (s, 6H, $2CH_3$)	1.49 (s, 6H, $2CH_3$)	1.50 (s, 6H, $2CH_3$)
R	7.12 (t, 2H, Ar-H), J = 8.9 Hz 8.43 (q, 2H, Ar-H), J_1 = 5.6 Hz, J_2 = 9.1 Hz	7.39–8.35 (q, 4H, Ar-H)	1.33 (s, 9H, Ar-*tert*-butyl), 7.45–8.32 (q, 4H, Ar-H)	2.38 (s, 3H, Ar-CH_3), 7.23–8.29 (q, 4H, Ar-H)	2.33 (s, 3H, Ar-CH_3), 7.20–7.31 (m, 4H, Ar-H)

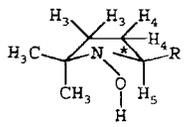
[a] The chemical shifts (δ) are in ppm relative to internal TMS in $CDCl_3$.
[b] For the ^{1}H NMR data for **1a**, see Ref. 34.
[c] Data from a recently published paper.[33]

Further recrystallization was carried out and some nice large crystals were generated. This sample was of EPR grade. For instance, the EPR spectrum of a 0.02 mol l^{-1} benzene solution showed no impurity signals when the spectrometer receiver gain was set at 1×10^6, or even 1×10^7.

Although nitrone **1b** has some excellent characteristics such as smaller steric bulk (2-methyl *vs.* 2-*tert*-butyl in **1c**), and its hydroxyl adduct is more persistent than that of DMPO,[25] the problem with this spin trap is the strong impurity triplet EPR signal which is produced during the synthesis and/or purification by vacuum distillation.[26] Recent investigations by mass spectrometry uncovered a dimer *N*,*N*-dialkylhydroxylamine of **1b** which could be produced by an enamine type of addition between two molecules of **1b**.[27] Once the dimer *N*,*N*-dialkylhydroxylamine of **1b** is formed, air oxidation would produce the corresponding dimer aminoxyl.[27]

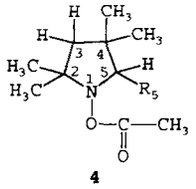
The various cyclic *N*,*N*-dialkylhydroxylamines (2a–k)

The extent of reduction of nitrones **1a–e** by sodium borohydride strongly depends on the substituent group linked to the 2-position of the pyrroline ring. Reactions of **1a** and **1b** with a fivefold molar excess of sodium borohydride for 30–60 min at room temperature produced 61% of **2a** and 6% of **2b**. Overnight reactions of **1c, d** and **e** with a 5–20-fold molar excess of sodium borohydride gave *ca.* 13% of **2c**, 19% of **2d** and 14% of **2e**. Sodium borohydride is too mild for the reduction of 2-aryl substituted DMPOs. The use of lithium aluminum hydride makes the reduction more complete. The ^{1}H NMR data for the various cyclic *N*,*N*-dialkylhydroxylamines (**2a–k**) are collected in Table 2.



2a-k

The cyclic *N*,*N*-dialkylhydroxylamine **2a** possesses no chiral center (apart from the pyrrolidine nitrogen atom). Inversion of this nitrogen (along with the attached groups including the nitrogen lone pair) must be fast on the ^{1}H NMR time-scale (in solution at room temperature) since the protons at position 2, 3, 4 and 5 (**2a**) (Table 2) form groups of magnetically equivalent nuclei. In contrast, the presence of a chiral center (C-5) in the cyclic *N*,*N*-dialkylhydroxylamines **2b–k** (indicated by an asterisk) causes the methyl groups (position 2) (**2b–k**) and methylene hydrogens (position 4) (**2c–k**) to become magnetically inequivalent [i.e. exhibit distinct chemical shifts (δ)] (Table 2). The ^{1}H NMR data for 1-acetoxyl-5-phenyl-2,2,4,4-tetramethylpyrrolidine (**4**) (see Experimental) confirm the assignment of the protons (at position 4) (Table 2). Peaks corresponding to the two 4-H protons in pyrrolidines **2c–k** (Table 2) disappear owing to methyl substitution at C-4 in **4**.



$R_5 = C_6H_5$

4

Table 2. ^{1}H NMR data for the various cyclic *N,N*-dialkylhydroxylamines (1-hydroxypyrrolidines) 2a–k[a]

Position	2a	2b	2c	2d	2e
1	~4.2–4.7 (bs, 1H, NOH)	~4.2–4.7 (bs, 1H, NOH)	4.22 (bs, 1H, NOH)	4.62 (bs, 1H, NOH)	4.44 (bs, 1H, NOH)
2	1.14 (s, 6H, $2CH_3$)	1.04 (s, 3H, CH_3), 1.22 (s, 3H, CH_3)	1.07 (s, 3H, CH_3), 1.16 (s, 3H, CH_3)	1.13 (s, 3H, CH_3), 1.17 (s, 3H, CH_3)	1.13 (s, 3H, CH_3), 1.20 (s, 3H, CH_3)
3	1.63 (t, 2H, H) J = 7.5 Hz	1.57 (dd, 2H, H) J_1 = 9.0 Hz, J_2 = 7.2 Hz	1.38–1.56 (m, 2H, CH_2)	1.73 (m, 2H, CH_2)	1.74 (m, 2H, CH_2)
4	1.76 (m, 2H, H)	1.87 (m, 2H, H)	1.38–1.56 (m, 1H, H), 1.84 (m, 1H, H)	1.62 (m, 1H, H), 2.18 (m, 1H, H)	1.62 (m, 1H, H), 2.18 (m, 1H, H)
5	3.12 (bs, 2H, H)	3.07 (m, 1H, H)	2.75 (dd, 1H, H), J_1 = 4.6 Hz, J_2 = 10.7 Hz	3.99 (t, 1H, H), J = 8.9 Hz	4.01 (dd, 1H, H), J_1 = 8.2 Hz, J_2 = 9.4 Hz
R	—	1.22 (d, 3H, CH_3), J = 6.4 Hz	0.91 (s, 9H, *tert*-butyl)	7.35 (m, 5H, Ar-H)	—

