Free Radical Research, January 2006; 40(1): 59–65

Hydrazyl-nitrones, novel hybrid molecules in free radical research

PETRE IONITA

Institute of Physical Chemistry, Spl. Independentei 202, Bucharest, Romania

Accepted by Professor M. Davies

(Received 10 May 2005; in revised form 22 June 2005)

Abstract
This work describes the synthesis and characterisation of some novel hybrid molecules which contains in the same molecule a free radical moiety of hydrazyl type and a spin-trap moiety of nitrone type. The new compounds synthesized have multiple and easy to follow spectroscopic properties, making them useful as sensors or probes in radical chemistry. The new class of hydrazyl-nitrone molecules can act, in a single step process, as both generator and spin-trap of short-lived radicals. The hybrid molecules can be also involved in acid–base or redox processes, and the chemical processes can be easily monitored by visible or electron paramagnetic resonance spectroscopy. The excellent generator and trap properties recommend them as valuable sensors and probes in radical chemistry.

Keywords: *Nitrone, spin-trap, radical, hydrazyl, EPR*

Introduction

Four decades ago the technique of spin-trapping emerged in free radical chemistry. The pioneering work of Jansen [1], Blackburn [2], Lagercrantz [3] and Perkin [4] has been developed such that accurate identification of short-lived radicals from different chemical processes can now be made, and this technique has also been used extensively *in vivo* [5,6]. There are mainly two types of compounds used in EPR spin-trapping, namely nitroso-compounds and nitrones. Interestingly, while nitroso-compounds show considerable toxicity in living systems, nitrones do not, and have been shown to be valuable medicines in many diseases caused or induced by radicals, prolonging the life span of subject [6].

It is well known that reactive oxygen species (ROS) are implicated in a large number of diseases arising from oxidative damage in cells [7]. Consequently, the use of nitrones in conjunction with EPR spectroscopy plays an important role in understanding biological reactions in complex systems [8].

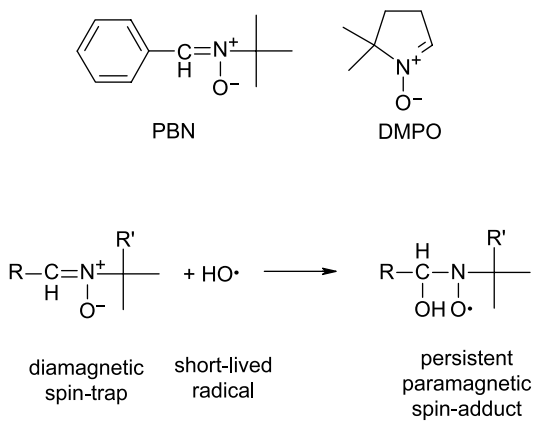
The most widely known nitrone is *t*-butyl-phenyl-nitrone (PBN, Scheme 1) synthesized in 1957 [9]. This was used for first time to trap radicals in chemical systems in 1969 [2], and in biological systems in 1975 [10]. Since 1985, there have been an increasing number of reports regarding its protective biological activity [11–14], and in the last decade PBN has been extensively evaluated as a therapeutic probe in ageing processes [14,15].

There is a considerable contemporary interest [11,16,17] in the preparation and properties of novel nitrones. For *in vivo* use, nitrones require a fast rate of addition of the transient radical, as well as rapid uptake and long lifetimes at a high concentration in biological fluids. A study of the tissue distribution of radio-labeled PBN has demonstrated its rapid absorbance [18]. The excretion and metabolism is rather slow, with 30% of the initial dose remaining in animals after 3 days [18].

Besides nitrones, other free radical scavenging agents like mono- or poly-nitroxide radicals have been proposed as potential candidates for stroke therapy, because of the hypothesised role of free

Correspondence: P. Ionita, Department of Chemistry, University of York, York YO10 5DD, UK. Fax: 44 1904432516. E-mail: pi4@york.ac.uk; E-mail: pionita@chimfiz.icf.ro

ISSN 1071-5762 print/ISSN 1029-2470 online © 2006 Taylor & Francis
DOI: 10.1080/10715760500385699



Scheme 1. Chemical structure of PBN and DMPO spin-traps (top); trapping of hydroxyl radical by a nitrone spin-trap (bottom).

radicals in the progression of strokes and ischemia induced neurodegeneration [19].

