Free Rad. Res. Comms., Vol. 19 Supplement, pp. S23-S32
Reprints available directly from the publisher
Photocopying permitted by license only

β-PHOSPHORYLATED FIVE MEMBERED RING NITROXIDES. SYNTHESIS AND EPR STUDY

L. DEMBKOWSKI, J.P. FINET, C. FRÉJAVILLE, F. LE MOIGNE, R. MAURIN, A. MERCIER, P. PAGES, P. STIPA and P. TORDO*

Université de Provence, CNRS URA 1412, Radicaux Libres et Synthèse, case 521, Av. Escadrille Normandie-Niemen, 13397 Marseille Cedex 13 France

A series of stable β-phosphorylated five membered ring nitroxides was prepared by intramolecular aminomercuration of alkenyl α-aminophosphonates. The structure of these nitroxides was deduced from their ^{13}C and ^{31}P coupling constants and from force field calculations.

KEY WORDS: β-phosphorylated nitroxides; α-aminophosphonates; intramolecular aminomercuration; 1-pyrroline-N-oxides; EPR.

INTRODUCTION

Stable aminoxyl radicals continue to play a central role in a wide variety of spin-labelling[1-2] and spin trapping applications[2,3]. More recently novel applications have arisen in which nitroxides are being investigated as contrast-enhancing agents for magnetic resonance imaging (MRI)[1d,1f,2,4]. In order to prevent a disproportionation reaction[5] almost all the reported stable nitroxides bear quaternary sp^3 hybridized carbon atoms adjacent to the nitroxide group. In non viscous solution these nitroxides exhibit isotropic three line EPR spectra, resulting from the coupling of the unpaired electron with the nitrogen atom. For a spin-labelled system most of the information is gained by analyzing the changes in the EPR spectrum of the attached nitroxide relative to its isotropic spectrum[1c]. These changes depend on the motion of the nitroxide moiety characterized by its correlation time, and the correlation time domain which is opened depends on the anisotropy of the nitrogen hyperfine tensor[1c].

Stable nitroxides exhibiting an extra large hyperfine splitting with a one-half spin nucleus should possess interesting new potentialities in spin-labelling investigations and in different applications based on their relaxivity[4,6]. We have recently described[7,8] some preliminary results on the synthesis of β-phosphorylated five membered ring nitroxides (Scheme 1), and in this paper we would like to describe the preparation of new molecules and to discuss the characteristics of their EPR spectra.

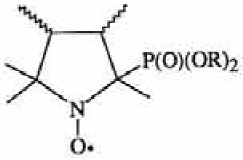
MATERIALS AND METHODS

Synthesis

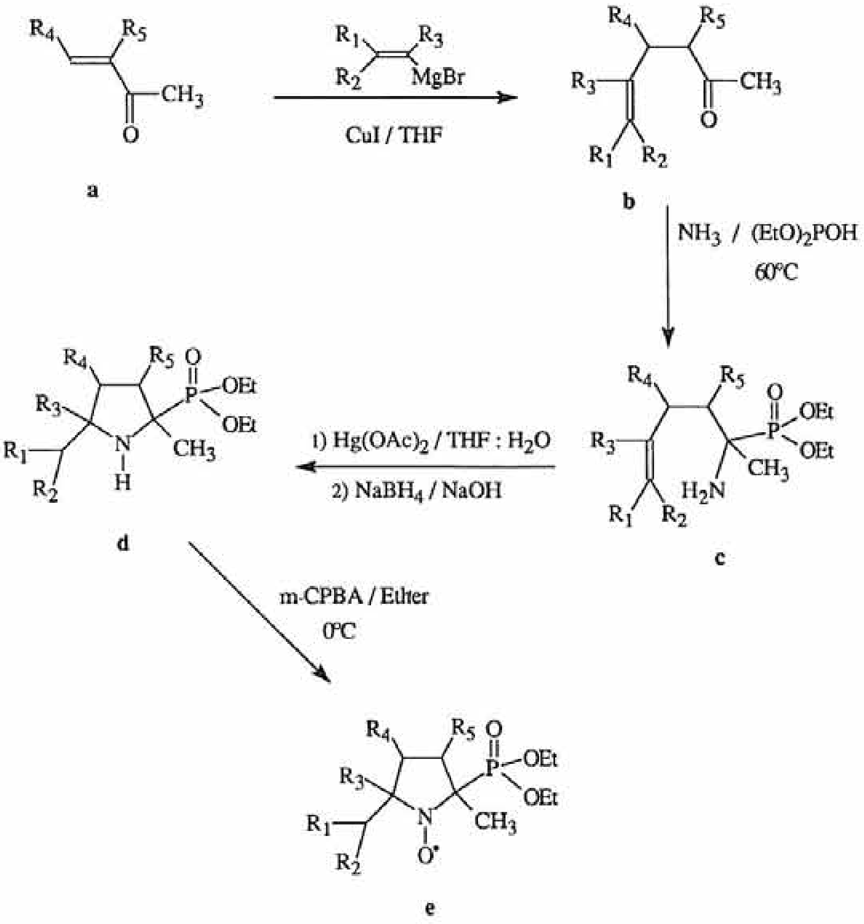
The general synthetic strategy used to prepare the β-phosphorylated five membered ring nitroxides is shown on Scheme 2. The γ,δ-ethylenic ketones **2b**, **3b**, **5b**–**7b** were

