Spin Trapping

Synthesis and Spin-Trapping Properties of a Trifluoromethyl Analogue of DMPO: 5-Methyl-5-trifluoromethyl-1-pyrroline *N*-Oxide (5-TFDMPO)**

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In memory of François Le Moigne

Abstract: The 5-diethoxyphosphonyl-5-methyl-1-pyrroline *N*-oxide superoxide spin adduct (DEPMPO–OOH) is much more persistent (about 15 times) than the 5,5-dimethyl-1-pyrroline *N*-oxide superoxide spin adduct (DMPO–OOH). The diethoxyphosphonyl group is bulkier than the methyl group and its electron-withdrawing effect is much stronger. These two factors could play a role in explaining the different half-life-times of DMPO–OOH and DEPMPO–OOH. The trifluoromethyl and the diethoxyphosphonyl groups show similar elec-tron-withdrawing effects but have different sizes. We have thus synthesized and studied 5-methyl-5-trifluoromethyl-1-pyrroline *N*-oxide (5-TFDMPO), a new trifluoromethyl analogue of DMPO, to compare its spin-trapping performance with those of DMPO and DEPMPO. 5-TFDMPO was prepared in a five-step sequence by means of the Zn/AcOH reductive cyclization of 5,5,5-trifluoro-4-methyl-4-nitropentanal, and the geometry of the molecule was estimated by using DFT calculations. The spin-trapping properties were investigated both in toluene and in aqueous buffer solutions for oxygen-, sulfur-, and carbon-centered radicals. All the spin adducts exhibit slightly different fluorine hyperfine coupling constants, thereby suggesting a hindered rotation of the trifluoromethyl group, which was confirmed by variable-temperature EPR studies and DFT calculations. In phosphate buffer at pH 7.4, the half-life of 5-TFDMPO–OOH is about three times shorter than for DEPMPO–OOH and five times longer than for DMPO–OOH. Our results suggest that the stabilization of the superoxide adducts comes from a delicate balance between steric, electronic, and hydrogen-bonding effects that involve the β group, the hydroperoxyl moiety, and the nitroxide.

Introduction

In the last three decades, much effort has been devoted to the development of methods to detect and characterize the superoxide radical, and among these methods EPR/spin-trapping techniques have proven to be one of the most reliable.^[1] 5,5-Dimethyl-1-pyrroline *N*-oxide (DMPO, **1**; Figure 1) is one of the

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Figure 1. Spin-trap structures.

most popular spin traps, and EPR features of its spin adducts are well known.^[2] However, major limitations, such as a low superoxide trapping rate constant and a poor stability ($t_{1/2} \approx 1$ min at physiological pH) of the DMPO–OOH spin adduct restrict the use of DMPO in trapping the superoxide anion (O_2 [•]), one of the most important free-radical species formed in aerobic organisms.^[3,4] We have shown that the replacement of a methyl group of DMPO with a diethoxyphosphonyl group to generate 5-diethoxyphosphonyl-5-methyl-1-pyrroline *N*-oxide

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(DEPMPO, 2; Figure 1) dramatically improved the spin-trapping properties.^[5] Under the same experimental conditions, the halflifetime of the DEPMPO-OOH adduct is at least 15 times longer than that of DMPO-OOH. Furthermore, unlike DMPO-OOH, the DEPMPO-OOH adduct does not decay to the corresponding hydroxyl adduct in phosphate buffer.^[5,6] In the course of our studies on the parameters that control the spinadduct stability, we investigated the spin-trapping properties of the 5-diethoxyphosphonyl-5-phenyl-1-pyrroline N-oxide spin trap (DEPPPO, 3; Figure 1).^[7] The replacement of a methyl group with a phenyl was shown to change the stereoselectivity of the trapping reaction and the conformations of the nitroxide spin adducts,^[8] as illustrated by the significant changes observed for the hyperfine coupling constants. However, no significant change was observed for the half-lifetime of the DEPPPO-OOH spin adduct relative to DEPMPO-OOH (DEPPPO-OOH $t_{1/2} = (13.1 \pm 0.1)$ min and DEPMPO-OOH $t_{1/2} =$ (14.8 ± 1.4) min at pH 7.4).^[7] Additionally, we reported an EPR/ spin-trapping study of the 5-ethoxycarbonyl-5-methyl-1-pyrroline *N*-oxide spin trap (EMPO, **4**; Figure 1) in which one β substituent is an ethoxycarbonyl group.^[9] The half-lifetime of the EMPO-OOH spin adduct was estimated from 4.8^[9] to 8.6 min.^[10] Several articles reporting the spin-trapping properties of EMPO derivatives were subsequently published by Kalyanaraman et al.,^[11] Nohl et al.,^[10,12] Rosen et al.,^[13] and Stolze et al.^[14] From these studies, the superoxide spin-adduct stability appeared to depend on the chain length of the alkoxy moiety and/or on the pyrrolidine ring substitution.^[11-14] Since these works, numerous studies have been devoted to the development of new pyrroline N-oxide-based spin traps with improved superoxide trapping properties,^[15-20] and DEPMPO derivatives remain one of the most efficient structures for spin traps for in vitro and ex vivo trapping of oxygen-centered radicals.

