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Deuterated analogues of the free radical trap DEPMPO: synthesis and EPR studies

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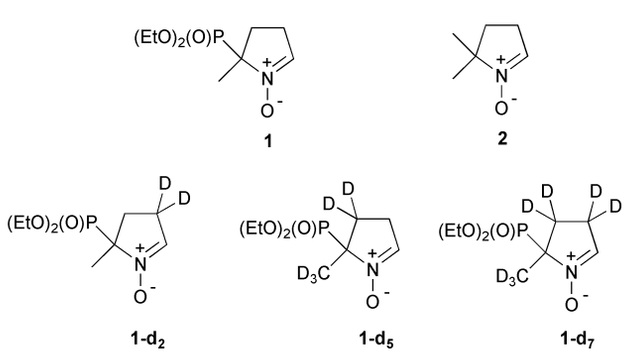
Three analogues of 5-diethoxyphosphoryl-5-methyl-1-pyrroline *N*-oxide (DEPMPO, **1**) labelled with two (**1-d$_2$**), five (**1-d$_5$**) or seven (**1-d$_7$**) ^{2}H were synthesized and used to trap the *tert*-butylperoxyl radical. The EPR spectra of **1-d$_2$**-OOBu^t and **1-d$_7$**-OOBu^t spin adducts exhibited more straightforward patterns and better signal to noise ratio than those obtained with **1** or **1-d$_5$**. The use of the easily available **1-d$_2$** as spin trap could help significantly the analysis of the EPR signals when the signal of either superoxide or alkylperoxyl spin adduct is superimposed with the signals of other spin adducts.

Introduction

Combined with EPR spectroscopy, the spin-trapping technique has been extensively used for the detection and study of free radicals generated in biological milieu. A variety of cyclic nitrones, especially pyrroline *N*-oxides, exhibit particularly interesting properties as spin traps.[1] Among them, 5-diethoxyphosphoryl-5-methyl-1-pyrroline *N*-oxide (DEPMPO, **1**) (Scheme 1) is gaining wider acceptance as a spin trap because of the very characteristic EPR spectra that are observed upon reaction with free radicals.[2] The phosphorus coupling affords valuable information on the trapped radical and gives greater reliability to the EPR spectrum assignments. In biological milieu, the trapping of superoxide (namely the couple $O_2^{\bullet -}$/$HO_2^{\bullet}$, pK_a 4.8[3]) remains a topic of continuing interest for the development of new specific and efficient spin traps. DEPMPO **1** is recognized as presently the most efficient spin trap for superoxide, particularly because of the greater stability of the DEPMPO–OOH adduct compared to the analogous phosphorus free DMPO–OOH adduct obtained with DMPO **2**.[4] Moreover peroxyl radicals are effectively trapped by DEPMPO in water and distinction between peroxyl and alkoxyl spin adducts can be made unambiguously.[5] However the signal patterns observed for alkylperoxyl adducts and also for the superoxide adduct are complex and can hamper the analysis of EPR spectra when different spin adducts are superimposed.



Scheme 1

Addition of the peroxyl radical can occur on both faces of DEPMPO, leading to *trans* (denoted ⇓) or *cis* (denoted ↓) diastereoisomers (Fig. 1). The expected twelve EPR lines of the main signal, the *trans* diastereoisomer resulting from addition of the alkylperoxyl radical on the less hindered face of DEPMPO, exhibit an alternating line width effect and show the

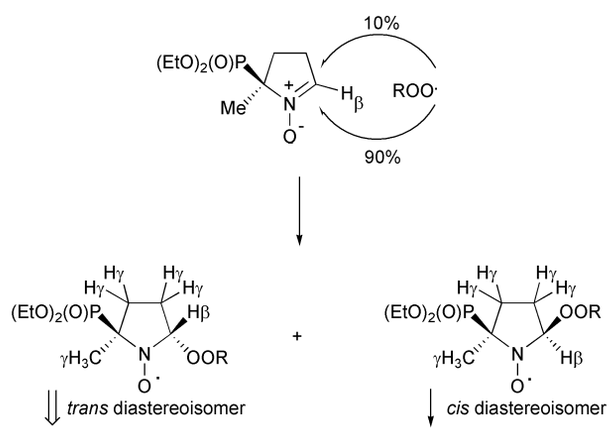


Fig. 1 Radical addition of peroxyl radicals on DEPMPO.

resolution of the long range γ-hydrogen splittings, from the pyrrolidine ring. This phenomenon was assumed to be the result of a conformational exchange (see below). The same type of spectrum was also observed when the superoxide radical anion was trapped by DEPMPO. In order to improve the utility of DEPMPO in spin trapping experiments for the various types of peroxyl or alkylperoxyl radicals, we decided to simplify the EPR spectra of their adducts by replacement of the γ-hydrogen by deuterium. Furthermore, the isotopic substitution of hydrogen by deuterium results in an enhancement of the signal to noise ratio.[6,7] Herein, we report the synthesis of three ^{2}H regiospecifically labelled DEPMPO analogues (Scheme 1) and an EPR study of their use for the spin trapping of *tert*-butylperoxyl radical in toluene.