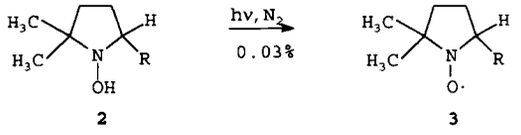
	2f[b]	2g	2h	2i	2j	2k
1	—	4.62 (bs, 1H, NOH)	4.45 (bs, 1H, NOH)	4.70 (bs, 1H, NOH)	4.60 (bs, 1H, NOH)	4.36 (bs, 1H, NOH)
2	—	1.12 (s, 3H, CH_3), 1.14 (s, 3H, CH_3)	1.13 (s, 3H, CH_3), 1.18 (s, 3H, CH_3)	1.08 (s, 3H, CH_3), 1.13 (s, 3H, CH_3)	1.17 (s, 3H, CH_3), 1.24 (s, 3H, CH_3)	1.17 (s, 3H, CH_3), 1.24 (s, 3H, CH_3)
3	—	1.70 (m, 2H, CH_2)	1.72 (m, 2H, CH_2)	1.68 (m, 2H, CH_2)	1.73 (m, 2H, CH_2)	1.73 (m, 2H, CH_2)
4	—	1.58 (m, 1H, H), 2.16 (m, 1H, H)	1.55 (m, 1H, H) 2.15 (m, 1H, H)	1.68 (m, 1H, H), 1.15 (m, 1H, H)	1.47 (m, 1H, H) 2.15 (m, 1H, H)	1.47 (m, 1H, H), 2.25 (m, 1H, H)
5	—	3.97 (t, 1H, H), J = 8.8 Hz	3.97 (dd, 1H, H), J_1 = 8.1 Hz, J_2 = 10.1 Hz	3.97 (dd, 1H, H), J_1 = 8.1 Hz, J_2 = 10.1 Hz	3.96 (t, 1H, H), J = 9.5 Hz	4.28 (t, 1H, H), J = 9.0 Hz
R	—	7.02 and 7.35 (m, 4H, Ar-H)	7.32 (m, 4H, Ar-H)	1.32 (s, 9H, Ar-*tert*-butyl), 7.35 (m, 4H, Ar-H)	7.13 and 7.27 (m, 4H, Ar-H)	7.15, 7.24 and 7.62 (m, 4H, Ar-H)

[a] The chemical shifts (δ) are in ppm relative to internal TMS in $CDCl_3$.
[b] Data not available.

Aminoxyls 3a–k and their EPR spectra

As mentioned before, reduction of **1b–e** with sodium borohydride is not complete. However, the presence of the unreduced nitrone does no interfere with the EPR detection of the corresponding aminoxyl (**3a–e**). After extraction of the reduction mixture with dichloromethane and evaporation, the residue obtained consisted of the unreacted nitrone, the *N,N*-dialkylhydroxylamine and a trace amount of the aminoxyl radical (0.03 wt.%). Samples **2a–k** afforded by reduction of **1a–k** with lithium aluminum hydride also contain aminoxyl components (**3a–k**) (*ca.* 0.01–0.04 wt.%). In fact, these aminoxyls are probably intermediates through which the *N,N*-dialkylhydroxylamines are converted into other species such as nitrones. Although reduction and extraction procedures were carried out in an atmosphere of nitrogen, the absolute absence of oxygen is very difficult to arrange. On the other hand, because of the presence of trace amounts of the aminoxyls, dilute solutions of **2a–k** (0.02 mol l^{-1}) may provide the opportunity to measure conveniently EPR hfscs of **3a–k** in various aqueous or organic solutions.

Aminoxyls **3a–k** are also partly generated by the photolysis of the cyclic *N,N*-dialkylhydroxylamines. Irradiation of the 0.02 mol l^{-1} solution of the crude **2a** or **2b** in water or benzene can initially increase the concentration of the aminoxyl **3a** or **3b**. Eventually, the concentration of the aminoxyl will reach a maximum and decrease linearly with time thereafter if irradiation is continued. It was noticed that the initial generation of the aminoxyl is only *ca.* 0.03% of the weight of the crude **2** used. Therefore, the generation of the aminoxyl **3** is only a side-product of the decomposition of **2** caused by irradiation with ultraviolet light [Eqn (4)].