Hydrazyl radicals (Figure 1) have also been used as scavengers of short-lived radical species [20–22]. The stable hydrazyl free radical 2,2-diphenyl-1-picryl hydrazyl (DPPH) has been used as an indicator for measuring antioxidant capacity in many systems, including human plasma [23]. Although DPPH has been known since 1922 [24], it is still widely used as an EPR standard and as colorimetric reagent for redox processes. Because DPPH can be kept indefinitely with no decomposition and because it neither dimerizes nor reacts with oxygen, it has proved to be useful in a variety of scenarios, such as polymerization inhibition or radical chemistry, the determination of antioxidant properties of amines, phenols or natural compounds (vitamins, plant extracts, medicinal drugs) and for inhibiting hemolytic reactions [25–27]. DPPH is also well known as a good hydrogen abstractor yielding DPPH-H as byproduct. This is a redox type process and was first mentioned by Goldschmidt and Renn in DPPH oxidation of hydroquinone to benzoquinone [24]. More generally, the process was proved to be a homolytic cleavage by the DPPH of a H–X bond (X=C, O, N, S, Cl, Br) present in hydrocarbons, alcohols, phenols, thiols, amines, enols, hydroxylamines, *N*-alkoxynitroanilines, hydracids [28–32].

Some of our previous work has shown that hydrazyl radicals can act as one-electron oxidants [32–34],

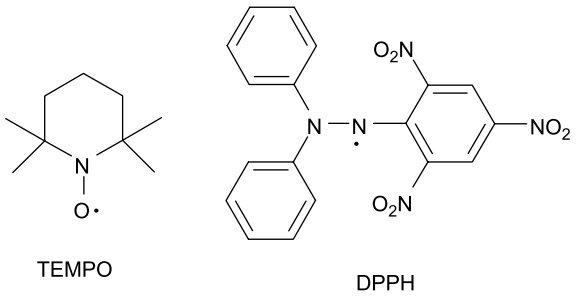


Figure 1. TEMPO (nitroxide) and DPPH (hydrazyl) stable free radicals.

leading to the formation of short-lived radicals, which can be subsequently trapped by spin-traps such as PBN or DMPO (Scheme 1). In order to follow these processes, the reaction mixture should contain both the free stable radical and the spin-trap. It was also observed *in vivo* that, under specific conditions, DPPH can sensitise or protect against ionisation radiation [35]. Spin-traps have also been developed which can target cell membranes [36].

In this paper, we describe the development of new compounds that contain in the same molecule a stable free radical moiety of the hydrazyl type, and a spin-trap moiety of the nitrone type. These hybrid molecules may find direct applications as sensors or probes in EPR spectroscopy, as well as in chemical or biological systems.

Materials and methods

Solvents and starting materials were purchased from Lancaster or Aldrich. The UV–VIS spectra were recorded at ambient temperature in dichloromethane (DCM) using a Hitachi U-3000 spectrophotometer. EPR spectra were normally recorded at room temperature (293 K) in deoxygenated DCM using both JEOL-RE1X (typical settings: microwave frequency 9.45 GHz, power 1 mW, sweep time 60 s, time constant 0.1 s, modulation frequency 100 kHz, gain 200, modulation width 0.1 mT), and Bruker (typical settings: microwave frequency 9.49 GHz, power 1 mW, modulation frequency 100 kHz, modulation amplitude 0.1 mT, receiver gain 5×10^4, conversion time 80 ms, time constant 160 ms) ESP 300 spectrometers. Comparing with DPPH standard, g values were determined ($g = 2.0037$). TLC was performed on silica gel plates (Merck). Synthesis of **1** is described elsewhere [32]. Compounds **2** and **3** were obtained from **1** by nitration with nitrous acid in a biphasic system [31–33], and were separated by column chromatography (silica gel/DCM). **2**: ESI-MS (M^+) 422; **3**: ESI-MS (M^+) 467. The purity of all new compounds was checked by analytical TLC (single spot).