---

*Author to whom all correspondence should be addressed.



SCHEME 1



1 $R_1 = R_2 = R_4 = R_5 = H$; $R_3 = Me$

2 $R_1 = R_2 = R_5 = H$; $R_3 = Me$; $R_4 = Ph$

3 $R_1 = R_2 = R_5 = H$; $R_3 = Me$; $R_4 = CH_2OCH_2Ph$

4 $R_1 = R_2 = R_5 = H$; $R_3 = Me$; $R_4 = CH_2OH$

5 $R_1 = R_3 = Me$; $R_2 = R_5 = H$; $R_4 = Ph$

6 $R_1 = R_2 = H$; $R_3 = Me$; R_4, $R_5 = (CH_2)_4$

7 $R_1 = R_3 = Me$; $R_2 = H$; R_4, $R_5 = (CH_2)_4$

SCHEME 2

obtained by 1,4-addition of the appropriate vinyl cuprate on the corresponding α,β-unsaturated ketones **2a, 3a, 5a–7a** (vinyl magnesium bromide-copper iodide – THF, 0°C, 12 hr, 43–89% yield). The ketone **1b** was purchased from Aldrich. Aminophosphorylation of the ketones **1b–3b, 5b–7b** was performed by gentle bubbling of ammonia into a solution of the ketone in diethylphosphite (1.1 equiv.) at 60°C for 3–12 hours[8] in 61 to 87% yield, except for **3c** (40%) and were pure enough to be used in the next step without further purification. **1c–3c, 5c–7c** were cyclized by mercuric acetate-promoted intramolecular aminomercuration[8] [$Hg(OAc)_2$, 1 eq.; THF – H_2O 1:1 or CH_2Cl_2,R.T.] and the resulting acetoxymercurio derivatives were reduced *in situ* with sodium borohydride, either in basic aqueous solution ($NaBH_4$, 0.75–1 eq., in 10% aqueous sodium hydroxide) or in phase-transfer catalyzed reaction (benzyltriethyl ammonium chloride, 3.3 eq., $NaBH_4$ (0.75–1 eq.) in 10% aqueous sodium hydroxide, CH_2Cl_2). Usual work-up and removal of the solvent afforded crude mixtures containing 65 to 95% of the desired **1d–3d, 5d–7d** pyrrolidin-2-yl-phosphonates and non-reacted starting material. The pyrrolidin-2-yl-phosphonate **4d** was obtained in 78% yield from **3d** by reductive debenzylation (Pd/C 10%, HCl, EtOH, R.T). The pyrrolidin-2-yl-phosphonates **2d–7d** were obtained as a mixture of two diastereomers. The conditions to obtain the pure diastereomers **2d$_1$** and **2d$_2$** were found for **2d** (see below).

Oxidation of **1d–7d** with *meta*-chloroperbenzoic acid (1.7 equiv.) in ethereal solution at 0°C for 3 hours gave the corresponding stable nitroxides **1e–7e** which were isolated as yellow oils in 25 to 50% yield after preparative TLC purification. For nitroxides **3e–7e** small amounts of the pure diastereomers can be obtained by flash column chromatography on silica gel 60 H, eluting in 10% petroleum ether-diethyl ether or ether/acetone 10:1 v/v. The purity of each diastereomer was checked by EPR.