Results and discussion

Synthesis

The synthesis of DEPMPO **1** can be performed by three routes which have already been reported.[8,9] Two of them proceed *via* the same intermediate, diethyl (2-methylpyrrolidin-2-yl)-phosphonate **3**, which is directly oxidized into DEPMPO. This intermediate **3** can be prepared either by aminophosphonylation of the commercially available 5-chloropentan-2-one **4** or by addition of the appropriate dialkyl phosphite on 2-methylpyrroline **5** (Scheme 2).[8] In the third route, pyrroline *N*-oxides can be prepared through reductive cyclisation of γ-nitroaldehydes with zinc dust in the presence of NH_4Cl or CH_3COOH.[10] Thus reduction and cyclisation of the appropriate diethyl

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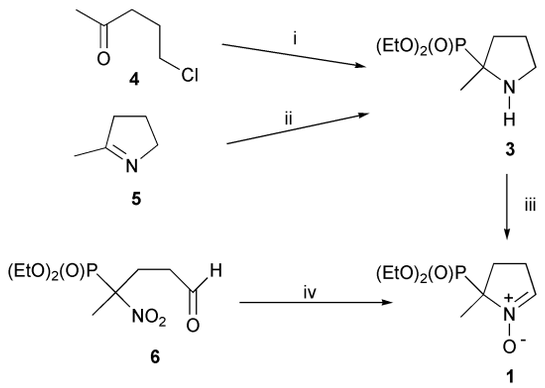
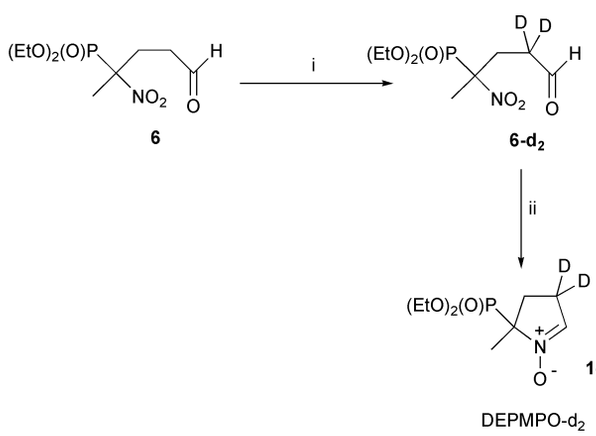


Scheme 2 i: $HP(O)(OEt)_2$, NH_3 gas in EtOH 2.5 h, 60 °C, 60%; ii: $HP(O)(OEt)_2$, 7 d, 25 °C, 98%; iii: *m*-CBPA or PSPO or DMD or oxone or $H_2O_2/NaWO_4$, 39–83%; iv: Zn, CH_3COOH in $EtOH/H_2O$, 3 h, 10 °C, 82%.

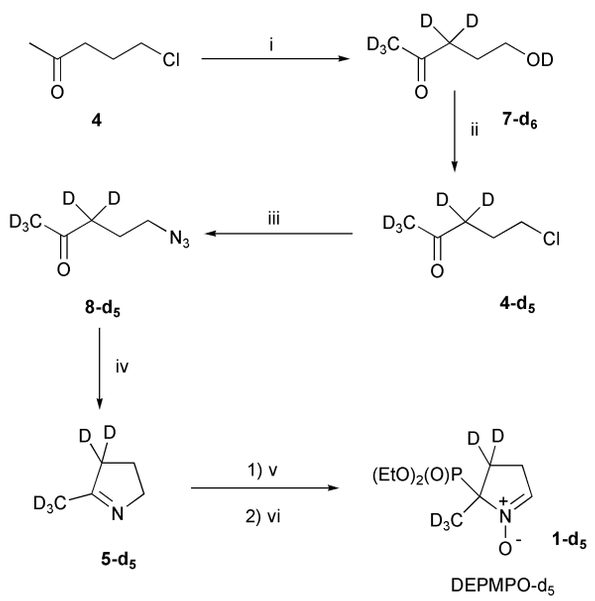
(5-oxo-2-nitropentan-2-yl)phosphonate **6** lead to DEPMPO in good yields (Scheme 2).[9] This flexibility is important for our purpose, as selective hydrogen–deuterium exchange on properly chosen starting materials can lead to different selectively deuterated nitrones. To remove the long range couplings, the various possibilities are to prepare DEPMPO derivatives which will contain deuterium atoms on the C-3, C-4 positions as well as on the 5-methyl substituent. On the other hand, it was considered important to keep present the β-hydrogen atoms on the C-2 centre (see Fig. 1) to avoid the dilution of the EPR signal and to conserve the splitting information obtained in spin-trapping experiments.[7] Therefore, our selection of deuterated nitrones was : a) **1-d$_2$**, the C-3 deuterated compound, b) **1-d$_5$**, the analogue deuterated on C-4 and on the methyl group on C-5, and c) **1-d$_7$**, the γ-perdeuterated compound.

To prepare **1-d$_2$**, the d_2-DEPMPO analogue selectively deuterated on the C-3 carbon, we applied the route involving reductive cyclisation of γ-nitroaldehydes. The required phosphonylated γ-nitroaldehyde **6** was prepared by Michael addition of 1-nitro-1-diethylphosphonoethane on acrolein.[9,11] Then, reaction with deuterium oxide in the presence of pyridine, acting both as co-solvent and a base, led to deuteration of the enolizable α-protons of the aldehyde function. Finally, the ^2H labelled γ-nitroaldehyde **6-d$_2$** was reduced by zinc in the presence of deuterated acetic acid in $EtOD/D_2O$ to afford directly [3-$^2H_2$]DEPMPO (**1-d$_2$**) in good yield (79%; Scheme 3).