*General procedure for synthesis of **4–6***

To a solution of aldehydes **1**–**3** (500 mg) in 250 ml DCM was added 250 mg *t*-butyl-hydroxylamine hydrochloride in 50 ml pyridine and the reaction mixture left for few days at room temperature. The mixture was then washed with saturated aqueous solution of sodium hydrogencarbonate and the separated organic layer was dried and removed under vacuum. The resulting crude product was chromatographied on a silica gel column, with DCM as eluent. **4**: ESI-MS: 449 (M^+). λ_{max} (DCM) = 324 nm (yellow color); in a presence of a base λ_{max} = 655 nm (green color). ^{1}H-NMR (chloroform-d, δ ppm): 1.58 (s, 9H, methyl), 7.10–7.13 (m, 4H, phenyl), 7.26–7.31 (m, 5H, phenyl), 7.52 (s, 1H, CH=N), 9.03 (s, 2H, CH-*meta*), 9.72 (s, 1H, NH).

TLC (silica gel/DCM) $R_f = 0.2$. **5**: ESI-MS 493 (M^+). λ_{max} (DCM) = 330 nm (yellow color); in a presence of a base $\lambda_{max} = 495$ nm (red–purple color). ^{1}H-NMR (chloroform-d, δ ppm): 1.49 (s, 9H, methyl), 7.10–7.21 (m, 4H, phenyl), 7.28–7.42 (m, 6H, phenyl), 7.58 (s, 1H, CH=N), 9.07 (s, 2H, CH-*meta*), 9.79 (s, 1H, NH). TLC (silica gel/DCM) $R_f = 0.18$. **6**: ESI-MS 538 (M^+). λ_{max} (DCM) = 332 nm (yellow color); in a presence of a base $\lambda_{max} = 492$ nm (red color). ^{1}H-NMR (chloroform-d, δ ppm): 1.56 (s, 9H, methyl), 7.10–7.13 (m, 4H, phenyl), 7.26–7.34 (m, 6H, phenyl), 7.58 (s, 1H, CH=N), 9.05 (s, 2H, CH-*meta*), 10.01 (s, 1H, NH). TLC (silica gel/DCM) $R_f = 0.15$.

General procedure for synthesis of 7–9

Oxidation of **4–6** with excess lead dioxide or potassium permanganate in DCM, for about 3 h, in the presence of anhydrous sodium sulfate, afforded almost quantitatively the hydrazyl-nitrones **7**–**9**. No further purification is necessary; for good elemental analysis an overnight drying *in vacuo* at 40°C is requested. **7**: λ_{max} (DCM) = 524 nm (violet color). Nitrogen elemental analysis: requested 15.62%, found 15.12%. $a_{N1} = 0.95$ mT, $a_{N2} = 0.7$ mT, $g = 2.0031$. TLC (silica gel/DCM) $R_f = 0.24$. **8**: λ_{max} (DCM) = 517 nm (violet color). Nitrogen elemental analysis: requested 17.03%, found 16.62%. $a_{N1} = 1.03$ mT, $a_{N2} = 0.58$ mT, $g = 2.0034$. TLC (silica gel/DCM) $R_f = 0.29$. **9**: λ_{max} (DCM) = 508 nm (violet color). Nitrogen elemental analysis: requested 18.21%, found 15.77%. $a_{N1} = 1.05$ mT, $a_{N2} = 0.58$ mT, $g = 2.0037$. TLC (silica gel/DCM) $R_f = 0.26$.

General procedure for spin-trapping experiments involving nitrones 4–6

A solution of **4**–**6** (5 ml, 10^{-3} M) in DCM was stirred with an aqueous solution of ferrous sulphate (5 ml, 10^{-3} M), to which a small amount (~50 mg) of hydrogen peroxide (30%) was added. After few seconds the separated organic phase was then investigated by EPR (after drying on anhydrous sodium sulphate).

General procedure for experiments involving hydrazyl-nitrones 7–9

To a solution of **7**–**9** (10^{-3} M) in DCM was added diphenylphosphine (10^{-2} M) and the mixture directly investigated by EPR.

Results and discussion

Synthetic strategy

In order to obtain a hybrid hydrazyl-nitrone molecule, our starting point was the known compound **1**, synthesised as described previously [32]. By nitration with sodium nitrite and hydrochloric acid in a biphasic system, compounds **2** and **3** were obtained in good yields and isolated by preparative chromatography.