In our continuing efforts to understand the influence of the diethoxyphosphonyl group on the stabilization of the DEPMPO-OOH adduct, we prepared the 5-methyl-5-trifluoromethyl-1-pyrroline N-oxide spin trap (5-TFDMPO, 5; Figure 1) and performed spin-trapping experiments for oxygen-, carbon-, and sulfur-centered radicals. It has been previously reported that the trifluoromethyl group has a similar electronwithdrawing effect to the diethoxyphosphonyl group although its size is significantly smaller.^[21a] It can be assumed that the general pattern of the spin-adduct EPR spectra of 5-TFDMPO should be similar to those of DMPO adducts. However, further splittings owing to the fluorine atoms $(m_l = 1/2)$ can be expected. For instance, it has been reported that the *i*-amyloxy adduct of an isoindoline-N-oxide spin trap that bears a -CF₃ group exhibited additional fluorine hyperfine splittings.^[21b] A DMPO derivative that bears a $-CF_3$ group on the nitronyl double bond (2-TFDMPO, 7; Figure 1) has been previously described by Janzen et al.^[22] For 2-TFDMPO (7), EPR spectra of most spin adducts exhibited a three-line signal further split into 1:3:3:1 quartets owing to the γ coupling with fluorine atoms. No indication of the half-lifetime of 2-TFDMPO-OOH was reported. However, nitrone spin traps that bear a substituent on the nitronyl double bond (1,1,3-trimethylisoindole-N- oxide (TMINO) and M_3PO , **6**) were reported to lead to superoxide adducts with short lifetimes (< 1 min), thus highlighting the destabilizing effect of C-2 substitution and the decrease in the trapping rate.^[23]

Results and Discussion

Synthesis

According to the method described by Shepard and Sciaraffa,^[24] we prepared 1,1,1-trifluoroacetone oxime (**9**) starting from commercially available 1,1,1-trifluoroacetone (**8**). Nitro compound **10** was obtained by means of oxidizing oxime **9** with H_2O_2 in the presence of HNO₃ and NH₄NO₃ as reported by Bissel and Fields.^[25] Treatment of nitro compound **10** with acrolein in the presence of triethylamine afforded nitroaldehyde **11**, and reductive cyclization of **11** yielded nitrone **5** (5-TFDMPO) as a colorless oil after distillation. 5-TFDMPO samples stored at -20 °C were stable for several months, and we never observed any additional paramagnetic signal when they were used for spin-trapping studies either in phosphate buffers or in organic solvents (Scheme 1).



Scheme 1. Synthesis of the 5-TFDMPO spin trap.

DFT molecular geometry of DEPMPO, EMPO, and 5-TFDMPO

To obtain information on the molecular geometry of 5-TFDMPO, EMPO, and DEPMPO, the energetically favored conformations were calculated for each molecule. The optimized geometries of their most stable conformer are shown in Figure 2, and the main geometrical features are reported in Table 1. The net atomic charges were calculated using the CHelpG scheme.^[26]



Figure 2. DFT-calculated geometries of the most stable conformer of a) 5-TFDMPO, b) DEPMPO, and c) EMPO.

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Table 1. Main geometrical features of DEPMPO, 5-TFDMPO, and EMPO.						
	C₅–R	N–O	C=N	C₂NC₅R	C₂NC₅C₄	Charge ^[a]
	[Å]	[Å]	[Å]	[°]	[°]	C ₅ (R)
$\label{eq:constraint} \begin{array}{ c c } \mbox{DEPMPO} (R = P(O)(OEt)_2) \\ \mbox{5-TFDMPO} (R = -CF_3) \\ \mbox{EMPO} (R = -CO_2Et) \end{array}$	1.865	1.272	1.306	101.1	14.4	0.243
	1.538	1.263	1.310	103.7	13.1	0.234
	1.541	1.264	1.307	102.6	12.4	0.182
[a] The net atomic charges were calculated using the CHelpG scheme. ^[27]						

All the calculations were performed with the Gaussian 98 molecular-orbital package.^[27] The geometry optimizations were carried out without constraints at the B3LYP/6-31G(d) level. For DEPMPO and 5-TFDMPO, very close values of net charge at the C-5 position (the carbon atom that bears the electron-withdrawing group) have been obtained by calculation, thus indicating that $-CF_3$ and $-P(O)(OEt)_2$ groups have similar electronic effects on the nitronyl function. For EMPO, the net charge is smaller at the C-5 position, thus indicating a less important electron-withdrawing effect. Vibrational frequencies were calculated at the B3LYP/6-31G(d) level to determine the nature of the located stationary points.