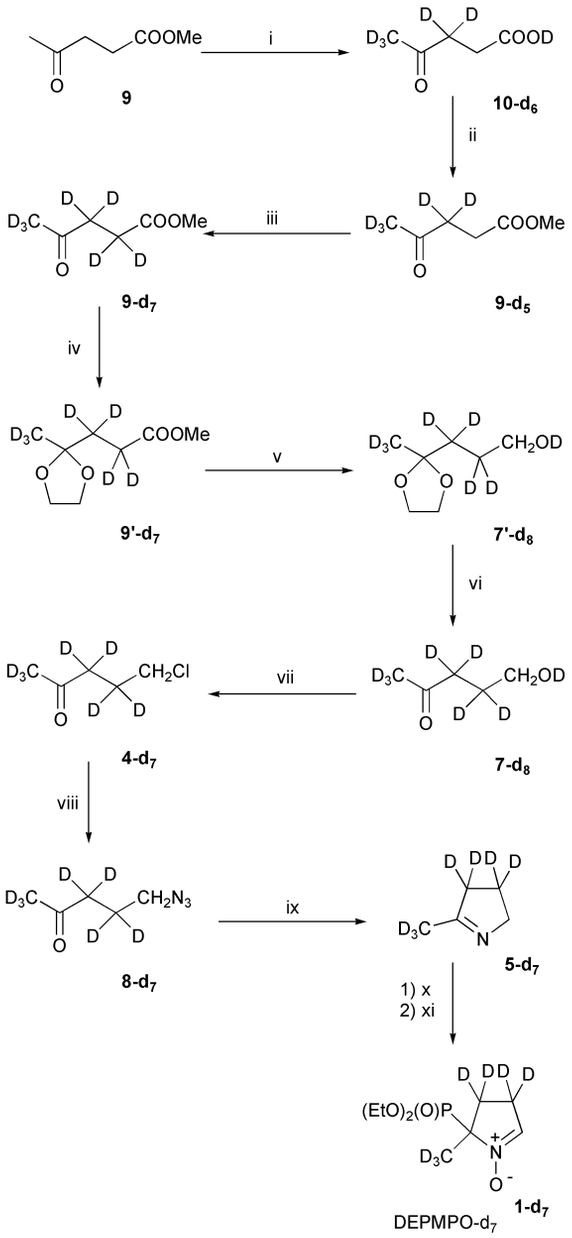
Scheme 3 i: D_2O, pyridine, 18 h, 20 °C, 84%; ii: Zn/CH_3COOD in $EtOD/D_2O$, 3 h, 10 °C, 79%.

For the synthesis of the pentadeutero analogue (**1-d$_5$**), a different strategy was followed. Deuterium exchange performed on 5-chloropentan-2-one **4** will give the correctly deuterated carbon skeleton, which then needs further straightforward modifications to reach the target. Complete ^2H exchange on the α-positions of the keto function of 5-chloropentan-2-one occurred by treatment of **4** in boiling deuterium oxide in the presence of K_2CO_3. The reaction was repeated three times to ensure satisfactory isotopic purity. However, under the conditions used for the exchange reaction, the haloketone **4** was converted progressively into the deuterated ketoalcohol **7-d$_6$**. Chloration of the alcohol **7-d$_6$** by reaction with PPh_3 and CCl_4 led to ^2H labelled 5-chloropentan-2-one **4-d$_5$** in a 26% overall yield without diminution of the isotopic purity.[12] Subsequently, synthesis of the deuterated 2-methylpyrroline **5-d$_5$** was achieved in two steps. In the first one, the chlorine group was replaced by an azido group through nucleophilic substitution by NaN_3 in heterogeneous medium in the presence of a catalytic amount of tetra-*n*-butylammonium chloride.[13] In the second step, the ketoazide **8-d$_5$** was treated with triphenylphosphane in an "Aza–Wittig" type reaction to give the pyrroline **5-d$_5$**.[14] DEPMPO-d_5 was then obtained in two steps by addition of deuterated diethyl phosphite [$(EtO)_2$POD] followed by oxidation of the α-aminophosphonate **3-d$_6$** with dimethyldioxirane (DMD) in acetone (Scheme 4).



Scheme 4 i: K_2CO_3 in D_2O, 15 h, reflux, 3 times, 37%; ii: PPh_3 in CCl_4, 30 min, 80 °C, 70%; iii: NaN_3 in DME, cat. *n*-Bu_4NCl, 2 h, reflux, 98%; iv: PPh_3 in Et_2O, 4 h, reflux, 61%; v: $DP(O)(OEt)_2$, 7 d, 25 °C, 97%; vi: DMD in acetone, 2 h, 0 °C, 65%.

To prepare the third compound **1-d$_7$**, the γ-perdeuterated DEPMPO compound, the 2-methylpyrroline **5** was again considered as the key intermediate. To obtain this conveniently deuterated cyclic imine **5-d$_7$**, the sequence started with methyl 4-oxopentanoate **9** which has all the seven protons in enolizable positions. However to achieve perdeuteration of the α-positions of an ester, the bases need to be stronger than those required for the enolization–deuteration of a ketone or an aldehyde. In the first step, the protons α to the ketone function were replaced by deuterium by treatment of **9** in alkaline deuterium oxide at 60 °C (Scheme 5). However, under these conditions, saponification of the methyl ester was an unavoidable side reaction. Thus, the ketoacid **10-d$_6$** was esterified with MeOD to give the pentadeuterated ketoester **9-d$_5$**. Reaction of **9-d$_5$** with sodium methylate in MeOD gave the perdeuterated methyl levulinate **9-d$_7$**. To reach the chloroketone **4-d$_7$**, the keto group of **9-d$_7$** was protected as a dioxolanyl derivative, and the ester group was reduced with $LiAlH_4$. Deprotection of the carbonyl by treatment with DCl/D_2O led to alcohol **7-d$_8$** which was converted by PPh_3 and CCl_4 to the heptadeuterated haloketone **4-d$_7$**. The last steps afforded selectively the deuterated 2-methylpyrroline which led, after addition of deuterated diethyl phosphite followed by oxidation with dimethyldioxirane, to the γ-perdeuterated compound DEPMPO-d_7 (**1-d$_7$**).



Scheme 5 i: NaOD in D_2O, 12 h, 60 °C, 3 times, 92%; ii: CH_3OD, D_2SO_4, 24 h, reflux, 75%; iii: Na in CH_3OD, 1 h, reflux, twice, 60%; iv: $DOCH_2$–CH_2OD in benzene, cat. PTSA, 12 h, reflux; v: $LiAlH_4$ in THF, 3 h, reflux, 97%; vi: DCl in D_2O, 12 h, 20 °C, 95%; vii: PPh_3 in CCl_4, 30 min, 80 °C, 68%; viii: NaN_3 in DME, cat. Bu_4NCl, 2 h, reflux, 99%; ix: PPh_3 in Et_2O, 4 h, reflux, 65%; x: $DP(O)(OEt)_2$, 7 d, 25 °C, 96%; xi: DMD in acetone, 2 h, 0 °C, 63%.