Compounds **1**–**3** react with *N*-*t*-butyl-hydroxylamine at room temperature, resulting in good yields of the nitrones **4**–**6**. Oxidation with lead dioxide or potassium permanganate affords almost quantitatively the final hybrid hydrazyl-nitrone products **7**–**9** (Figure 2).

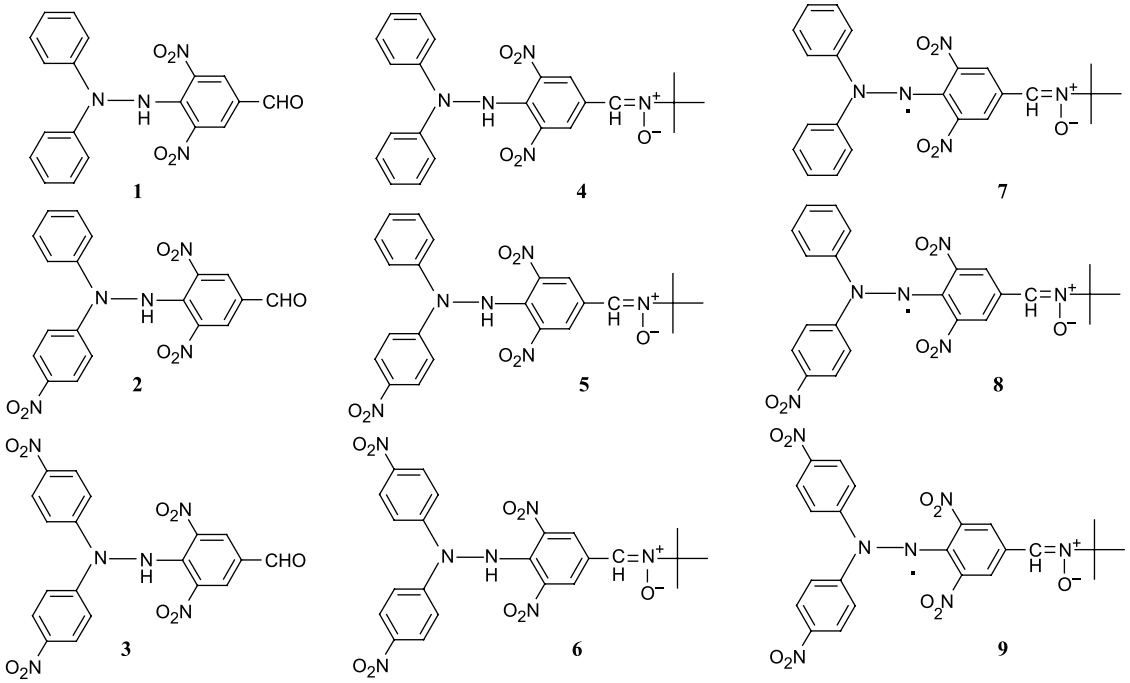
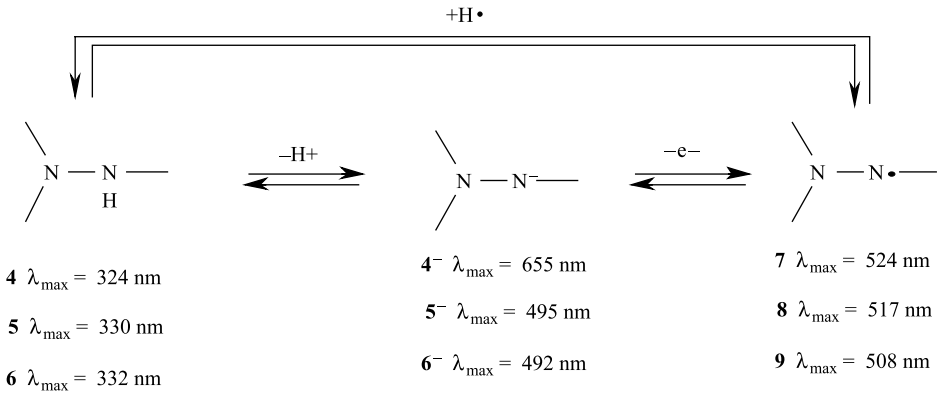


Figure 2. Chemical structure of compounds **1**–**9.**



Scheme 2. Acid–base and redox interconversion of compounds **4**–**9**: nitrones **4**–**6** are yellow in solution, and by oxidation become violet hydrazyls **7**–**9** (in basic media nitrones **4**–**6** are green or red, as anions $\mathbf{4}^-$–$\mathbf{6}^-$).