The three spin traps display very similar geometries: the conformation of the ring is an envelope (E₄), and the measured C₂NC₅C₄ dihedral angles are 14.4, 13.1, and 12.4° for DEPMPO, 5-TFDMPO, and EMPO, respectively. It is noteworthy that the ring conformation imposes a pseudoaxial position of the dieth-oxyphosphonyl, trifluoromethyl, and carboxyethyl moieties (C₂NC₅P and C₂NC₅C₆ dihedral angles are 101.1, 103.7, and 102.6° for DEPMPO, 5-TFDMPO, and EMPO, respectively). Thus, in contrast to DMPO in which the *gem*-dimethyl groups have a bisecting conformation, the pseudoaxial position of the $-CF_{3}$, $-CO_2Et$, and $-P(O)(OEt)_2$ groups strongly increases the steric crowding of one face of the molecule. Thus a stereoselective trapping reaction is expected, with the *trans* spin adducts formed primarily, most notably for DEPMPO because of the larger size of the diethoxyphosphonyl group.

EPR studies

Spin trapping of hydroxyl radicals

The hydroxyl radical spin adduct (5-TFDMPO–OH) was generated by incubating 5-TFDMPO (50 mM) with H₂O₂ (1 mM) and FeSO₄ (0.3 mM) in 0.1 M phosphate buffer at pH 7.4. A persistent and intense EPR signal (Figure 3a) was observed that exhibited a general 1:2:2:1 pattern (a_N = 1.38 mT; a_H = 1.34 mT) but with each line of the quartet being further split into a quartet due to coupling with three nonequivalent fluorine atoms (a_F = 0.217, 0.203, and 0.159 mT; Table 3 and Figure 3a). The same EPR signal was also observed either by Fe³⁺-catalyzed nucleophilic addition of water^[28] or by UV photolysis of a solution that contained 5-TFDMPO (50 mM) and H₂O₂ (1%) in phosphate buffer at pH 7.4. The 5-TFDMPO–OH adduct signal was inhibited by adding catalase (50 U ml⁻¹) in the Fenton system incubation mixture.



Figure 3. EPR spectra of the hydroxyl and superoxide spin adducts of 5-TFDMPO. a) UV photolysis of a solution containing 5-TFDMPO (50 mM) and H_2O_2 (1%) in phosphate buffer (0.1 M, pH 7.4). b) Incubation mixture containing hypoxanthine (0.4 mM), xanthine oxidase (0.4 Uml⁻¹), DTPA (1 mM), and 5-TFDMPO (100 mM) in phosphate buffer (0.1 M, pH 7.4). The computer simulations are below the experimental spectra. Spectrometer settings: microwave power 10 mW, modulation amplitude a) 0.05 mT and b) 0.1 mT, time constant 0.128 s, gain 5×10^4 , scan range 10 mT, scan time a) 120 s and b) 240 s.

Spin trapping of superoxide radical anions and tert-butylperoxyl radicals

Spin-trapping of superoxide with 5-TFDMPO was performed at pH 7.4 and 5.6 using hypoxanthine (HX; 0.4 mm)/xanthine oxidase (XOD; 0.4 U ml⁻¹) as the superoxide-generating system. A complex EPR spectrum was observed (Figure 3b), which was cancelled in the presence of superoxide dismutase (SOD; 1250 Uml⁻¹), thus indicating that the signal can be assigned to the 5-TFDMPO-OOH spin adduct. The same signal was observed by UV photolysis of a solution that contained 5-TFDMPO and H₂O₂ (30%) in phosphate buffer. During the decay or after the total disappearance of the 5-TFDMPO-OOH spin adduct, no 5-TFDMPO-OH signal was observed. The experimental spectrum (Figure 3b) was satisfactorily calculated (Figure 3b, bottom and Table 2) by assuming the superimposition of the signals of the cis (7.4%) and trans (92.6%) diastereomeric spin adducts. The best fit was obtained by assuming three nonequivalent fluorine atoms for each diastereoisomer. 5-TFDMPO-OOtBu was generated by irradiation of a solution of tBuOOH in benzene (1.5 m) in the presence of 5-TFDMPO. For both 5-TFDMPO–OOH (Figure 3b) and 5-TFDMPO–OOtBu (see the Supporting Information) spin-adduct signals, a complex and similar spectral pattern was obtained. The 5-TFDMPO–OOtBu signal was calculated by considering the presence of two diastereoisomers (cis and trans addition, 28.4 and 71.6%, respectively) that exhibited different coupling constants (Table 2). As for 5-TFDMPO-OOH, the major species is supposed to be the trans diastereoisomer owing to the steric crowding of the face that displays the -CF₃ moiety.