EPR studies

When Bu^{t}OOH was photolysed in the presence of DEPMPO **1** in degassed toluene, the spectrum shown in Fig. 2a was obtained. This spectrum is a superimposition of two different signals which can be attributed to the spin adducts arising from the addition of Bu^{t}OO$^\bullet$ on both faces of the nitrone **1** (see Fig. 1, R = Bu^t). The major signal (⇓) was attributed to the *trans* diastereoisomer which results from addition of Bu^{t}OO$^\bullet$ to the less hindered face of the molecule and the minor signal (↓) was attributed to the *cis* diastereoisomer. Both diastereoisomers should exhibit twelve equivalent EPR lines resulting from couplings with the nitrogen, hydrogen and phosphorus atoms. The signal of the *cis* diastereoisomer (noted ↓) is indeed composed of twelve lines (a_N = 1.28, a_P = 3.92, a_H = 0.99 mT), which are further splitted by a weak a_{H_γ} coupling with a pyrrolidine ring hydrogen (a_{H_γ} = 0.16 mT). The EPR signal of the *trans* diastereoisomer (⇓) also consists of twelve main lines and,

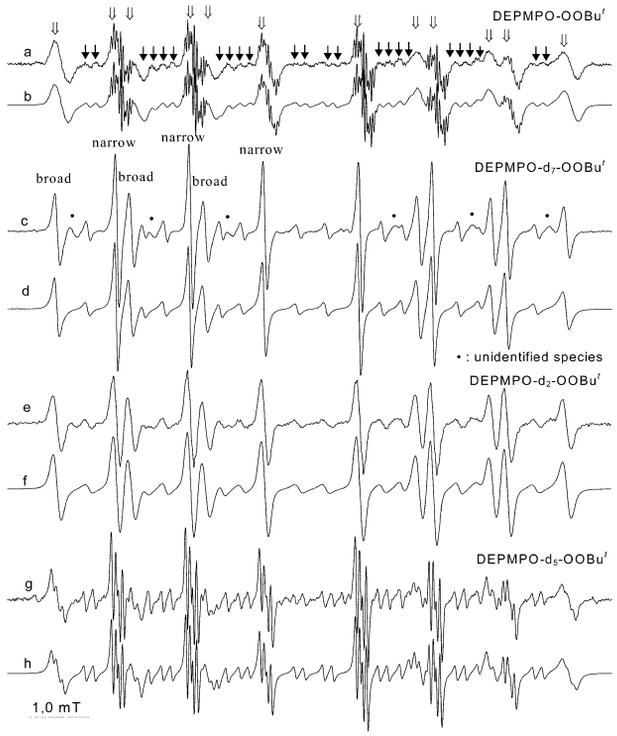


Fig. 2 EPR spectra of DEPMPO-OOBu^t spin adducts formed by photolysis of Bu^{t}OOH in toluene, (a) in the presence of DEPMPO, (b) calculated spectrum; (c) in the presence of DEPMPO-d_7, (d) calculated spectrum; (e) in the presence of DEPMPO-d_2, (f) calculated spectrum; (g) in the presence of DEPMPO-d_5, (h) calculated spectrum. Settings: modulation amplitude 0.01 mT, time constant 0.128 s, (a), (e), (g) scan time 240 s, (c) scan time 480 s, gain 5×10^4, microwave power 10 mW.

on the other hand, it shows an alternating line width and only the narrow lines exhibit a clear resolution of long range hydrogen couplings. This pattern has been observed for the spin adducts of either Bu^{t}OO$^\bullet$ or superoxide with all the DEPMPO analogues that we have studied.[9,15] We suggest that this alternating line width is generated by a hindered rotation around the O–O peroxyl bond, since this phenomenon was observed only in the case of the ROO$^\bullet$ (R = H, alkyl) spin adducts. Rotation around the peroxyl bond induces conformational changes within the five-membered ring, resulting in the existence of two chemical sites which are composed of fastly exchanging conformers or mean conformers and which exhibit significantly different mean values of the coupling constants. A satisfactorily computed spectrum was only obtained when assumption of a two site chemical exchange imitating the conformational exchange was considered (Fig. 2b) (for parameters see Table 1).[16] The detailed conformational study of this chemical exchange is now in progress and will be reported in a forthcoming paper. The introduction of all the γ-hydrogen splittings was required for an adequate simulation of the signal of the major *trans* diastereoisomer spin adduct **1-OOBu^t**. The main γ-coupling was slightly different for the two exchanging sites (0.11–0.12 mT) while the other couplings were kept unchanged (5 a_{H_γ} = 0.04 mT; 1 a_{H_γ} = 0.06 mT).

The spectrum of the DEPMPO-d_7-OOBu^t adduct (Fig. 2c), observed after photolysis of Bu^{t}OOH in the presence of perdeuterated **1-d$_7$** (DEPMPO-d_7), shows a significant simplification of the EPR signal. The EPR spectra of the two diastereoisomers (↓ *cis*, ⇓ *trans*) are now clearly distinguishable (Fig. 2c). The important decrease of the γ-splittings resulting from ^2H isotope substitution leads to the expected decrease of the width of the EPR lines and to a significant improvement of the signal-to-noise ratio. The minor signal attributed to the *cis* diastereoisomer exhibits 12 equivalent EPR lines due to the