2 3 (4)

^{14}N hfscs and ^{1}H hfscs of the various cyclic aminoxyls **3a–k** in ten solvents presented in Table 3. The a^N value of **3a** is on average 0.16 G greater than that of **3b**, which is 0.11 G larger than the value for **3c**. The magnitude of a^N for **3c** is 0.24 G larger than the value for **3d**, which is almost equal to those of **3e–k**. The a^N values of **3a–k** in the same solvent follow the sequence **3a** > **3b** > **3c** > **3d** ≈ **3e** ≈ **3f** ≈ **3g** ≈ **3h** ≈ **3i** ≈ **3j** ≈ **3k**. The decrease in the a^N value may be considered to be the consequence of a lower spin density located on the ^{14}N nucleus. The spin density on the nitrogen atom in **3a–k** should be in the sequence **3k** ≈ **3j** ≈ **3i** ≈ **3h** ≈ **3g** ≈ **3f** ≈ **3e** ≈ **3d** < **3c** < **3b** < **3a**. This sequence follows the electron-withdrawing abilities of the substituents separated from the aminoxyl function by two single bonds, namely YC_6H_4 > *tert*-butyl > methyl > hydrogen.

The β-H hfsc values of DMPO adducts are extremely

Table 3. EPR hyperfine splitting constants for the various cyclic aminoxyls (pyrrolidine-*N*-oxyl nitroxides) 3a–k in ten solvents[a]

			3a		3b		3c	
No.	Solvent	$E_{T(30)}$	a^N	a_β^H	a^N	a_β^H	a^N	a_β^H
1	*n*-Hexane	30.9	14.08	18.29	13.93	19.94	13.87	20.65
2	Benzene	34.5	14.44	18.91	14.30	20.58	14.14	20.99
3	Chloroform	39.1	15.05	19.96	14.91	21.16	14.81	21.16
4	Dichloromethane	41.1	14.88	19.65	14.74	21.02	14.64	21.07
5	Acetone	42.2	14.65	19.21	14.52	20.77	14.38	21.03
6	Ethanol	51.9	15.30	20.33	15.16	21.68	14.92	21.87
7	Acetic acid	51.9	15.90	21.49	15.65	22.09	15.52	22.35
8	95% Ethanol	53.7	15.42	20.63	15.23	21.75	15.11	21.92
9	Methanol	55.5	15.49	20.60	15.26	21.97	15.17	22.07
10	Water	63.1	16.55	22.48	16.37	23.41	16.25	23.22

			3d		3e		3f		
			a^N	a_β^H	a^N	a_β^H	a^N	a_β^H	a_α^{13C}
1	*n*-Hexane	30.9	13.58	18.35	13.66	18.33	13.62	18.34	5.94
2	Benzene	34.5	13.94	19.43	13.97	19.38	13.70	19.37	5.91
3	Chloroform	39.1	14.49	20.43	14.46	20.26	14.47	20.34	6.14
4	Dichloromethane	41.1	14.37	20.77	14.39	20.77	14.30	20.78	6.41
5	Acetone	42.2	14.17	20.23	14.15	20.38	14.38	20.42	6.04
6	Ethanol	51.9	14.81	21.36	14.76	21.26	14.65	21.36	6.11
7	Acetic acid	51.9	15.28	22.29	15.26	22.14	15.27	22.21	6.75
8	95% Ethanol	53.7	14.91	21.51	14.90	21.33	14.91	21.42	6.55
9	Methanol	55.5	14.94	21.87	14.96	21.85	14.95	21.86	6.41
10	Water	63.1	15.89	24.63	15.93	24.50	15.84	24.50	6.56

			3g		3h		3i	
			a^N	a_β^H	a^N	a_β^H	a^N	a_β^H
1	*n*-Hexane	30.9	13.66	18.57	13.58	18.56	13.69	18.48
2	Benzene	34.5	13.98	19.65	13.92	19.50	13.98	19.45
3	Chloroform	39.1	14.47	20.66	14.41	20.45	14.54	20.60
4	Dichloromethane	41.1	14.37	20.99	14.32	20.75	14.44	21.18
5	Acetone	42.2	14.16	20.50	14.10	20.38	14.18	20.38
6	Ethanol	51.9	14.78	21.59	14.71	21.39	14.85	21.49
7	Acetic acid	51.9	15.19	22.43	15.19	22.19	15.35	22.40
8	95% Ethanol	53.7	14.85	21.75	14.81	21.52	14.97	21.63
9	Methanol	55.5	14.96	22.16	14.85	21.93	14.99	22.03
10	Water	63.1	15.98	24.88	15.95	24.55	NA	NA

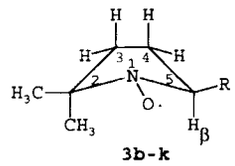
			3j		3k	
			a^N	a_β^H	a^N	a_β^H
1	*n*-Hexane	30.9	13.64	18.46	13.69	18.51
2	Benzene	34.5	13.99	19.52	13.97	19.08
3	Chloroform	39.1	14.54	20.63	14.51	19.86
4	Dichloromethane	41.1	14.42	21.02	14.39	20.27
5	Acetone	42.2	14.18	20.30	14.20	19.84
6	Ethanol	51.9	14.86	21.39	14.82	20.94
7	Acetic acid	51.9	15.32	22.42	15.28	21.62
8	95% Ethanol	53.7	14.94	21.54	14.92	21.05
9	Methanol	55.5	15.04	22.09	15.02	21.48
10	Water	63.1	15.99	24.75	15.97	24.05

[a] The EPR hyperfine splitting constants listed are in G (0.1 mT) and were calibrated against Fremy's salt (from Aldrich) in aqueous $NaHCO_3$ (a^N = 13.091 G).[30]

sensitive to the structure of the added radical (or radical addend), and this feature has been rationalized as due to changes in conformation.[28] For aminoxyl **3a** the two β-hydrogens are equivalent in solution at room temperature because of very rapid interconversion [Eqn (5)]. However, for other aminoxyls, a preferential conformation exists in solution and **3b–k** exhibit a_β^H values greater than that of **3a** in the same solvent, as shown in Table 3. Generally, the influence of the *tert*-butyl substituent on a_β^H values is the most significant, the effect of the methyl group is moderate and the effects of phenyl, d_5-phenyl and other substituted phenyl groups are minimal with respect to hydrogen.