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Table 2. Calculated EPR features of 5-TFDMPO–OOH and 5-TFDMPO–OOtBu.								
Radical	Source	Solvent	[%]	<i>a</i> _N [mT]	<i>а</i> _{нβ} [mT]	<i>a</i> _F [mT]		
ноо.	HX/XOD	water ^[a]	trans 92.6 ^[b] cis	1.279 1.290	1.120 0.863	0.237, 0.236, 0.183 0.251, 0.168,		
tBuOO*	tBuOOH/hν	benzene	7.4 ^[b] <i>trans</i> 71.6 ^[b]	1.156	0.950	0.168 0.266, 0.242, 0.213		
		_	cis 28.4 ^[b]	1.212	0.816	0.310, 0.192, 0.147		

[a] Phosphate buffer (0.1 м), pH 7.4. [b] Percentage contribution of diastereoisomer.

Kinetic decay of the superoxide adduct

5-TFDMPO-OOH was generated by incubating HX (0.4 mm), XOD (0.04 Uml⁻¹), DTPA (0.3 mм), and 5-TFDMPO (50 mм) in 0.1 M phosphate buffer (pH 7.4 and 5.6). After 7 min, a steadystate concentration of adduct was reached, and a large excess amount of SOD (1250 Uml⁻¹) was added to stop its formation. Decay of 5-TFDMPO-OOH was monitored by measuring the decrease in the low-field line intensity of the EPR spectrum. The kinetic decay was calculated as a pseudo-first-order process. Half-lifetimes were determined to be (4.5 ± 0.3) min at pH 7.4 and (14.3 ± 0.3) min at pH 5.6. Under the same conditions, DEPMPO-OOH exhibited a longer half-lifetime, with values of (14.8 ± 1.4) min at pH 7.4 and 30.4 min at pH 5.6.^[5,29] It was not possible to perform an accurate comparison with DMPO under the same experimental conditions because of the very short half-lifetime of DMPO–OOH ($t_{1/2} \approx 50$ s at pH 7.4). Study of 5-TFDMPO-OOH decay was performed at two different pH values (5.6 and 7.4). At pH 5.6, the 5-TFDMPO-OOH adduct was more persistent than at pH 7.4 ((14.3 \pm 0.3) and (4.5 \pm 0.3) min, respectively), but the signal intensity was weaker. It is interesting to mention that unlike DMPO-OOH, 5-TFDMPO-OOH adduct decays by giving diamagnetic byproducts. Thus, 5-TFDMPO exhibited a similar performance to EMPO, even if the --CF3 group is more electron-withdrawing than the --CO2Et group. Both spin adducts have similar half-lifetimes ($t_{1/2}$ = (4.5 \pm 0.3) min for 5-TFDMPO–OOH and (4.8 \pm 1.1) min for EMPO–OOH at pH 7.4).^[9]

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tylperoxide (0.15 M) in *tert*-butylbenzene at 298 K. In the presence of 5-TFDMPO, the 5-TFDMPO–OtBu adduct spectrum was observed as a very persistent signal and was best calculated as a single diastereoisomer adduct (only *trans* addition) with three nonequivalent fluorine atoms (Figure 4a). The hyperfine coupling constants are reported in Table 3.

Sulfur-centered radicals

Sulfur-centered radicals were generated by UV photolysis of a degassed solution that contained different disulfides (RS–SR, 1.5 M in benzene). Spin adducts obtained by trapping of thiyl radicals were persistent, and their corresponding spectra were calculated by assuming

the presence of only one diastereoisomer (only *trans* addition)



Figure 4. EPR spectra from spin trapping of *tert*-butoxyl radical with 5-TFDMPO at different temperatures. a) UV photolysis of a solution containing 5-TFDMPO (50 mM) and *t*BuOOtBu (1.5 M) in *tert*-butylbenzene at 298 K. b) Same as in (a) but at 373 K. The computer simulations are below the experimental spectra. Spectrometer settings: microwave power 10 mW, modulation amplitude 0.015 mT, time constant 0.064 s, gain 5×10^4 , scan range 10 mT, scan time 168 s.