Table 1 Hyperfine coupling constants for Bu^{t}OO$^\bullet$ adducts in toluene

Adduct	Isomer	a_N/mT	a_P/mT	$a_{H\beta}$/mT	$a_{H\gamma}$/mT	$a_{H\gamma}$/mT	%	%	Exchange Cst. × 10^{-6}/s^{-1}
DEPMPO-OOBu^t	*trans*	1.22 1.21	5.04 4.78	1.0 0.82	0.11 0.12	0.04 × 3; 0.04 × 2; 0.06 × 1	72.6 27.4	93.5	5.81
	cis	1.28	3.92	0.99	0.16			6.5	
DEPMPO-d$_2$-OOBu^t	*trans*	1.22 1.22	5.06 4.74	1.0 0.85	— —	0.04 × 3; 0.04 × 2; —	77.2 22.8	88.8	8.68
	cis	1.27	3.92	0.94	—			11.2	
DEPMPO-d$_5$-OOBu^t	*trans*	1.23 1.21	5.06 4.78	1.00 0.84	0.11 0.12	—; —; 0.07 × 1	69.4 30.6	92.8	8.43
	cis	1.27	3.94	0.94	0.15			7.2	
DEPMPO-d$_7$-OOBu^t	*trans*	1.22 1.21	5.06 4.77	1.0 0.89	— —	—; —; —	71.9 28.1	90.3	8.54
	cis	1.27	3.94	0.92	—			9.7	

following couplings : a_N = 1.27 mT, a_P = 3.94 mT, $a_{H\beta}$ = 0.92 mT without further Hγ splitting. The line width effect stated above for the major *trans* diastereoisomer is easily observable and pointed out in Fig. 2c. Careful examination of the EPR spectrum (Fig. 2c) shows also the presence of an unidentified species (marked ·). In order to avoid a loss in the isotopic purity, the DEPMPO-d$_7$ was not purified by chromatography. This contaminant species (·) may result from the presence of a by-product of over-oxidation formed during the oxidation of **3-d$_8$** to **1-d$_7$**, which remained as a trace in DEPMPO-d$_7$.

The EPR spectrum of DEPMPO-d$_2$-OOBu^t (Fig. 2e) obtained under the same conditions is similar to the one obtained with the perdeuterated DEPMPO-d$_7$. An important simplification is gained from the ^{2}H substitution of the hydrogen atoms at the C-3 position. However, the observed line widths are larger as a result of the presence of the remaining γ-hydrogen couplings (methyl group and two hydrogen atoms at C-4 position).

By contrast, no significant improvement was obtained in the case of the EPR signal of the DEPMPO-d$_5$-OOBu^t adduct (Fig. 2g). Although the disappearance of the five γ-hydrogen splittings gave narrower EPR line width, the EPR signal remained still as complex as the one obtained with the non-deuterated DEPMPO.

Conclusion

In the present work we have synthesized three different selectively deuterated DEPMPO derivatives in order to simplify the EPR spectra of the peroxyl adducts of DEPMPO. No major simplification was obtained for the EPR signal of DEPMPO-d$_5$-OOBu^t. Despite the fact that disappearance of the five γ-proton splittings diminished the EPR line width, the signal was still complex and diluted. By contrast, the use of DEPMPO-d$_7$ greatly simplified the EPR spectrum of the corresponding *tert*-butylperoxyl adduct. Moreover total ^{2}H substitution also can lead to a considerable increase in the EPR signal intensity of the superoxide adduct which can be useful for *in vivo* detection. Interestingly, ^{2}H substitution of only the two γ-hydrogen atoms at the C-3 position removes two important couplings and allows a significant simplification of the EPR spectrum of the DEPMPO-d$_2$-OOBu^t adduct. The use of the easily available **1-d$_2$** could help significantly the analysis of EPR spectra when the signals of either superoxide or alkylperoxyl spin adduct are superimposed with the signals of other spin adducts.

Experimental

All chemicals and organic solvents were commercially available and used without further purification. Melting points were measured on a Büchi 535 apparatus and are uncorrected. ^{31}P-NMR spectra were recorded on a Bruker AC 100 at 40.53 MHz, with 85% H_3PO_4 as an external reference. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker AC 100 or AC 200 at 100 or 200 MHz and 25 or 50 MHz respectively. Chemical shifts (δ) are reported in ppm for solutions in $CDCl_3$, unless otherwise stated, with Me_4Si as an internal reference and J values are given in Hz. EPR spectra were recorded on a Varian E 109 spectrometer. Spin-trapping of Bu^{t}OO$^\bullet$ has been achieved from photolysis of Bu^{t}OOH (1.5 M) in toluene in presence of the trap (100 mM). The solutions were degassed by freeze–pump–thaw cycles before photolysis. The spectrometer settings are given in the relevant figure.

Analysis of deuteration

The decrease of the intensities of the ^{1}H-NMR signal of exchanging protons has been considered as a probe for exchanging rate. Quantitative estimations were carried out by comparing non-exchanging proton signal intensities with those of exchanging proton. The isotopic purity was considered as sufficient (estimated >95%) when exchanging proton signals disappeared from the ^{1}H-NMR spectrum. Absence of ^{13}C signals for carbon bearing deuterium was another probe used for non-quantitative determination of deuteration.

Diethyl phosphite-d$_1$

A mixture of triethyl phosphite (11.1 g, 66.8 mmol) and D_2O (2 g, 100 mmol) in dry CH_3CN (10 mL) was refluxed for 2 h. After concentration under reduced pressure, the residue was distilled to afford diethyl phosphite-d$_1$ (7 g, 75%); bp 50–51 °C/3 mmHg; δ_P 6.1 (t, J_{P-D} 106); δ_H (100 MHz) 1.39 (6H, t, J 7.1), 4.13 (2H, q, J 7.0, J_{H-P} 7.1) and 4.22 (2H, q, J 7, J_{H-P} 7).

1,2-Ethanediol-d$_2$

A mixture of 2,2-dimethyl-1,3-dioxolane (7.6 g, 74.5 mmol) and DCl (5%) in D_2O solution (50 mL) was refluxed for 15 h. Water was removed under reduced pressure and the oily residue was distilled under reduced pressure (4 g, 83%); bp 100 °C/30 mmHg; δ_H (100 MHz) 3.70 (s).