The details of our general synthetic procedure are given below for the synthesis of compounds **2b, 2c, 2d** and **2e** and will be published elsewhere for the other compounds.

Synthesis of 5-methyl-4-phenyl-5-hexen-2-one 2b

2-Bromopropene (0.2 mol, 24.2 g) in THF was slowly added to a suspension of powdered magnesium (0.2 eq. g, 4.86 g) in dry THF. The mixture was left at room temperature. Copper(I)iodide (5%, 1.9 g) was added. At 0°C, a THF solution of 4-phenyl-3-buten-2-one (0.1 mol, 14.6 g) was slowly added to the mixture, which was refluxed for 0.5 hour, left overnight at room temperature and then hydrolyzed on ice/ammonium chloride under vigorous stirring. Extraction with dichloromethane, drying over magnesium sulfate and removal of the solvent gave the crude product which was distilled under reduced pressure to yield 43% (8.15 g) of **2b** (73°C, 4.10^{-1} mbar). ^{1}H-NMR ($CDCl_3$)δ: 1.55 (3H, s), 2.0 (3H, s), 2.85 (2H, m), 3.75 (1H, t), 4.80 (2H, s), 7.2 (5H, m). MS (70 eV) m/e: 188 (M^+), 145 ($M^+ - CH_3C(O)$), 131 ($M^+ - CH_3C(O)CH_2$), 43 ($CH_3C(O)^+$).

Synthesis of Alkenylaminophosphonates 2c$_1$ and 2c$_2$

Ammonia was bubbled into a mixture of **2b** (43 mmol, 8.15 g) and diethylphosphite (44 mmol, 6.08 g) at 60°C for about 12 hours; the progress of reaction was monitored by ^{1}H-NMR. Then the mixture was acidified with diluted hydrochloric acid and extracted with ether to remove non reacted starting materials. The aqueous layer was poured over potassium carbonate, extracted with ether, washed with a saturated aqueous sodium chloride solution and dried over sodium sulfate; removal of the solvent afforded 8.58 g (61% yield) of a mixture of diastereomers **2c**$_1$ and **2c**$_2$, which was pure enough for further uses. ^{31}P-NMR: (CDCl$_3$) δ**2c**$_1$ 30.22 ppm (62%), **2c**$_2$ 30.13 ppm (38%). ^{1}H-NMR (CDCl$_3$) δ: 1.10 and 1.20 (3H, d, J_{P-H} = 16 Hz), 1.30 (6H, t, J = 7.1 Hz), 1.68 (5H, s), 2.0–2.4 (2H, m), 3.2–3.8 (1H, m), 4.05 (4H, m), 4.7–5.1 (2H, m), 7.05 (5H, m).

Aminomercuration of Alkenylaminophosphonates: Synthesis of 2d$_1$ and 2d$_2$

At room temperature, a suspension of mercuric acetate (12.5 mmol, 3.98 g) in dichloromethane (20 ml) was slowly added to the mixture of diastereomers **2c**$_1$ and **2c**$_2$ (12.5 mmol, 4.06 g). The resulting organomercury coumpound was added to a solution of benzyltriethylammonium chloride (3.75 mmol, 8.54 g) in water (50 ml). Sodium borohydride (10 mmol, 0.37 g) in 10% aqueous sodium hydroxyde solution (6 ml) was then added. After 1 hour, the mixture was saturated with sodium chloride and extracted with dichloromethane. The organic layer was dried over magnesium sulfate. Removal of the solvent yielded 85% (10.6 mmol, 3.47 g) of a mixture of diastereomers **2d**$_1$ and **2d**$_2$ (^{1}H and ^{31}P-NMR: 65% **2d**$_1$, 35% **2d**$_2$), (^{31}P-NMR CDCl$_3$) δ: **2d**$_1$ 29.42, **2d**$_2$ 28.55), which were separated by flash column chromatography in pentane/acetone 1/1 v/v on Merck silica gel 60. ^{1}H-NMR (400 MHz) characteristics are given in Table 1.

Oxidation of Aminophosphonates 2d$_1$ and 2d$_2$ into Nitroxides 2e$_1$ and *2e$_2$*

At 0°C, oxidation of **2d**$_1$ and **2d**$_2$ (13 mmol) with *m*-CPBA (85%, 21 mmol) in ether (70 ml) led to nitroxides **2e**$_1$ and **2e**$_2$[8]. For preparative purposes, these nitroxides were obtained from the mixture of diastereomers of aminophosphonates; separation of diastereomeric nitroxides was performed in 50% yield by preparative TLC (Merck silica gel 60 F 254 plates) using ether/acetone 10:1 v/v.