*а*_н [mT]

1.341

0.813

2.233

1.981

1.921

1.739

1.070

1.127

1.339

1.522

*a*₅ [mT]

0.217, 0.203, 0.159

0.224, 0.212, 0.184

0.250, 0.218, 0.174

0.272, 0.235, 0.187

0.284, 0.255, 0.225

0.266, 0.235, 0.208

0.230, 0.211, 0.186

0.225, 0.199, 0.176

0.235, 0.212, 0.192

0.278, 0.254, 0.212

*a*_№ [mT]

1.382

1.227

1.490

1.328

1.282

1.419

1.260

1.269

1.224

1.291

Spin trapping of other radicals

Other (C-, O-, S-centered) radicals were trapped with 5-TFDMPO in water and in organic solvents. The calculated EPR parameters of the spin adducts are reported in Table 3.

Oxygen-centered radical in organic solvent

tert-Butoxyl radical (tBuO') was produced by UV photolysis of a degassed solution of di-*tert*-bu-

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Radical

HO.

tBuO'

CH3.

CH₃.

Ph[.]

'CO2

EtS'

tBuS

PhS[•]

SO3

Source

 $H_{2}O_{2}/Fe^{2+}$

(tBuO)₂/hv

 $CH_3I/h\nu$

 $(EtS)_2/h\nu$

 $(PhS)_{2}/hv$

 $(tBuS)_{3}/hv$

Phl/hv

H₂O₂/DMSO/Fe²⁺

H₂O₂/Fe²⁺/HCO₂Na

H₂O₂/Fe²⁺/NaHSO₃

[a] Phosphate buffer (0.1 м), pH 7.4.

Table 3. EPR parameters of different 5-TFDMPO spin adducts at 298 K.

Solvent

water^[a]

water^[a]

benzene

benzene

benzene

benzene

benzene

water^[a]

water^[a]

tert-butylbenzene

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*а*_{нү} [mT]

0.094

0.090, 0.120

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with three nonequivalent fluorine atoms (see the Supporting Information). 5-TFDMPO–SO₃H spin adduct was obtained by using the Fenton reaction in the presence of NaHSO₃ (0.1 μ).

EPR parameters of 5-TFDMPO-thiyl spin adducts are reported in Table 3.

Carbon-centered radicals

Very intense signals (Figure 5) were detected for the trapping of Ph[•] and [•]CH₃ generated by UV photolysis of a benzene solution of the corresponding iodides, and for [•]CO₂⁻, [•]CH₃, and [•]CH-(CH₃)OH generated by Fenton reaction in water in the presence of sodium formate, dimethylsulfoxide, and ethanol, respectively. The EPR signals consisted of six lines ($a_N < a_H$) further split into four lines because of the presence of three slightly nonequivalent fluorine atoms (Table 3).



Figure 5. EPR spectra from spin trapping of 'CO₂⁻ radical with 5-TFDMPO. 'CO₂⁻ was generated by means of a Fenton standard system (2 mM H_2O_{27} , 1 mM FeSO₄) in the presence of sodium formate (0.2 M) and 5-TFDMPO (50 mM). The black line below represents a computer simulation of the experimental spectrum. Spectrometer settings: microwave power 10 mW, modulation amplitude 0.05 mT, time constant 0.064 s, gain 5×10^4 , scan range 10 mT, scan time 240 s.

Stereoselectivity of the trapping reaction

Two diastereoisomers (*cis* and *trans*) are expected when trapping radicals with 5-TFDMPO because of the chirality of the C-5 atom. For DEPMPO in which the chiral C-5 atom is linked to an EPR-active phosphorus nucleus (m_i =1/2), when they are observed the two diastereoisomers exhibit different EPR spectra owing to the large value and the conformational dependence of the β -phosphorus coupling constant. For the 5-TFDMPO spin adducts, the two diastereoisomers are expected to exhibit close EPR signals because of the small values of the γ -fluorine hyperfine coupling constants (a_F). For all the spin adducts, except for 5-TFDMPO–OOH and 5-TFDMPO–OOtBu (Table 2), we obtained a good fit between the experimental and calculated EPR spectra, assuming the presence of only one diastereoisomer. For all the calculated spectra, a better fit was

obtained by assuming the presence of three nonequivalent fluorine hyperfine coupling constants. In his previous work on the 2-TFDMPO spin trap (**6**), Janzen and co-workers reported nonequivalent fluorine atoms in the case of carbon dioxide anion, phenyl, and alkyl adducts.^[22] Our calculations suggest that the trapping of radicals with 5-TFDMPO is highly stereoselective. The high stereoselectivity observed could be explained by the pseudoaxial position of the $-CF_3$ group (Figure 2) and its steric hindrance, which, because of the solvent sphere, is reported to be in between that of isopropyl and *tert*-butyl groups.^[30] The observation of a small amount (7.4%) of *cis* adduct for 5-TFDMPO–OOH could be accounted for by the presence of a network of hydrogen bonds from water molecules that link the $-CF_3$ group, the nitroxide, and the peroxyl moiety (Figure 6).