Characteristic visible absorption spectra and interconversion

Like other hydrazines of similar type, nitrones **4**–**6** show acid–basic and redox properties [32]. The hybrid nitrone-radicals **7**–**9** are very stable in solution, as well as in the solid state, without evidence of decomposition at room temperature for months. In basic media the N–H group of the compounds **4**–**6** deprotonated, giving the corresponding anions; these processes are accompanied by color change from yellow to green or red (Scheme 1). Addition of an acid converts the anions back into the parent hydrazines (Scheme 1). On oxidation, the yellow hydrazine-nitrone precursors **4**–**6** are converted into the corresponding hydrazyl-nitrones **7–9** with a characteristic purple–violet color. These radical-nitrones **7**–**9** can be easily reduced by ascorbic acid, hydrazine or hydroxylamine to the parent nitrones **4**–**6.** All these acid–base and redox processes are reversible and are accompanied by color changes, as summarised in Scheme 2.

EPR spectra

Spectra of hydrazyl-nitrones 7–9. Oxidation of **4**–**6** by solid potassium permanganate or lead dioxide in DCM gave the corresponding persistent radicals **7**–**9** (color changes from yellow to violet, Scheme 2), with EPR spectra as shown in Figure 3 (EPR hyperfine coupling constants values for **7** $a_{N1} = 0.95$ mT, and $a_{N2} = 0.7$ mT, $g = 2.0031$; for **8** $a_{N1} = 1.03$ mT and $a_{N2} = 0.58$ mT, $g = 2.0034$; for **9** $a_{N1} = 1.05$ mT, and $a_{N2} = 0.58$ mT, $g = 2.0037$). As expected, the EPR spectra changes from **7** to **8** with the addition of a supplementary nitro-group (as shown by hyperfine coupling constants and g values), as happens with other congener hydrazyl radicals [31–33]; interestingly on going from **8** to **9** there is not a big change in the EPR spectra; probably the second nitro-group is not able to induce as marked a perturbation in spin-densities as the first one, so the a_N values do not change by much, though the g value shifts.

*Nitrones **4**–**6** as classical spin-traps (trapping of hydroxyl radical).* Active oxygen species such as hydrogen peroxide, hydroxyl radical and superoxide anion radical, are readily generated in cells by metabolic processes such as respiration, ischemia/reperfusion, and oxidation of fatty acids, and they are highly toxic to cells by damaging components such as DNA, lipids and enzymes [13,17,19]. Cells can be reversibly injured, or irreversibly damaged when the concentration of active oxygen species exceeds the cellular antioxidant capacity. In this study we chose hydroxyl radicals as a representative damaging agent for spin-trapping studies. The new nitrones **4**–**6** can be used instead of classical PBN, in any spin-trapping processes in which a nitrone is required. For example, hydroxyl radicals generated *via* a Fenton reaction

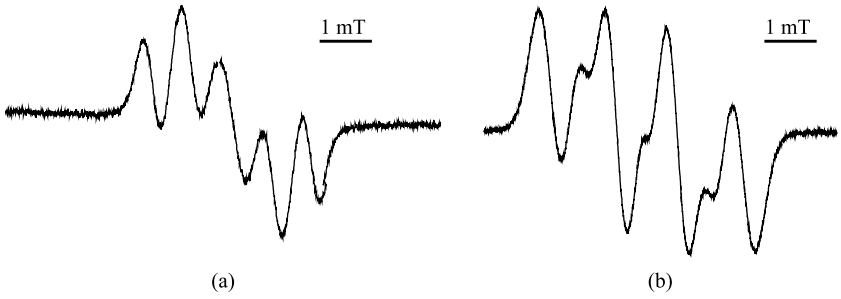


Figure 3. EPR spectra of compound **7** (a) and **8** (b); compound **9** has almost an identical spectra with **8** (not shown).