EPR STUDY-RESULTS AND DISCUSSION

The EPR features of nitroxides **1e**–**7e**, are reported in Table 2. The diasteromeric nitroxides **2e**$_1$, **2e**$_2$–**7e**$_1$, **7e**$_2$ exhibit very similar nitrogen coupling constants but very different phosphorus coupling constants (17.3 G ≥ ΔAp ≥ 12.9 G).

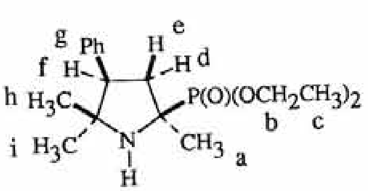
In the case of **2e**, oxidation of the diastereomer **2d**$_2$, exhibiting its phenyl and diethoxyphosphoryl groups in a trans conformation, yielded the diastereomer **2e**$_2$ with the largest phosphorus coupling.

The mixture of diastereomeric nitroxides **2e**$_1$, **2e**$_2$ was also obtained[7] by reacting the (2-^{13}C, methyl)-5,5-dimethyl-4-phenyl-1-pyrroline-N-oxide with diethylphosphite in presence of air. As expected, the major component was the nitroxide **2e**$_2$ (^{13}C) which corresponds to the approach of the diethylphosphite on the less hindered face of the pyrroline ring (Scheme 3).

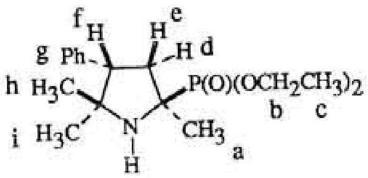
The hyperfine splitting constants of **2e**$_1$ and **2e**$_2$ are also reported in Table 2. The A ^{13}C coupling constants are significantly different and the lowest ^{13}C coupling is associated with the highest ^{31}P coupling.

We have shown that the phosphorus coupling in **2e**$_1$ and **2e**$_2$ is almost independent of the temperature (ΔAp ≈ 1 G in the temperature range 148–353°K), and this result

TABLE 1
^{1}H-NMR (400 MHz, $CDCl_3$) characteristics of diastereomers **2d$_1$** and **2d$_2$**.



2d$_1$



2d$_2$

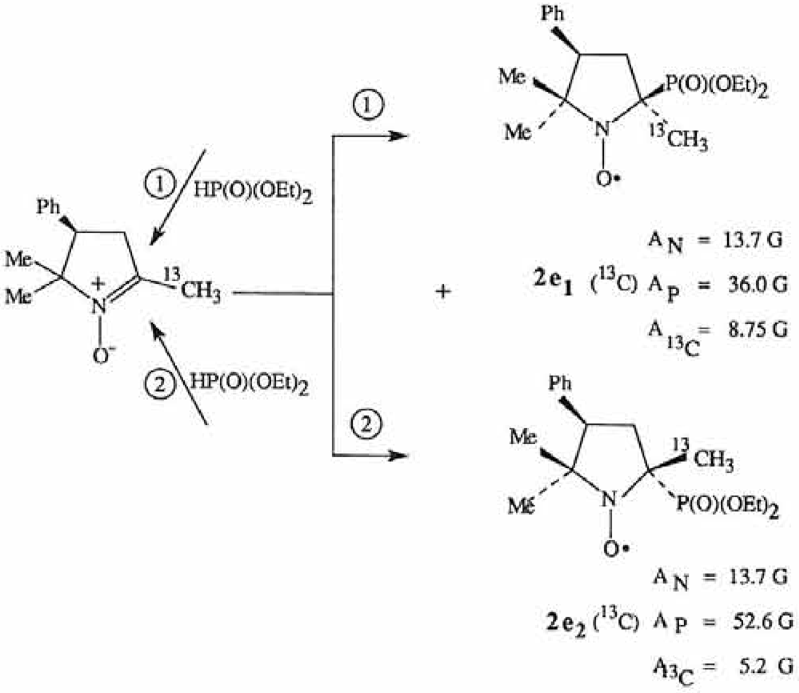
H(2d$_1$)	δ(ppm)		J(Hz)	H(2d$_2$)	δ(ppm)		J(Hz)
a	1.447	d	$J_{Ha\text{-}P} = 15.7$	a	1.530	d	$J_{Ha\text{-}P} = 15.4$
b	4.20		non-resolved multiplet	b	4.15 4.20		non-resolved multiplet
c	1.331	t	$J_{Hc\text{-}Hb} = 7.1$	c	1.314 1.337	t t	$J_{Hc\text{-}Hb} = 7.1$ $J_{Hc\text{-}Hb} = 7.1$
d	2.883	m	$J_{Hd\text{-}P} = 17.3$ $J_{Hd\text{-}He} = 12.9$ $J_{Hd\text{-}Hf} = 12.9$	d	2.274	m	$J_{Hd\text{-}P} = 27.6$ $J_{Hd\text{-}He} = 13.4$ $J_{Hd\text{-}Hf} = 13.4$
e	1.905	m	$J_{He\text{-}Hd} = 12.2$ $J_{He\text{-}Hf} = 5.8$ $J_{He\text{-}P} = 1.5$	e	2.547	m	$J_{He\text{-}P} = 15.8$ $J_{He\text{-}Hd} = 13.6$ $J_{He\text{-}Hf} = 6.8$
f	3.065	m	$J_{Hf\text{-}Hd} = 13.7$ $J_{Hf\text{-}He} = 6.0$	f	3.376	m	$J_{Hf\text{-}Hd} = 13.2$ $J_{Hf\text{-}He} = 6.8$
g	7.2		non-resolved multiplet	g	7.2		non-resolved multiplet
h	0.778	s	-	h	1.179	s	-
i	1.240	s	-	i	0.840	s	-