Synthesis of 5-diethoxyphosphoryl-5-methyl-[4,4-$^2H_2$]-1-pyrroline *N*-oxide 1-d_2 (DEPMPO-d_2)

Diethyl 5-oxo-[4,4-$^2H_2$]-2-nitropentan-2-ylphosphonate 6-d_2. A mixture of **6**[9] (2 g, 7.5 mmol), pyridine (2 g, 25.3 mmol) and D_2O (6 mL) was stirred for 18 h at room temperature. The mixture was then acidified at pH 5–6 with D_2SO_4 and rapidly extracted with dry Et_2O (3 × 15 mL). The combined organic phases were dried over Na_2SO_4 and then concentrated under reduced pressure. The title compound **6-d_2** was obtained as a yellow oil (1.7 g, 84%); δ_P (C_6D_6) 15.9; δ_H (C_6D_6, 100 MHz) 1.0 (6H, t, *J* 6.9), 1.49 (3H, d, J_{H-P} 14), 2.22 (1H, dd, *J* 10, J_{H-P} 15.6), 2.60 (1H, dd, *J* 10, J_{H-P} 15.6), 3.7–4.1 (4H, m) and 9.07 (1H, d, *J* 1).

5-Diethoxyphosphoryl-5-methyl-[3,3-$^2H_2$]-1-pyrroline *N*-oxide 1-d_2 (DEPMPO-d_2). Acetic acid-d_1 (1.6 g, 26.2 mmol) was slowly added over 1.5 h at 10 °C to a mixture of **6-d_2** (1.72 g, 6.4 mmol) and zinc (0.844 g, 12.9 mmol) in ethanol-d_1/D_2O (19 mL) (18/1). The mixture was then stirred for 1.5 h at 25 °C. CH_2Cl_2 (50 mL) was added, the mixture was filtered off and concentrated under reduced pressure. The residue was poured into D_2O (20 mL), washed with dry Et_2O (4 × 15 mL), saturated with NaCl and extracted with dry $CHCl_3$ (3 × 15 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was dissolved in D_2O (20 mL). The aqueous phase was saturated with NaCl, extracted with dry Et_2O (4 × 10 mL) and extracted with dry $CHCl_3$ (3 × 10 mL). DEPMPO-d_2 was obtained as a yellow oil (1.2 g, 79%). The resulting organic phases were combined and dried over Na_2SO_4 and concentrated under reduced pressure to give DEPMPO-d_2; δ_P 21.6; δ_H (100 MHz) 1.36 (6H, t, *J* 7.1), 1.69 (3H, d, *J* 14.2), 1.9–2.1 (1H, m), 2.6–3.1 (1H, m), 3.9–4.5 (4H, m) and 6.91 (1H, d, J_{H-P} 2.8).

Synthesis of 5-diethoxyphosphoryl-5-([$^2H_3$]methyl)-[3,3-$^2H_2$]-1-pyrroline *N*-oxide (DEPMPO-d_5) 1-d_5

[1,1,1,3,3-$^2H_5$]-5-Deuteroxypentan-2-one 7-d_6. A mixture of 5-chloropentan-2-one **4** (18 g, 149 mmol) and K_2CO_3 (10.5 g, 76 mmol) was refluxed in D_2O (20 g, 1 mol) for 15 h. The aqueous solution was then cooled at 25 °C, saturated with NaCl and extracted with dry CH_2Cl_2 (4 × 40 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The isotopic exchange was repeated again twice. **7-d_6** was obtained as a yellow oil (6 g, 37%); δ_H (200 MHz) 1.82 (2H, t, *J* 6) and 3.7 (2H, t, *J* 6.1).

[1,1,1,3,3-$^2H_5$]-5-Chloropentan-2-one 4-d_5. A solution of **7-d_6** (6 g, 55.6 mmol) in dry CCl_4 (13 mL) was added dropwise to a solution of triphenylphosphane (16.3 g, 62.2 mmol) in dry CCl_4 (5 mL). At the beginning of the addition the mixture was heated at 65 °C and temperature was then left to reach 80 °C. The mixture was heated at 80 °C for 30 min and then cooled and concentrated under reduced pressure. Dry pentane (80 mL) was added and the mixture was stirred for 30 min and filtered off. The precipitate was washed with dry pentane (2 × 15 mL). The organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. Distillation under reduced pressure led to **4-d_5** (4.9 g, 70%); bp 70 °C/19 mmHg; δ_H (100 MHz) 2.04 (2H, t, *J* 6.1) and 3.58 (2H, t, *J* 6.2).

[1,1,1,3,3-$^2H_5$]-5-Azidopentan-2-one 8-d_5. A mixture of NaN_3 (5.5 g, 84.6 mmol), **4-d_5** (4.9 g, 39 mmol), tetra-*n*-butylammonium chloride (0.2 g, 0.72 mmol) in dry DME (30 mL) was refluxed for 2 h. After cooling, dry Et_2O (30 mL) was added and the mixture was filtered off. The resulting precipitate was washed with dry Et_2O (2 × 10 mL). The combined organic phases were concentrated under reduced pressure to give **8-d_5** (5.1 g, 99%) as a colourless oil; δ_H (100 MHz) 1.86 (2H, t, *J* 6.6) and 3.34 (2H, t, *J* 6.6).

2-[$^2H_3$]Methyl-[3,3-$^2H_2$]-1-pyrroline 5-d_5. Triphenylphosphane (11.3 g, 43.1 mmol) in dry Et_2O (75 mL) was added dropwise to a solution of **8-d_5** (5.1 g, 38.6 mmol) in dry Et_2O (5 mL). The mixture was refluxed for 4 h. After cooling, dry pentane was added (50 mL), the mixture was stirred for 30 min and filtered off. The precipitate was washed with dry pentane (2 × 10 mL). The organic phases were collected, dried over Na_2SO_4 and the solvents distilled under reduced pressure. The resulting oily residue was distilled under reduced pressure to lead to **5-d_5** (2.1 g, 61%); bp 50–51 °C/115 mmHg; δ_H (200 MHz) 1.86 (2H, t, *J* 7.6) and 3.79 (2H, t, *J* 7.4).