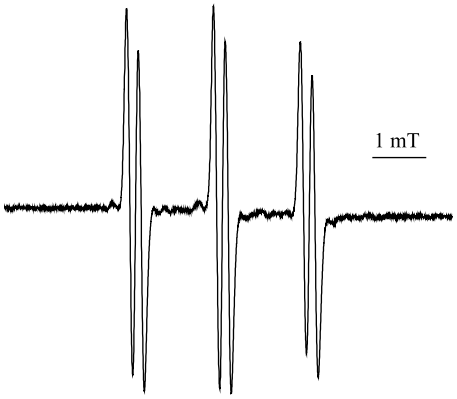


Figure 4. EPR spectra of hydroxyl radical spin adduct of **4** ($a_N = 1.35$ mT, $a_H = 0.18$ mT, $g = 2.0059$).

(hydrogen peroxide/iron sulphate) were detected in a straightforward manner (Figure 4). A standard test was performed to confirm the identity of the spin-adduct: PBN was used in a typical iron sulphate/hydrogen peroxide system (in water), and the PBN–OH spin-adduct extracted into DCM and compared with nitrones **4**–**6** spin-adducts; the a_N and g values were identical: $a_N = 1.35$ mT, $a_H = 0.18$ mT, $g = 2.0059$.

Hybrid hydrazyl-nitrones **7**–**9** *as simultaneous generators and traps for short-lived radicals*. As was previously demonstrated, hydrazyl radicals can generate, via one-electron oxidation, short-lived radicals, starting from several anions or compounds which can loose one electron or a hydrogen atom [34]. Adding diphenylphosphine to any of the hybrid molecules **7**–**9**, the initial EPR spectra of hydrazyl radicals (shown in Figure 3) turned to a very different ones, shown in Figure 5. Diphenylphosphine is oxidized by the hydrazyl moiety to the corresponding short-lived radical $Ph_2P^{\cdot}$, which is subsequently trapped by the nitrone moiety. This process is identical to that described in systems which used DPPH as oxidant

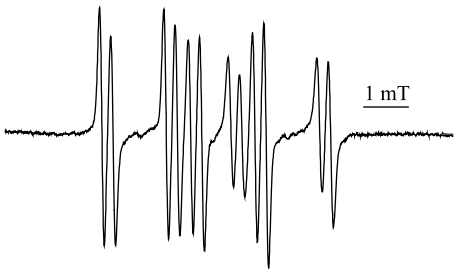


Figure 5. EPR spectra of Ph_2P-**9** spin adduct ($a_N = 1.44$ mT, $a_H = 0.25$ mT, $a_P = 1.98$ mT, $g = 2.0058$).

and PBN, DMPO and DEPMPO as spin-traps [34]. The EPR spectra recorded using this new hydrazyl-nitrone is shown in Figure 5. The overall reaction is detailed in Scheme 3.

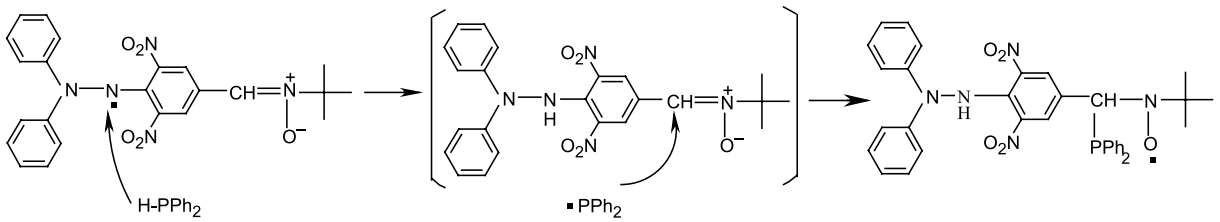
Thus, the hybrid compounds **7**–**9** could find application in EPR studies of the generation and identification of some species (self-generated by hydrazyl oxidation), the short-lived radicals obtained in this way being trapped by the nitrone moiety. Besides this, not only EPR spectra are changing dramatically, but also the UV–Vis spectra (from violet to green or red, see also Scheme 2). EPR characteristics of the new radicals involved in this study are summarized in Table I.