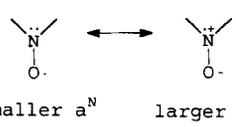
3a **3a'** (5)

The only exception is in water, where the sequence is exactly reversed (i.e. the influence of the phenyl group is the most significant and *tert*-butyl the least). Perhaps a π-electron interaction between the p-orbitals of the aromatic ring and the aminoxyl function (favored by an equatorial C_6H_5) is more important in more polar solvents. This might also account for the observed lower a^N values for the phenyl (**3d–k**) ($a^N \approx 15.9$ G) *vs.* the *tert*-butyl (**3c**) ($a^N \approx 16.3$ G) derivatives. The strong steric interactions of the *tert*-butyl group with the *cis*-H at C-3 and C-4 make the bulky group tend to take on an equatorial position. This results in the C—H_β bond taking on a more axial position, namely more parallel with the p-orbital of nitrogen atom. Because the dihedral angle is smaller the interaction of H_β with spin density on the nitrogen atom is greater. The methyl group has a smaller steric effect than the *tert*-butyl group. The steric effect for the phenyl ring appears even smaller.



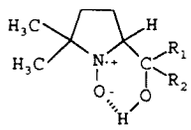
3b-k

The a^N values for each of **3a–k** in ten solvents (Table 3) follow the sequence $H_2O > CH_3COOH > CH_3OH > 95\%$ $CH_3CH_2OH > CHCl_3 > CH_2Cl_2 > CH_3C(O)CH_3 > C_6H_6 > n\text{-}C_6H_{14}$. There are two factors affecting the a^N value. One effect is the solvent polarity: the more polar the solvent, the larger is the a^N value.[19] This feature can be accounted for by considering the contribution of the two major resonance structures for the aminoxyl function[18–22] [Eqn (6)].



smaller a^N larger a^N (6)

The other factor is the H-bonding between the N—O function of the aminoxyl spin adduct and some solvents such as water, carboxylic acids, alcohols and chloroform,[29] or intramolecular H-bonding when the structure of the adduct is suitable (cf. **5**).[1,30]



5

The ^1H hfscs of each of **3a–k** (Table 3) also follow the sequence $H_2O > CH_3COOH > CH_3OH > 95\%$ $CH_3CH_2OH > CHCl_3 > CH_3C(O)CH_3 > C_6H_6 > n\text{-}C_6H_{14}$, except for **3d–k** in chloroform. However, the solvent effect on the ^1H hfsc can be more complicated because the value may be related to the conformation of the aminoxyl. The question is how to estimate whether the ring conformation is significantly changed in different solvents. The a^N value is usually considered to be proportional to the spin density on nitrogen and to have little dependence on the conformation of the pyrrolidine ring. However, the a_β^H value depends on both the spin density on nitrogen and the dihedral angle between C—H_β bond and the p-orbital on nitrogen, which is related to the ring conformation. For an individual spin adduct, changes in spin density on nitrogen may result in changes in both a^N and a_β^H values. However, a conformational variation is expected to result in a change of a_β^H value mostly. Therefore, a better way to estimate whether the ring conformation is different in various solvents is the ratio of a^N to a_β^H instead of a^N or a_β^H separately. Li and Chignell[22] found that the ratio is a useful parameter for the identification of DMPO adducts. The range of the ratio may be due to slight changes in the adduct conformation when different solvents are used. As the ratio range for the various cyclic aminoxyls **3a–k** in Table 4 shows, the ratio value for each of **3a–c** in ten solvents is almost the same (i.e. 0.736–0.770 for **3a**, 0.695–0.708 for **3b** and 0.672–0.700 for **3c**). We conclude that each of **3a–c** exhibit similar ring conformations in the ten solvents. Spin adducts **3d–k** display a larger numerical average for NoH (or a^N/a_β^H) (i.e. 0.085) (Table 4). Hence, the conformations of the **3d–k** group may also share a common conformation in the ten solvents.

Correlations with the solvent polarity parameter $E_{T(30)}$[31]

Plots of the N hyperfine splitting (e.g. **3d**) *vs.* the solvent polarity parameter [$E_{T(30)}$] exhibit some linearity ($r^2 = 0.9055$) (Fig. 1). For **3a–k** the average r^2 value for a^N *vs.* $E_{T(30)}$ is 0.8895 ± 0.05. Likewise, a plot of the β-H hfsc of 2-phenyl-DMPO-H$^\cdot$ (or DMPO-phenyl$^\cdot$) (**3d**) *vs.* $E_{T(30)}$ yields some linear character ($r^2 = 0.9115$) (Fig. 2). For **3a–k** the average r^2 value is 0.9037 ± 0.05.