suggests that for these nitroxides only one conformation is significantly populated in solution.

In order to get more information on the preferred conformation of these nitroxides we generated the nitroxides **8C** and **8T** as shown on Scheme 4. The β-^{13}C hyperfine coupling constants in aminoxyl radicals with a planar nitroxyl moiety were shown to follow a simplified McConnell relations[9]:

$$A_C^{13} = B_{13_C} \cdot \cos^2\theta_C \qquad (1)$$

The angle θ_C is the angle between the planes $NC\alpha^{13}C_\beta$ and $C\alpha N2p_z$ (Scheme 5). If we assume that in **8C** and **8T** the presence of the phenyl substituent strongly favors one conformation, we will have θ_C **(8C)** + θ_C **(8T)** = 60° and we can write the equations (2), the solution of which gives $\theta_{13_C} = 15.6°$, $\theta_{12_C} = 44.4°$ and $B_{13_C} = 9.6$ G.

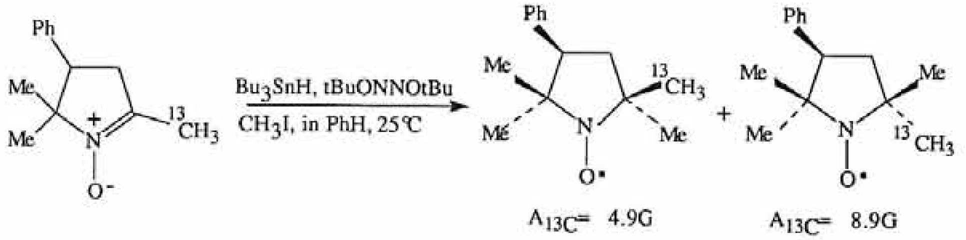


SCHEME 3

TABLE 2
EPR features for nitroxides **1e**–**7e** in benzene at room temperature.

Nitroxide	A_N(G)	A_P(G)	g	other
1e	13.7	50.0	2.0059	
2e$_1$	13.7	36.0	2.0060	8.75 (1 ^{13}C)^i
2e$_2$	13.7	52.6	2.0061	0.45 (6H) 0.43 (3H) 7.6 (2 ^{13}C) 5.2 (1 ^{13}C)^i
3e$_1$	14.0	36.5	2.0059	
3e$_2$	13.7	53.5	2.0061	
4e$_1$	13.9	37.2	2.0059	
4e$_2$	13.6	52.8	2.0061	
5e$_1$	13.8	36.4	2.0059	
5e$_2$	14.1	49.3	2.0060	
6e$_1$	14.1	34.7	2.0059	
6e$_2$	14.0	50.3	2.0060	
7e$_1$	13.9	34.4	2.0059	
7e$_2$	14.1	51.7	2.0060	

i: from the methyl attached to the carbon bearing the diethoxyphosphoryl group.