Distribution of generated radicals between two STs. Another interesting finding in the spin-trapping properties of our hybrid molecules **7**–**9** arises from the very short distance between the hydrazyl center (which generates the short-lived radical) and the nitrone moiety (which traps the generated radical). We believe that the radical generated at hydrazyl center does not leave the solvation shell of the hybrid molecule, so it is expected that there will be a high yield of trapping, as well as the possibility to trap species with low life time. In order to demonstrate this, we set up an experiment in which we used an equimolecular mixture of our hybrid molecule **9** and

Table I. EPR characteristics of the new radicals detected in this study (solvent: dichloromethane).

Radical	Type	Obtained from	Hyperfine coupling constants (±0.005) (mT)	*g* value (±0.00005)	Line-width (±0.005) (mT)
7	Hydrazyl	Oxidation of **4**	$a_{N1} = 0.95$ $a_{N2} = 0.7$	2.0031	0.3
8	Hydrazyl	Oxidation of **5**	$a_{N1} = 1.03$ $a_{N2} = 0.58$	2.0034	0.35
9	Hydrazyl	Oxidation of **6**	$a_{N1} = 1.05$ $a_{N2} = 0.58$	2.0037	0.35
9-OH⋆	Spin-adduct	**9** and HO· (H_2O_2/Fe^{2+})	$a_N = 1.35$ $a_H = 0.18$	2.0059	0.05
9-PPh_2⋆	Spin-adduct	**9** and H-PPh_2	$a_N = 1.44$ $a_H = 0.25$ $a_P = 1.98$	2.0058	0.05

⋆ **7** or **8** spin-adducts of $HO^{\cdot}$ or $^{\cdot}PPh_2$ radicals have the same EPR characteristics as in the case of **9**.



Scheme 3. Detailed spin-trapping process of $^{\bullet}PPh_2$: the first step consists of a hydrogen atom abstraction induced by the hydrazyl moiety, and the second step consists of the spin-trapping of the short-lived radical generated in the first step by the nitrone moiety.

another nitrone, DMPO (Scheme 1). Although both spin-traps contain the same nitrone moiety, DMPO spin-adducts have different EPR hyperfine coupling constants [34,37], thereby allowing differentiation between the two spin-adducts formed. Thus, $Ph_2P^{\bullet}$ radicals generated by hydrazyl moiety might be captured both by DMPO and the nitrone part of the hybrid molecule. Experimental data showed that 94% of the generated $Ph_2P^{\bullet}$ was captured by the nitrone moiety contained in compound **9**, and only 6% by DMPO (see Figure 6). This unexpected result can be explained by the very close spatial vicinity of the spin-trap moiety next to the point in which the $Ph_2P^{\bullet}$ radical is generated (hydrazyl group). A test experiment was also performed, using an equimolecular mixture of standard PBN and DMPO, to which Ph_2PH and DPPH was added. DPPH oxidized Ph_2PH to $Ph_2P^{\bullet}$, and this is trapped by PBN and DMPO. Simulation of the spectra as a mixture of these spin-adducts has shown a ratio of about 35% (PBN–PPh_2) and 65% (DMPO–PPh_2). Comparing this ratio (35/65) with the previous one (94/6, in which **9** and DMPO were used), it is clear that these hybrid hydrazyl-nitrones might be advantageous over the use of the two separate compounds.