Diethyl 2-[$^2H_3$]methyl-[3,3-$^2H_2$]pyrrolidin-2-ylphosphonate 3-d_6 (DEPMP-d_6). Diethyl phosphite-d_1 (1.58 g, 11.3 mmol) and **5-d_5** (1 g, 11.3 mmol) were stirred for 7 days at 25 °C. The crude mixture was poured into D_2O (20 mL), acidified to pH 1 with a DCl (20%) aqueous solution. The aqueous phase was washed with dry CH_2Cl_2 (2 × 20 mL), saturated with NaCl, alkalinised with dry K_2CO_3 to pH 9 and extracted with dry $CHCl_3$ (4 × 30 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure to afford **3-d_6** as a colourless oil (2.5 g, 97%); δ_P 30.2; δ_H (200 MHz) 1.27 (6H, t, *J* 7), 1.5–1.9 (2H, m), 2.8–3.1 (2H, m) and 4.11 (4H, m, *J* 7, J_{H-P} 7).

5-Diethoxyphosphoryl-5-[$^2H_3$]methyl-[4,4-$^2H_2$]-1-pyrroline *N*-oxide 1-d_5 (DEPMPO-d_5). An acetone solution of dimethyldioxirane (75 mL, 0.06 mol L^{-1}) was added dropwise at 0 °C over 2 h to a solution of **3-d_6** (0.5 g, 2.25 mmol) in acetone (10 mL). The mixture was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by column chromatography (CH_2Cl_2/EtOH, 90/10) to give DEPMPO-d_5 as a yellow oil (0.35 g, 65%); δ_P 21.6; δ_H (C_6D_6, 200 MHz) 0.76 (3H, t, *J* 6.8), 0.88 (3H, t, *J* 6.8), 1.3–1.5 (1H, m), 1.9–2.1 (1H, m), 3.5–3.8 (2H, m), 4.0–4.3 (4H, m) and 6.91 (1H, q, *J* 2.8, J_{H-P} 2.8).

Synthesis of 5-diethoxyphosphoryl-5-[$^2H_3$]methyl-[3,3,4,4-$^2H_4$]-1-pyrroline *N*-oxide 1-d_7 (DEPMPO-d_7)

4-Oxo-[3,3,5,5,5-$^2H_5$]pentanoic acid 10-d_6. A mixture of methyl 4-oxopentanoate **9** (11.4 g, 100 mmol), NaOD/D_2O (10 mL of a 8.65 M solution) in D_2O (80 mL) was stirred at 60 °C for 12 h. The aqueous solution was cooled and concentrated under reduced pressure. The isotopic exchange was repeated again twice (D_2O: 50 mL and 30 mL). The aqueous solution was acidified to pH 1 with D_2SO_4 and continuously extracted with dry Et_2O (150 mL) for 12 h. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to give **10-d_6** as a white powder (9.2 g, 92%); mp 33 °C; δ_H (100 MHz) 2.6 (2H, s); δ_C (25.1 MHz) 27.6, 177.9 and 207.6.

Methyl 4-oxo-[3,3,5,5,5-$^2H_5$]pentanoate 9-d_5. 10-d_6 (11.2 g, 91.7 mmol) in CH_3OD (50 mL) in the presence of D_2SO_4 (0.5 mL) was refluxed for 24 h. CH_3OD was distilled and the residue was poured in dry CH_2Cl_2 (150 mL). The organic phase was washed with brine (NaCl/D_2O) (30 mL) containing K_2CO_3 (5%), dried over Na_2SO_4 and concentrated under reduced pressure to give **9-d_5** as a colourless oil (9.3 g, 75%); δ_H (100 MHz) 2.57 (2H, s) and 3.68 (3H, s).

Methyl 4-oxo-[2,2,3,3,5,5,5-$^2H_7$]pentanoate 9-d_7. Metallic sodium (0.6 g, 26 mmol) was carefully added to CH_3OD (50 mL). After complete dissolution, **9-d_5** (8 g, 59.2 mmol) was added and the mixture was refluxed for 1 h. After cooling, a DCl/D_2O solution (20%) was added until neutralisation. The resulting mixture was concentrated under reduced pressure, saturated with NaCl and extracted with dry Et_2O (50 mL). The organic phase was washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure. The isotopic exchange

was repeated once with CH_3OD (30 mL) to afford **9-d$_7$** as a colourless oil (4.9 g, 61%); δ_H (100 MHz) 3.65 (s); δ_C (25.1 MHz) 51, 172.6 and 206.5.

Methyl 4-(1,3-dioxolan-2,2-diyl)-[2,2,3,3,5,5,5-$^2H_7$]pentanoate 9′-d$_7$. A mixture of **9-d$_7$** (4.9 g, 35.7 mmol), $DOCH_2$–CH_2OD (2.5 g, 39.3 mmol) and a catalytic amount of *para*-toluenesulfonic acid in dry benzene (20 mL) was refluxed for 12 h in a Dean–Stark apparatus. The solvent was removed under reduced pressure and the residue was distilled. The product **9′-d$_7$** was obtained as a colourless oil (4.5 g, 69%); bp 78–80 °C/8 mmHg; δ_H (100 MHz) 3.67 (3H, s) and 3.93 (4H, s).

[1,1,1,3,3,4,4-$^2H_7$]-5-Deuteroxypentan-2-one ethylene acetal 7′-d$_8$. A solution of **9′-d$_7$** (4.5 g, 24.8 mmol) in dry THF (45 mL) was slowly added to a mixture of $LiAlH_4$ (1.13 g, 29.7 mmol) in dry THF (25 mL), the temperature being kept below 40 °C. The mixture was then refluxed for 3 h. After cooling at 0 °C, a 1 : 1 mixture of D_2O/THF (30 mL) was carefully added and the resulting mixture was extracted with Et_2O (3 × 100 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The acetal **7′-d$_8$** was obtained as a colourless oil (3.7 g, 97%); δ_H (100 Mz) 3.62 (2H, s) and 3.96 (4H, s).