8C **8T**

SCHEME 4

5a **5b**

SCHEME 5

$$8.9 = B_{13_C}\cdot\cos^2\theta_C;\ 4.9 = B_{13_C}\cdot\cos^2(60-\theta_C) \qquad (2)$$

Force field calculations[10] performed on **8** supported the existence of a predominant conformation exhibiting a half-chair geometry (Figure 1). The nitroxide moiety was found planar and the dihedral angle values $\theta_{13_C} = 16°$, $\theta_{12_C} = 42.9°$, were in good agreement with those deduced from equations (2).

We have previously shown[11] that for β-phosphorylated five membered ring nitroxides the phosphorus coupling also follows a M^{c}Connell relation:

$$Ap = Bp\cos^2\theta p \qquad (3)$$

Bp was shown to depend on the electronegativity of the phosphorus substituents and a value close to 55 G was proposed for the $-P(O)(OEt)_2$ group. In order to confirm this Bp value, we characterized by EPR a bicyclic β-phosphorylated nitroxide **9** exhibiting a blocked geometry, which was generated from a mixture of **4e₁** + **4e₂** as shown on Scheme 6. Warming *in situ* at 120°C a mixture of **4e₁** (65%) and **4e₂** (35%) in *t*-butylbenzene, in the presence of catalytic amounts of NaH, led to the disappearance of the signal of nitroxide **4e₁**, while a new nitroxide signal appeared with an Ap of 57.8 G. The same nitroxide was obtained according to Scheme 7.

The lack of reactivity of nitroxide **4e₂** bearing the hydroxymethyl and diethoxyphosphoryl groups in a trans conformation (Scheme 6), and the very high phosphorus coupling observed for **9**, strongly support its bicyclic structure. However until now, we were not able to get a full characterization of the bicyclic amine **9H**, although a flash column chromatography on alumina, eluting with pentane, afforded

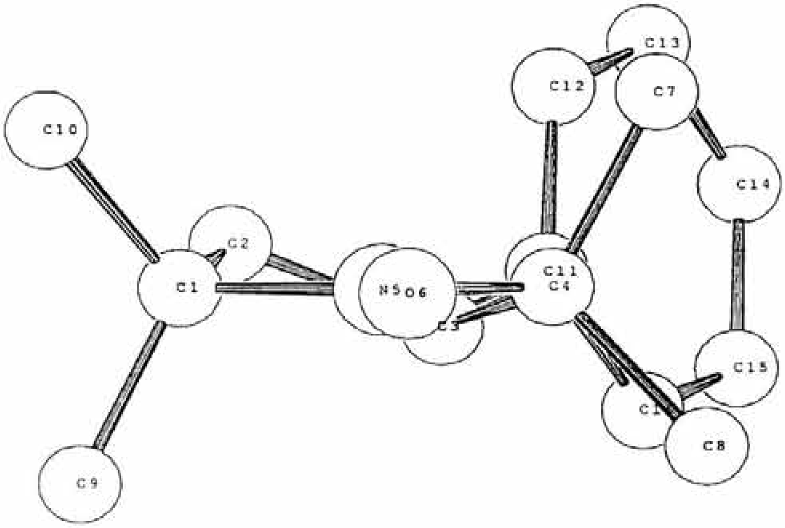
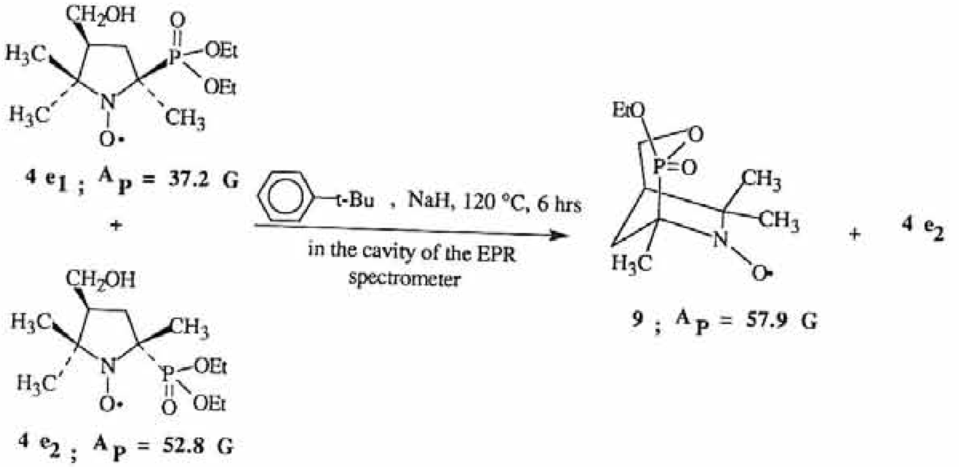
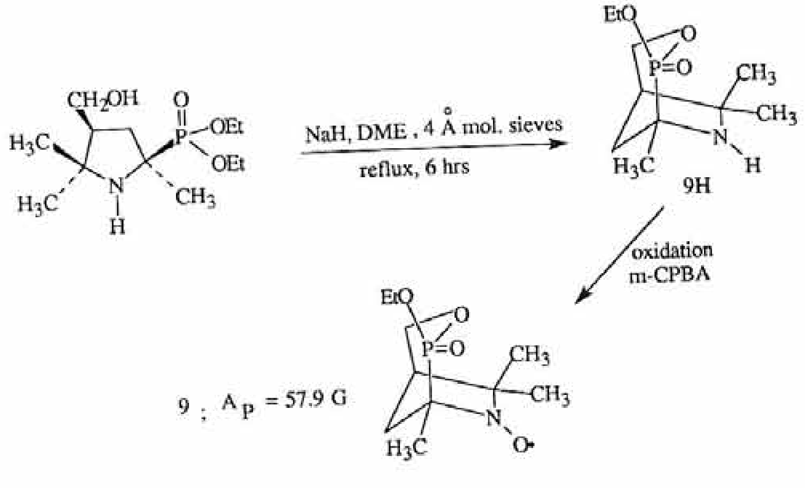