When one plots a_β^H *vs.* a^N in ten solvents one obtains a correlation with considerable linearity. For instance, **3d** shows an r^2 value of 0.9716 (Fig. 3). For **3a–k** the average r^2 value is 0.9700 ± 0.050. The nitrogen and β-H hfscs respond to differences in solvent polarity so similarly (Fig. 3) that conformational arguments for differences in hfscs may not alone explain the observed

Table 4. Ratios a^N/a_β^H for the various cyclic aminoxyls (pyrrolidine-*N*-oxyl nitroxides) 3a–3k in ten solvents

No.	Solvent	3a	3b	3c	3d	3e	3f
1	*n*-Hexane	0.770	0.699	0.672	0.740	0.745	0.743
2	Benzene	0.764	0.695	0.674	0.717	0.721	0.707
3	Chloroform	0.754	0.705	0.693	0.709	0.714	0.711
4	Dichloromethane	0.757	0.701	0.695	0.692	0.693	0.688
5	Acetone	0.763	0.699	0.684	0.700	0.694	0.704
6	Ethanol	0.753	0.699	0.682	0.693	0.694	0.686
7	Acetic acid	0.740	0.708	0.694	0.686	0.677	0.688
8	95% Ethanol	0.746	0.700	0.689	0.693	0.699	0.696
9	Methanol	0.752	0.695	0.687	0.683	0.685	0.684
10	Water	0.736	0.699	0.700	0.645	0.650	0.646
	Range	0.736–0.770	0.695–0.708	0.672–0.700	0.645–0.740	0.650–0.745	0.646–0.743
		3g	3h	3i	3j	3k	
1	*n*-Hexane	0.736	0.732	0.741	0.739	0.740	
2	Benzene	0.711	0.714	0.719	0.717	0.732	
3	Chloroform	0.700	0.705	0.706	0.705	0.731	
4	Dichloromethane	0.685	0.690	0.682	0.686	0.710	
5	Acetone	0.691	0.692	0.696	0.699	0.716	
6	Ethanol	0.685	0.688	0.691	0.695	0.708	
7	Acetic acid	0.677	0.685	0.685	0.683	0.705	
8	95% Ethanol	0.683	0.688	0.692	0.694	0.709	
9	Methanol	0.675	0.677	0.680	0.681	0.699	
10	Water	0.642	0.650	NA	0.646	0.664	
	Range	0.642–0.736	0.650–0.732	0.680–0.741	0.646–0.739	0.664–0.740	

trends. Specifically, the β-H hfsc may mostly be responding to different amounts of spin density on the aminoxyl (nitroxide) nitrogen [cf. Eqn (6)] owing to the two resonance forms.

In Fig. 4 the α-^{13}C hfsc of **3f** is plotted *vs.* its nitrogen hfsc in ten solvents. There is some linearity in this plot ($r^2 = 0.7$) although it is not as linear as a comparable plot of the β-H hfsc *vs.* its nitrogen hfsc ($r^2 = 0.97$, Fig. 3). This is perhaps to be expected for the mechanism of spin transfer to the α-^{13}C and β-H nuclei may be different. Spin polarization is possible for the former while spin polarization and hyperconjugation are possible for the latter.

EXPERIMENTAL

Melting points were measured with a MEL-TEMP melting point device and are not corrected. ^{1}H NMR spectra were recorded on a Varian XL-300 NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Mass spectra were determined using a VG QUATTRO mass spectrometer. Aminoxyls **3a–k** were detected using 0.02 mol l^{-1} of the solution of the reduction product of **2a–k** in various solvents on a Bruker ESP-300E or Bruker ER 200D spectrometer.

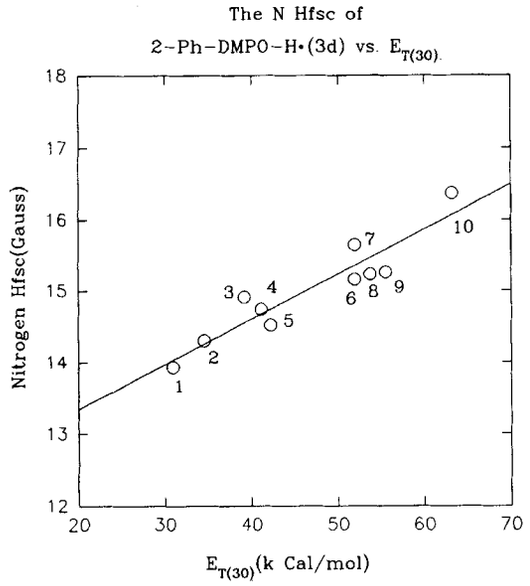


Figure 1. Plot of the nitrogen hfsc of 2-Ph-DMPO-H• (**3d**) *vs.* the solvent polarity parameter $E_{T(30)}$ in ten solvents.