Figure 6. Calculated DFT structures for *cis* and *trans* adducts of 5-TFMPO– OOMe at the UB3LYP/SVP level with PCM modeling water. The isosurface depicting the spin density of the radical is given at 0.005 eÅ⁻³. Hydrogenbond lengths are given in Å. The *cis* adduct is 2.15 kcal mol⁻¹ more stable than the *trans* adduct.

Nonequivalence of the fluorine atoms

For all the spin adducts, the best EPR-calculated parameters were obtained by assuming three nonequivalent fluorine coupling constants. To verify the possible $-CF_3$ hindered rotation at the origin of the nonequivalency of the three fluorine atoms, quantum mechanical (QM) simulations and EPR variable-temperature experiments were performed. To assess the hypothesis of a conformational restraint induced by water, quantum chemical calculations were carried out on a model system including, specifically, a water molecule (Figure 6). The level of calculation chosen (UB3LYP/SVP, with implicit water solvent modeled by the polarizable continuum model (PCM)) is sufficient to describe this type of radical.^[31]

The simulation for both diastereoisomers (*cis* and *trans*) indicates that a water molecule can simultaneously establish hydrogen bonding between the nitroxide and the trifluoromethyl part of the molecule. In both configurations, the distance between water and the nitroxide was found to be 1.90 Å, whereas the hydrogen-bond length between water and $-CF_3$ is around 2.4 Å. In the *cis* adduct, a second water molecule can establish a bridge between the trifluoromethyl group and the peroxide (hydrogen-bond lengths: 2.33 and 2.02 Å). Thus, the hydrogen-bonding network can slow the rotation of the $-CF_3$ group and affect the EPR spectra. Variable-temperature EPR ex-

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Table 4. 5-TFDMPO–OtBu spin adduct in <i>tert</i> -butylbenzene. Evolution of fluorine coupling constants (a_r) with temperature.								
<i>T</i> [K]	298	323	353	373	403			
a _F [mT]	0.224, 0.212, 0.184	0.223, 0.208, 0.186	0.224, 0.205, 0.188	0.224, 0206, 0.187	0.214, 0.214, 0.200			

periments were also performed to monitor the evolution of the EPR spectra of 5-TFDMPO–OtBu (Figure 4) from 298 to 403 K, and the fluoride hyperfine coupling constant (a_F) was examined (Table 4).

At 403 K, the three hyperfine coupling constants of the fluorine atoms become nearly identical ($\Delta a_{\rm F} = 0.014$ mT), thus indicating that at 400 K the rotation of the $-{\rm CF_3}$ group is fast in regard to EPR.

Conclusion

5-TFDMPO is a new trifluoromethyl analogue of DMPO. It is very stable for several months when stored at low temperature and does not give any paramagnetic impurity. 5-TFDMPO has shown efficient trapping of oxygen-, carbon-, and sulfur-centered radicals and a higher persistency than DMPO concerning the superoxide spin adduct ($t_{1/2} = 4.5$ min and 50 s at pH 7.4 for 5-TFDMPO-OOH and DMPO-OOH, respectively). The superoxide spin adduct does not decompose spontaneously to give the hydroxyl adduct or any paramagnetic byproduct. Despite extra couplings owing to the presence of three fluorine atoms, 5-TFDMPO spin adducts could be easily characterized. Relative to DEPMPO and EMPO superoxide adducts, the persistency of the 5-TFDMPO-OOH adduct is lower or close, respectively. This result indicates that the electron-withdrawing effect plays a role in the stabilization of the superoxide spin adducts, but it does not constitute a key parameter like the conformational effect and the hydrogen-bond network do.

Experimental Section

Synthesis and characterization

¹H NMR spectra were recorded at 100 and 400 MHz in CDCl₃ using TMS as an internal reference. ¹³C NMR spectral measurements were performed at 100.6 MHz using CDCl₃ and C₆D₆ as internal standards. ¹⁹F NMR spectral measurements were performed at 94.21 MHz in CDCl₃ and C₆D₆ using CClF₃ as an external standard. Chemical-shift (δ) values are given in ppm, and *J* values in Hz. Elemental analyses were carried out at the Spectropole of the Aix-Marseille University.

1,1,1-Trifluoroacetone oxime (9)

Compound **8** (22.4 g, 0.20 mol) was added to a stirred solution of hydroxylamine hydrochloride (27.80 g, 0.40 mol) and sodium acetate (65 g, 0.79 mol) in water (300 mL) at 0 °C. The mixture was then stirred at room temperature for 2 d and finally heated to reflux for 18 h. The mixture was extracted with diethyl ether (2× 100 mL), and the organic phase was washed with water (50 mL) saturated with sodium bicarbonate, with water (50 mL), and dried over Na₂SO₄, then the organic phase was distilled to yield the oxime (19 g, 75%). B.p. 101–104 °C; ¹H NMR (100 MHz, CDCl₃, TMS): δ = 1.76 (s; CH₃), 7.36 ppm (s; NOH); ¹⁹F NMR (94 MHz, CDCl₃): δ = -71.4 ppm.