FIGURE 1 Predominant half-chair conformation of nitroxide **8** according to force field calculations.



SCHEME 6

spectroscopically (EPR) pure samples of **9**. Force field calculations on **9** indicated a planar nitroxyl group and a θp dihedral angle value of 5.6° which, according to equation (3), corresponds to $Bp = 58.3$ G in close agreement with our previously reported value. Very consistent values were obtained when the sum of the dihedral angles $\theta_{13_C} + \theta p$ in nitroxides **2e₁** (^{13}C) and **2e₂** (^{13}C) were calculated assuming the existence of a largely predominant conformer and using the values $Bp = 58.3$ G and $B_{13_C} = 9.6$ G found in this work ($\theta_{13_C} + \theta p = 60.8°$ for **2e₂** (^{13}C) and 55.6° for **2e₁**



SCHEME 7

(^{13}C)). According to the above discussion we can reasonably propose for this conformer a half chair geometry similar to that of **8**, with the $P(O)(OEt)_2$ occupying the position of the methyl labelled with carbon-13.

Acknowledgements

We are grateful to the Centre d'Etudes Nucléaires de Grenoble (LETI) for support of this work and to the ATOCHEM company for a travel grant to present this work at the 3rd International Symposium on Spin Trapping and Aminoxyl Radical Chemistry (Kyoto, Japan, November 22–24, 1991).