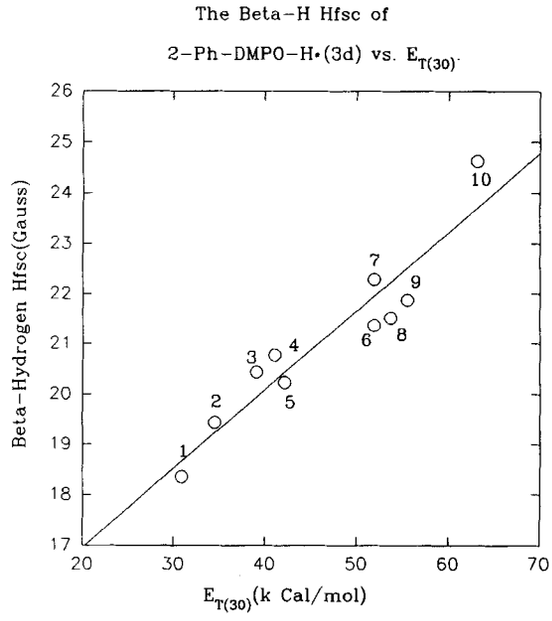


Figure 2. Plot of the β-hydrogen hfsc of 2-Ph-DMPO-H• (**3d**) *vs.* the solvent polarity parameter $E_{T(30)}$ in ten solvents.

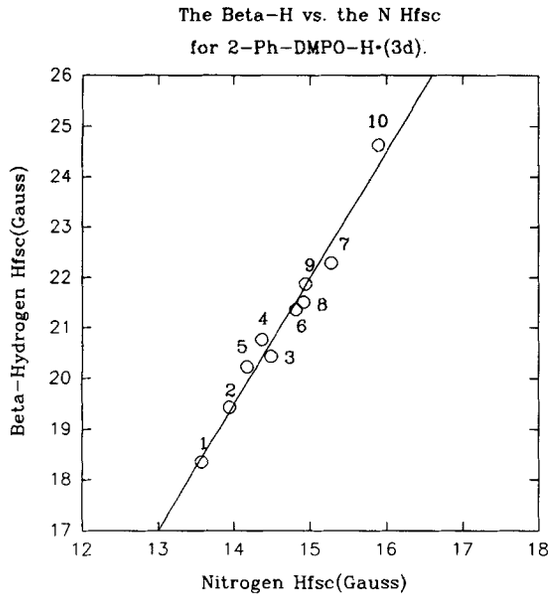


Figure 3. Plot of the β-hydrogen hfsc of 2-Ph-DMPO-H$^{\bullet}$ (**3d**) *vs.* its nitrogen hfsc in ten solvents.

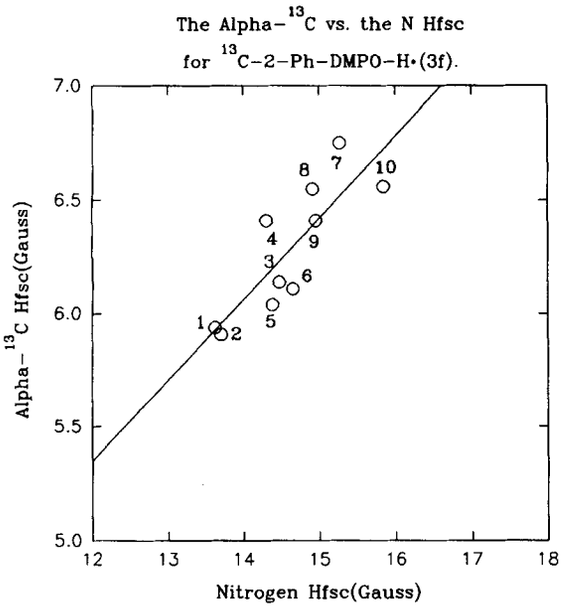


Figure 4. Plot of the α-^{13}C hfsc of ^{13}C-2-Ph-DMPO-H$^{\bullet}$ (**3f**) *vs.* its nitrogen hfsc in ten solvents.

The concentrations of **3a–k** were measured by comparison of the EPR spectra with that of a benzene solution (1.0×10^{-6} mol l^{-1}) of 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO). DMPO was obtained from Aldrich and also synthesized in our laboratories.[23] Elemental analyses were conducted by Galbraith Laboratories (Knoxville, TN, USA).

General procedure for the preparation and characterization (e.g. ^{1}H NMR, Table 1) of the various cyclic nitrones (1b–k)

A solution of DMPO (2.95 g, 26.1 mmol) in 80 ml of benzene was refluxed and evaporated to remove trace amounts of moisture. After cooling to room temperature, 50 ml of anhydrous diethyl ether were added. To this solution was added a diethyl ether solution of an appropriate Grignard reagent (50% excess) for 10 min and the mixture was left overnight at room temperature. A saturated aqueous solution of ammonium chloride (4 ml) was carefully added to stop the reaction. The mixture was filtered and washed with diethyl ether (2 × 50 ml). The filtrate was concentrated to give a solid residue which was dissolved in methanol (100 ml) in which copper(II) acetate monohydrate (0.2 g, 1.0 mmol) and a 28% solution of ammonia (1.5 ml) had been dissolved. The solution was aerated until a permanent blue coloration was restored. The solvent was removed to afford a crude solid product, which was purified at least by recrystallization (**1c** from pentane, **1d–k** from *n*-hexane), sublimation and recrystallization once more.