1,1,1-Trifluoro-2-nitropropane (10)

Compound **9** (10 g, 78.7 mmol) was added over 30 min at 10 °C to a stirred mixture of HNO₃ (13.20 g) and NH₄NO₃ (6.75 g). The mixture was stirred at 10 °C for 2 h. H₂O₂ (8 mL, 30%) was added over 15 min, and the mixture was stirred at 10 °C for 30 min, then at room temperature for 2.5 h. We added another 4 mL of H₂O₂ (30%). The mixture was poured into crushed ice and water. The organic phase was removed, and the aqueous layer was extracted with four portions of CH₂Cl₂ (10 mL). The organic layer was dried over Na₂SO₄. The solvent was then removed to yield a mixture (2.00 g, 2:1) of 1,1,1-trifluoro-2-nitropropane and 1,1,1-trifluoro-2,2dinitropropane after distillation. B.p. 102–103 °C; ¹H NMR (100 MHz, CDCl₃): δ = 1.78 (d, *J*=6.2 Hz; CH₃), 5.01 ppm (m; CH); ¹⁹F NMR (94 MHz, CDCl₃): δ = -74.7 ppm (d, *J*(F,H) = 7 Hz)

5,5,5-Trifluoro-4-methyl-2-nitropentanal (11)

Triethylamine (0.25 g, 2.45 mmol) was added at 0 °C to a cooled mixture of acrolein (3.30 g, 58.8 mmol) and the previous mixture of 1,1,1-trifluoro-2-nitropropane and 1,1,1-trifluoro-2,2-dinitropropane (3.55 g, 24.8 mmol) in acetonitrile (15 mL). The mixture was stirred for 3 h at room temperature, and the solvent was removed under reduced pressure. A colorless oil was obtained (1.88 g, 43%), which was further purified by distillation (42 °C at 0.1 mm Hg). ¹⁹F NMR (94 MHz, CDCl₃): $\delta = -76.2$ ppm.

5-Trifluoromethyl-5-methyl-1-pyrroline N-oxide (5)

A solution of acetic acid (2.00 g, 33.3 mmol) in ethanol (17 mL) was added to a mixture of 5,5,5-trifluoro-4-methyl-2-nitropentanal (3.55 g, 9.6 mmol) and zinc (1.00 g, 15.3 mmol) in ethanol (50 mL). The medium was allowed to warm to room temperature under stirring and was stirred for 4 h. After removal of the solvent and extraction by CH₂Cl₂ (2×50 mL), crude oil (1.12 g) was obtained and purified by distillation (60 °C at 10⁻¹ mm Hg) to yield 5-TFDMPO (0.56 g, 3.36 mmol, 35%). ¹H NMR (400 MHz, CDCl₃): δ = 1.72 (s, 3 H; CH₃), 2.19–2.77 (m, 4H; 2CH₂), 7.05 ppm (t, *J*=2.55 Hz, 1 H; CH); ¹³C NMR (50.25 MHz, C₆D₆): δ = 18.51 (s; CH₃), 24.53 (s; CH₂), 28.68 (s; CH₂), 76.62 (q, *J*=27.98 Hz; C–CF₃), 125.99 (q, *J*=284.90 Hz; CF₃), 133.48 ppm (s; CH); ¹⁹F NMR (94 MHz, CDCl₃): δ = -78.2 ppm; elemental analysis calcd (%) for C₆H₈F₃NO-0.25 H₂O (171.63): C 41.99, H 4.99, N 8.16; found: C 42.02, H 5.07, N 8.07.

Spin-trapping studies

Xanthine oxidase (XOD), bovine erythrocyte superoxide dismutase (SOD), and catalase were purchased from Boehringer Mannheim Biochemical Co. Glutathione (GSH), glutathione peroxidase (Gpx), diethylenetriaminepentaacetic acid (DTPA), di-*tert*-butyl peroxide, dibenzoyl peroxide, *tert*-butyl hydroperoxide, riboflavin, and other chemicals were from Sigma Chemical Co.