References

1. (a) C.F. Chignell (1984) Use of spin-labels as enzyme probes. In *Spin-Labelling in Pharmacology* (ed. J.L. Holtzman), Academic Press, pp. 131–156. (b) E.J. Rauckman, G.M. Rosen and L.K. Griffeth (1984) Enzymatic reactions of spin-labels, *ibid*, pp. 175–189. (c) L.J. Berliner (1976, 1979) *Spin-labelling. Theory and Applications, I and II*. Academic Press, New York. (d) G. Sosnovsky, N.U.M. Rao, S.W. Li and H.M. Swartz (1989) Synthesis of nitroxyl (aminoxyl) labeled probes for studies of intracellular environment by EPR and MRI *Journal of Organic Chemistry*, **54**, 3667–3674. (e) J.F.W. Keana (1984) *Spin-labelling in Pharmacology* (ed. J.L. Holtzman) Academic Press, London and New York, pp. 2–67. (f) E.G. Janzen and R.A. Towner (1992), Aminoxyl Radicals as MRI Contrast Agents, in *Bioactive Spin Labels* (Ed. R.I. Zhdanov), Springer-Verlag, Heidelberg, pp. 573–583.
2. J.F.W. Keana, L. Lex, J.S. Mann, J.M. May, J.H. Park, S. Pou, V.S. Prabhu, G.M. Rosen, B.J. Sweetman and Y. Wu (1990) Novel nitroxides for spin-labelling, -trapping, and magnetic resonance imaging applications. *Pure and Applied Chemistry*, **62**, 201–205.
3. (a) M.J. Perkins (1980) Spin Trapping. In *Advances in Physical Organic Chemistry* (Ed. V. Gold and D. Bethell), Academic Press, London, pp. 1–64. (b) E.G. Janzen (1980) A critical review of spin trapping in biological systems. In *Free Radicals in Biology* (Ed. W.A. Pryor), Academic Press, New York, pp. 115–154. (c) G.M. Rosen and E. Finkelstein (1985) Use of Spin Traps in Biological Systems. *Advances in Free Radical Biology and Medicine*, **1**, 345–375.
4. J.F.W. Keana, F.L. Van Nice (1984) Influence of structure on the reduction of nitroxide MRI contrast-enhancing agents by ascorbate. *Physiological Chemistry and Physics and Medical NMR*, **16**, 477–480.
5. D.F. Bowman, T. Gillan and K.U. Ingold (1971) Kinetic applications of electron paramagnetic resonance spectroscopy. III. Self reactions of dialkyl nitroxide radicals. *Journal of the American Chemical Society*, **93**, 6555–6561.

6. (a) W. Müller-Warmuth and K. Meise-Gresh (1983) Molecular motions and interactions as studied by dynamic nuclear polarization (DNP) in free radical solutions, *Advances in Magnetic Resonance* (Ed. J.S. Waught), Academic Press. (b) Y. Ayant, R. Besson, R. Casalagno (1980) Interprétation théorique de la forme des raies obtenues par polarisation dynamique dans les solutions de radicaux libres, *Journal de Physique*, **41**, 1183–1192. (c) Y. Ayant and R. Casalegno (1978) Effet du mode de relaxation sur le coefficient de polarisation dynamique dans les solutions de radicaux libres, *Journal de Physique*, **39**, 235–245. (d) E.H. Poindexter, J.R. Stewart and P.J. Captan (1967) Dynamic polarization of fluorine nuclei in solutions of selected free radicals, *Journal of Chemical Physics*, **47**, 2862–2873. (e)Y. Berchadsky, N. Kernevez, F. Le Moigne, A. Mercier, L. Secourgeon, P. Tordo (1990). Preparation of heterocyclic phosphono nitroxides, *Brit-UK Pat. Appl.* GB 2, 225, 015 (*Chemical Abstracts*, **113**, 191636w). (f)Y. Ayant, N. Kernevez, L. Secourgeon, P. Tordo (1990) Weak Field Dynamic Nuclear Polarization with Phosphorus Radicals in *Congress Ampere on Magnetic Resonance and Related Phenomena*, Ed. M. Mehring, J.U. Von Schütz, H.C. and Wolf, Springer-Verlag, 188–189.
7. A. Mercier, Y. Berchadsky, Badrudin, S. Pietri and P. Tordo (1991) β-phosphorylated five membered ring nitroxides, a new class of stable nitroxides. 1. Synthesis by reaction of dialkylphosphites with 1-pyrroline-N-oxides *Tetrahedron Letters*, **32**, 2125–2128.
8. F. Le Moigne, A. Mercier and P. Tordo (1991) β-phosphorylated cyclic nitroxides. 2. Synthesis of pyrrolidin-and piperidin-2-yl phosphonates and the corresponding stable nitroxides. *Tetrahedron Letters*, **32**, 3841–3844.
9. E.G. Janzen (1984) Electron spin resonance study of the hyperfine splitting constants of naturally abundant carbon-13 and nitrogen-15 in diphenylmethyl *t*-butyl aminoxyl (nitroxide). Solvent and temperature effects, *Canadian Journal of Chemistry*, **62**, 1653–1657.
10. F. Vila, G. Pepe, D. Siri and P. Tordo Force field calculations on five membered ring aminoxyl radicals, *Free Radical Research Communications*, in press.
11. P. Tordo, M. Boyer, A. Friedmann, O. Santero, and L. Pujol (1978) Phosphorus-substituted nitroxides. 3. Hyperconjugative ability of carbon-phosphorus bonds in five-membered-ring nitroxides, *Journal of physical chemistry*, **82**, 1742–1744.