General procedures for acquisition of the various cyclic *N*,*N*-dialkylhydroxylamines (2a–k) (and their characterization, e.g. ^{1}H NMR, Table 2) and EPR detection of the various cyclic aminoxyls (3a–k) (Tables 3 and 4)

Method I. To a solution of a nitrone (0.2 g) in anhydrous diethyl ether (10 ml) was added dropwise to a solution of lithium aluminum hydride (0.1 g) in anhydrous diethyl ether (3 ml) at 0–5 °C. The mixture was stirred for 1.5 h with cooling and afterwards ethanol (0.5 ml) was carefully added. The residue was filtered and washed with diethyl ether (2 × 10 ml). The residue left after evaporation of the filtrate was kept under nitrogen and stored in a refrigerator before ^{1}H NMR and EPR measurements. The *N*,*N*-dialkylhydroxylamine (*ca.* 1 mg) in an appropriate solvent (0.5 ml) may provide a sufficient concentration of the corresponding aminoxyl for detection by EPR spectroscopy. Most of the cyclic *N*,*N*-dialkylhydroxylamine samples (**2a–k**) were pure enough for collection of the ^{1}H NMR data (Table 2).

Method II. To a solution of the nitrone in water was added an aqueous solution of sodium borohydride (fivefold excess). The reaction mixture was stirred overnight at room temperature and then extracted with dichloromethane (3 × 35 ml) under a nitrogen atmosphere. The mixture was dried over anhydrous sodium sulfate and filtered. The solvent was rotary evaporated to give a crude residue which contained *ca.* 0.03 wt.% of the corresponding aminoxyl. The aminoxyl radical was detected with an EPR spectrometer without further purification.

EPR spectra of the various cyclic aminoxyls (**3a–f**) (Table 3) were recorded using the sample generated by both Methods I and II. EPR spectra of **3g–k** were obtained by Method I (Table 3). ^{1}H NMR spectra of **2b–k** were acquired using the samples from Method I whereas the ^{1}H NMR data for **2a** were obtained using the sample from Method II (Table 2).

Compounds

2,5,5-Trimethyl-1-pyrroline-*N*-oxide (1b). 5-Methyl-5-nitrohexan-2-one was prepared by a Michael addition of 2-nitropropane (60 g, 0.674 mol) to methyl vinyl ketone (35 g, 0.5 mol) with sodium methoxide (5.4 g, 0.1 mol) as a base in methanol (150 ml), yield 92%, b.p. 90 °C/2 mmHg, or with Triton B (benzyltrimethylammonium hydroxide) as a base catalyst in diethyl ether,[32] yield 67%, b.p. 72–74.5 °C/1.5 mmHg; ^{1}H NMR ($CDCl_3$), δ 2.49 (*t*, J = 7.8 Hz, 2H, CH_2), 2.20–2.15 (m, 2H, CH_2), 2.17 (s, 3H, CH_3), 1.59 (s, 6H, $2CH_3$). Nitrone formation I:[9] the nitro ketone (31.8 g, 0.2 mol) was reduced with zinc (27.5 g, 0.42 mol) and acetic acid (50 g, 0.84 mol) in 95% ethanol (350 ml) at $\leq$10 °C for 1.5 h and at 3 °C for 4 h to give an 80% yield of **1b** after the usual work-up (b.p. 66–68 °C/1 mmHg). Nitrone formation II:[23] the nitro ketone (31.8, 0.2 mol) was reduced with zinc (45 g, 0.688 mol) and ammonium chloride (9 g, 0.165 mol) in water (250 ml) to produce 88% yield of **1b** (b.p. 61.2 °C/1 mmHg). MS, *m/z* 128 (M + 1, 22), 127 (M^+, 100), 112 (37), 95 (23), 69 (23), 55 (22), 41 (22).

2-*tert*-Butyl-5,5-dimethyl-1-pyrroline-*N*-oxide (1c). Yield 22%; m.p. 81.5–82 °C (lit. [24] m.p. 80–81 °C). MS *m/z* 169 (M^+, 38), 154 (41), 127 (100), 112 (100). Analysis: calculated for $C_{10}H_{19}NO$, C 70.96, H 11.31, N 8.27; found, C 70.65, H 10.93, N 8.25%.

2-Phenyl-5,5-dimethyl-1-pyrroline-*N*-oxide (1d). Yield 90%; m.p. 100–100.5 °C (lit.[24] m.p. 101–102 °C). MS, *m/z* 190 (M + 1, 15), 189 (M^+, 100), 174 (14), 157 (17), 103 (16), 69 (23). Analysis: calculated for $C_{12}H_{15}NO$: C 76.16, H 7.99, N 7.40; found, C 75.94, H 7.94, N 7.38%.

2-d_5-Phenyl-5,5-dimethyl-1-pyrroline-*N*-oxide (1e). Yield 64%; m.p. 101–101.5 °C. MS, *m/z* 194 (M^+, 90), 179 (24), 162 (35), 109 (51), 108 (100), 82 (41), 69 (48), 55 (25). Analysis: calculated for $C_{12}H_{10}D_5NO$, C 74.21, N 7.21; found, C 74.40, N 7.14%.

2-Phenyl-5,5-dimethyl-1-pyrroline-*N*-oxide-nitronyl-^{13}C (1f). Details of its synthesis and EPR spin trapping applications have been published elsewhere.[33]

2-(4-Fluorophenyl)-5,5-dimethyl-1-pyrroline-*N*-oxide (1g). Yield 68%; m.p. 117.5 °C. MS, *m/z* 208 (M + 1, 15), 207 (M^+, 100), 192 (12), 190 (11), 175 (14), 148 (6), 121 (11). Analysis: calculated for $C_{12}H_{14}NOF$, C 69.55, H 6.81, N 6.76; found, C 69.04, H 7.16, N 6.27%.