EPR measurements

EPR spectra were recorded at room temperature using computercontrolled Varian E-3 and Bruker ESP 300 EPR spectrometers at 9.5 GHz (X-band) and employing 100 kHz field modulation. Reaction mixtures were prepared in a Chelex-treated phosphate buffer

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(0.1 M, pH 7.4). EPR spectra were simulated using EPR software developed by D. Duling from the Laboratory of Molecular Biophysics, NIEHS, USA (this software is available from the World Wide Web at http://epr.niehs.nih.gov/PEST.html) and by A. Rockenbauer from the Central Research Institute of Chemistry, Hungary.^[32] UV photolysis was performed by using a 1000 W Xe/Hg Oriel lamp.

Superoxide trapping hypoxanthine/xanthine oxidase system: Xanthine oxidase (0.4 Uml^{-1}) was added to a solution of 5-TFDMPO (0.1 m), diethylenetriaminepentaacetic acid (DTPA; 1 mm), and hypoxanthine (0.4 mm) in phosphate buffer (0.1 m, pH 7.4). The EPR spectrum was recorded 60 s after the addition of XOD.

HO' trapping Fenton system: Hydroxyl radical was generated by adding FeSO₄ (0.3 mm) to a solution of 5-TFDMPO (50 mm) and H₂O₂ (1 mm) in phosphate buffer (0.1 m, pH 7.4). The EPR spectrum of the hydroxyl adduct was recorded 60 s after the addition of ferrous sulfate. No EPR signal was observed in the presence of catalase (50 U ml⁻¹) in the incubation mixture

UV-photolysis system: A solution that contained 5-TFDMPO (50 mM) and H_2O_2 (1%) in phosphate buffer (0.1 M, pH 7.4) was irradiated with UV light, and the EPR spectrum was recorded after 15 s of irradiation.

Nucleophilic addition of water: The hydroxyl spin adduct was obtained by adding $FeCl_3$ (0.5 mm) to a solution of 5-TFDMPO (50 mm) in double-distilled water.

'CO₂ ('CO₂H), 'CH₃, and 'CH(CH₃)OH trapping: A Fenton system in the presence of HCO_2Na , DMSO, and ethanol was used to generate $'CO_2^{-}$ ('CO₂H), 'CH₃, and 'CH(CH₃)OH, respectively. FeSO₄ (0.5 mm) was added to a solution of 5-TFDMPO (50 mm) and H₂O₂ (1 mm), and HCO₂Na (0.1 m) and ethanol (10%), respectively, in phosphate buffer (0.1 m, pH 7.4). The EPR spectrum of the corresponding spin adduct was recorded 60 s after the addition of ferrous sulfate.

'CH₃ and Ph' trapping in organic solvent: 'CH₃ and Ph' were generated by UV photolysis of a solution of methyl and phenyl iodide (1 M), respectively, in the presence of 5-TFDMPO (50 mM) in benzene.

tBuOO' trapping: tBuOO' was produced by UV photolysis of a solution of *tert*-butyl hydroperoxide (1.5 м) and 5-TFDMPO (50 mм) in benzene.

Thiyl radical trapping: CH_3CH_2S' , PhS', and *t*BuS' were produced by UV photolysis of the respective dialkyl disulfide (1.5 m) and 5-TFDMPO (50 mm) in benzene.

(SO₃)' trapping: A Fenton system in the presence of NaHSO₃ was used to generate SO₃' ((SO₃H)'). FeSO₄ (0.5 mM) was added to a solution of 5-TFDMPO (50 mM), H₂O₂ (1 mM), and NaHSO₃ (0.1 M) in phosphate buffer (0.1 M, pH 7.4). The EPR spectrum of the corresponding spin adduct was recorded 60 s after the addition of ferrous sulfate.

tert-Butoxyl radical trapping: tBuO' was generated by UV photolysis of a solution of di-tert-butyl peroxide (0.15 μ) and 5-TFDMPO (50 mm) in toluene.

Kinetics of decay of superoxide spin adducts

We used the hypoxanthine/xanthine oxidase system described previously to generate superoxide in phosphate buffer (0.1 M, pH 7.4). The spin-trap concentration was 0.05 M for 5-TFDMPO. The superoxide generation was initiated by incubating xanthine oxidase in the reaction mixture for 7 min and suppressed by adding SOD (1250 U ml⁻¹). The spin-adduct decay was followed by monitoring the decrease in an appropriate line of the spin adduct. Computer simulations were performed using DAPHNIS Labs software developed by R. Lauricella. The signal amplitude at time t_n was calculated from the signal amplitude at time t_{n-1} by using the chosen rate equation. The standard least-squares method was then applied to fit the calculated curves with the experimental ones. In these calculations, the monitored EPR peak intensity is related to the actual radical concentration [SA] by a scale factor. The first-order constant k_a and the product k_b [SA]₀ are independent of this scale factor. Half-lifetimes are given as the mean \pm SD ($n \ge 3$).

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Keywords: EPR spectroscopy · fluorinated ligands · radicals · spin trapping · substituent effects

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