[1,1,1,3,3,4,4-$^2H_7$]-5-Deuteroxypentan-2-one 7-d$_8$. A solution of DCl in D_2O (3 mL of a 4% solution) was added to a solution of **7′-d$_8$** (3.7 g, 24 mmol) in THF (100 mL) and stirred at 20 °C for 12 h. The mixture was neutralised by adding a saturated solution of K_2CO_3 in D_2O. The resulting mixture was saturated with NaCl and extracted with dry Et_2O (50 mL). The organic phase was separated, dried over Na_2SO_4 and concentrated under reduced pressure to give **7-d$_8$** as a colourless oil (2.5 g, 95%); δ_H (100 MHz) 3.7 (s).

[1,1,1,3,3,4,4-$^2H_7$]-5-Chloropentan-2-one 4-d$_7$. 7-d$_8$ (2.5 g, 22.7 mmol) in dry CCl_4 (10 mL) was rapidly added to triphenylphosphane (6.75 g, 25.75 mmol) in dry CCl_4 (2 mL). The mixture was warmed at 65 °C at the beginning of the addition and then was left to reach 80 °C. After heating at 80 °C for 30 min and subsequent cooling at 25 °C, the solvent was removed under reduced pressure. Pentane (30 mL) was added and the mixture was stirred for 30 min and filtered off. The precipitate was washed with dry pentane (2 × 10 mL). The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. **4-d$_7$** was obtained as a colourless oil (2 g, 68%); δ_H (100 MHz) 3.58 (s).

[1,1,1,3,3,4,4-$^2H_7$]-5-Azidopentan-2-one 8-d$_7$. A mixture of NaN_3 (2 g, 31.4 mmol), **4-d$_7$** (2 g, 15.7 mmol) and tetra-*n*-butylammonium chloride (50 mg, 0.18 mmol) in dry DME (10 mL) was refluxed for 2 h. After cooling at 25 °C, dry Et_2O (15 mL) was added. The mixture was filtered off and the precipitate was washed with dry Et_2O (2 × 10 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. **8-d$_7$** was obtained as a colourless oil (2.05 g, 98%); δ_H (100 MHz) 3.34 (s).

2-[$^2H_3$]Methyl-[3,3,4,4-$^2H_4$]-1-pyrroline 5-d$_7$. A solution of triphenylphosphane (4.4 g, 16.9 mmol) in dry Et_2O (30 mL) was added dropwise to a solution of **8-d$_7$** (2 g, 15.1 mmol) in dry Et_2O (2 mL). After the addition, the mixture was refluxed for 4 h. After cooling at room temperature, dry pentane (20 mL) was added and the mixture stirred for 30 min and filtered off. The precipitate was washed with dry pentane (2 × 10 mL). The organic phase was dried over Na_2SO_4 and distilled. The oily residue was distilled under reduced pressure to yield **5-d$_7$** as a colourless oil (0.88 g, 65%); bp 50–51 °C/115 mmHg); δ_H (200 MHz) 3.77 (s).

Diethyl 2-[$^2H_3$]methyl-[3,3,4,4-$^2H_4$]pyrrolidin-2-ylphosphonate 3-d$_8$ (DEPMP-d$_8$). A mixture of diethyl phosphite-d$_1$ (1.35 g, 9.8 mmol) and **5-d$_7$** (0.88 g, 9.8 mmol) was stirred for 7 days at 25 °C. The mixture was poured into D_2O (20 mL), acidified to pH 1 with DCl (20%) D_2O solution. The aqueous phase was washed with dry CH_2Cl_2 (2 × 20 mL), saturated with NaCl, basified to pH 9 and extracted with dry $CHCl_3$ (4 × 30 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. **3-d$_8$** was obtained as a colourless oil (2.15 g, 96%); δ_P 30.2; δ_H (200 MHz) 1.32 (6H, t, *J* 6.8), 2.95 (1H, d, *J* 10), 3.09 (1H, d, *J* 10) and 4.16 (4H, m).

5-Diethoxyphosphoryl-5-[$^2H_3$]methyl-[3,3,4,4-$^2H_4$]-1-pyrroline *N*-oxide 1-d$_7$ (DEPMPO-d$_7$). An acetone solution of dimethyldioxirane (77 mL of a 0.055 M solution) was added dropwise at 0 °C for 2 h to **3-d$_8$** (0.5 g, 2.05 mmol) in acetone (10 mL). The mixture was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was poured into D_2O (10 mL) and washed with Et_2O (4 × 10 mL), saturated with NaCl and extracted with $CHCl_3$ (3 × 10 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. **1-d$_7$** was obtained as a yellow oil (0.310 g, 63%). The resulting residue was dissolved in D_2O (5 mL). The aqueous phase was saturated with NaCl, extracted with dry Et_2O (4 × 2 mL) and extracted with dry $CHCl_3$ (3 × 5 mL). The resulting organic phases were combined and dried over Na_2SO_4 and concentrated under reduced pressure to give DEPMPO-d$_7$; δ_P 21.6; δ_H (C_6D_6, 200 MHz) 0.77 (3H, t, *J* 6.7), 0.89 (3H, t, *J* 6.7), 3.5–3.8 (2H, m), 4.0–4.3 (2H, m) and 6.92 (1H, d, J_{H-P} 2.7).

Abbreviations

DMD	Dimethyldioxirane
PSPO	2-Phenylsulfonyl-3-phenyloxaziridine
DME	1,2-Dimethoxyethane
PTSA	*para*-Toluenesulfonic acid
m-CPBA	*m*-Chloroperbenzoic acid
Oxone	$2KHSO_5{\cdot}KHSO_4{\cdot}K_2SO_4$

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