2-(4-Chlorophenyl-5,5-dimethyl-1-pyrroline-*N*-oxide (1h). Yield 68%; m.p. 125 °C. MS, *m/z* 225 (33), 224 (M + 1, 18), 223 (M^+, 100), 222 (15), 208 (18), 206 (13), 191 (13). Analysis: calculated for $C_{12}H_{14}NOCl$, C 64.43, H 6.31, N 6.26; found C 64.66, H. 6.61, N 5.95%.

2-(4-*tert*-Butylphenyl)-5,5-dimethyl-1-pyrroline-*N*-oxide (1i). Yield 86%; m.p. 162.5 °C. MS, *m/z* 246 (M + 1, 20), 245 (M^+, 100), 244 (19), 230 (34), 228 (6), 213 (5), 172 (5), 160 (5). Analysis: calculated for $C_{16}H_{23}NO$, C 78.32, H 9.45, N 5.71; found, C. 78.37, H. 9.50, N 5.46%.

2-(4-Methylphenyl)-5,5-dimethyl-1-pyrroline-*N*-oxide (1j). Yield 92%; m.p. 109.5 °C. MS, *m/z* 204 (M + 1, 16), 203 (M^+, 100), 202 (20), 188 (10), 186 (10), 171 (12), 117 (10). Analysis calculated for $C_{13}H_{17}NO$, C 76.81, H 8.43, N 6.89; found, C 77.03, H 8.52, N 6.77%.

2-(2-Methylphenyl)-5,5-dimethyl-1-pyrroline-*N*-oxide (1k). Yield 78%; m.p. 66.0 °C. MS, *m/z* 204 (M + 1, 16), 203 (M^+, 100), 189 (14), 188 (92), 186 (7), 172 (6), 130 (5), 119 (8). Analysis: calculated for $C_{13}H_{17}NO$, C 76.81, H 8.43, N 6.89; found, C 77.01, H 8.46, N 6.88%.

2.2-Dimethyl-1-hydroxylpyrrolidine (2a). Method II. Yield 60%.

2,2,5-Trimethyl-1-hydroxypyrrolidine (2b). Method I. Yield 90%.

5-*tert*-Butyl-2,2-dimethyl-1-hydroxylpyrrolidine (2c).[24] Method I. Yield 76%.

5-Phenyl-2,2-dimethyl-1-hydroxylpyrrolidine (2d).[24] Method I. Yield 90%.

5-d_5-Phenyl-2,2-dimethyl-1-hydroxylpyrrolidine (2e). Method I. Yield not available.

5-Phenyl-5-^{13}C-2,2-dimethyl-1-hydroxylpyrrolidine (2f). Method II. The *N*,*N*-dialkylhydroxylamine **2f** was not isolated in the synthesis of the aminoxyl **3f**.[33]

5-(4-Fluorophenyl)-2,2-dimethyl-1-hydroxylpyrrolidine (2g). Method I. Yield 95%.

5-(4-Chlorophenyl)-2,2-dimethyl-1-hydroxylpyrrolidine (2h). Method I. Yield 95%.

5-(4-*tert*-Butylphenyl)-2,2-dimethyl-1-hydroxylpyrrolidine (2i). Method I. Yield 85%.

5-(4-Methylphenyl)-2,2-dimethyl-1-hydroxylpyrrolidine (2j). Method I. Yield 95%.

5-(2-Methylphenyl)-2,2-dimethyl-1-hydroxylpyrrolidine (2k). Method I. Yield 95%.

1-Acetoxyl-5-phenyl-2,2,4,4,-tetramethylpyrrolidine (4). To a solution of 3,3,5,5-tetramethyl-1-pyrroline-*N*-oxide (0.2 g, 1.4 mmol) in anhydrous diethyl ether (10 ml) was added dropwise a solution of phenylmagnesium bromide in diethyl ether (3.0 mol l^{-1} 1.5 ml, 4.5 mmol) at 5 °C over 15 min in a nitrogen atmosphere. The solution was stirred for 1 h at room temperature and refluxed for 30 min. After cooling and the addition of water (0.5 ml), the solid was extracted with diethyl ether (2 × 25 ml). The solvent was removed and the residue was dried, then dissolved in a mixture of dichloromethane (10 ml) and pyridine (1 ml, 13 mmol). Acetic anhydride (1 ml, 10 mmol) was added dropwise at 0–5 °C and the mixture was stirred for 1.5 h at 5 °C and 4 4 h at room temperature. The solution was left in a refrigerator for 2 days. Ammonia solution (28%, 1 ml), dichloromethane (50 ml) and water (40 ml) were added. After the usual work-up, the residue was chromatographed on a silica gel column (with dichloromethane as the eluent) to produce a 41% yield of **4**. ^{1}H NMR ($CDCl_3$), δ 7.42–7.20 (m, 5H, Ar-H), 4.14 (s, 1H, H_5), 1.82 (s, 3H, CH_3CO), 1.76 (s, 1H, H_3), 1.32 (s, 3H, 4-CH_3), 1.21 (s, 3H, 2-CH_3), 1.11 (s, 3H, 2-CH_3), 0.66 (s, 3H, 4-CH_3). MS, *m/z* 261 (M^+, 5), 246 (4), 220, (33), 219 (95), 205 (41), 204 (100), 201 (16), 186 (5), 171 (4), 147 (23), 146 (80), 98 (66).

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