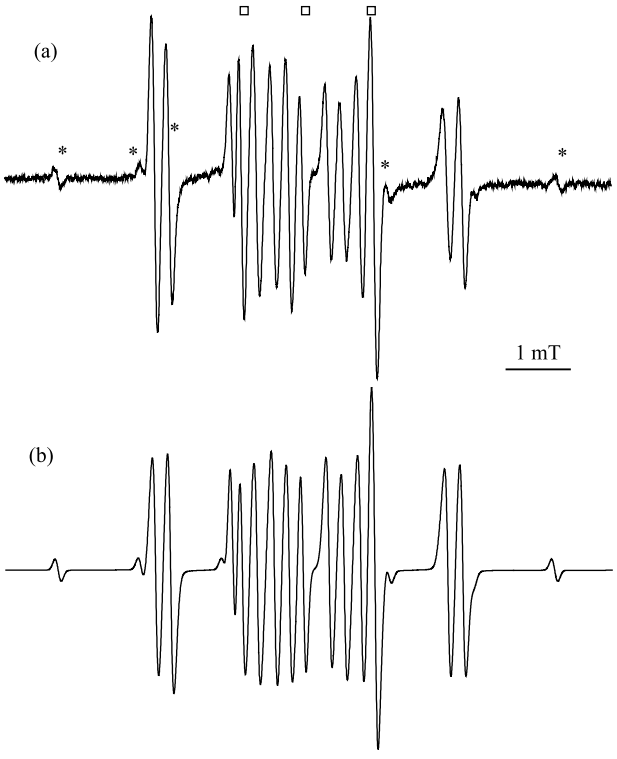


Figure 6. EPR spectra (a) and simulation (b) of a mixture of **9**-PPh_2 spin adduct (94%) and DMPO-PPh_2 spin-adduct (6%); the peaks marked ⋆ are due to the DMPO-PPh_2 spin-adduct ($a_N = 1.44$ mT, $a_H = 2.05$ mT, $a_P = 3.41$ mT, $g = 2.0058$); the peaks marked □ are due to a DMPO impurity, with $a_N = 1.12$ mT and $g = 2.0059$.

Conclusions

In this study, we have demonstrated that the new nitrones **4**–**6** and the novel hybrid hydrazyl-nitrone **7**–**9** have a high potential for direct applications in radical chemistry. Thus, nitrones **4**–**6** act as classical spin-traps for hydroxyl radical (generated by Fenton reaction), while the hybrid molecules **7**–**9** act simultaneously as generators and traps for phosphine radicals.

References

[1] Janzen EG, Blackburn BJ. Detection and identification of short-lived free radicals by an electron spin resonance trapping technique. J Am Chem Soc 1968;90:5909–5910.
[2] Janzen EG, Blackburn BJ. Detection and identification of short-lived free radicals by electron spin resonance trapping techniques (spin trapping)—photolysis of organolead, -tin, and -mercury compounds. J Am Chem Soc 1969;91: 4481–4490.
[3] Lagercrantz C, Forchult S. Trapping of free radicals formed by gamma-irradiation of organic compounds. Nature 1968; 218:1247–1248.
[4] Chalfont GR, Perkins MJ, Horsfield A. A probe for homolytic reactions in solution. 2. Polymerization of styrene. J Am Chem Soc 1968;90:7141–7142.
[5] Janzen EG, Haire DL. Advances in Free Radical Chemistry. Vol. 1. Greenwich: JAI Press; 1990. p 253–295.
[6] Floyd RA, Hensley K, Forster MJ, Kelleher-Anderson JA, Wood PL. Nitrones as neuroprotectants and antiaging drugs. Ann NY Acad Sci 2002;959:321–329.
[7] Villamena FA, Zweier JL. Detection of reactive oxygen and nitrogen species by EPR spin trapping. Antioxid Redox Signals 2004;6:619–629.
[8] Berliner LJ, Khramtsov V, Fujii H, Clanton TL. Unique *in vivo* applications of spin traps. Free Radic Biol Med 2001; 30:489–499.
[9] Hawthorne MF, Strahm RD. Kinetics of the thermal isomerization of 2-*tert*-butyl-3-phenyloxazirane. J Org Chem 1957;22:1263–1264.

[10] Harbour JR, Bolton JR. Superoxide formation in spinach-chloroplasts-electron-spin resonance detection by spin trapping. Biochem Biophys Res Commun 1975;64:803–807.
[11] Novelli GP, Angiolini P, Tani R, Consales G, Bordi L. Phenyl-*t*-butyl-nitrone is active against traumatic shock in rats. Free Radic Res Commun 1986;1:321–327.
[12] Floyd RA. Role of oxygen free-radicals in carcinogenesis and brain ischemia. FASEB 1990;4:2587–2597.
[13] Saito K, Yoshioka H, Cutler RG. A spin trap, *N*-*t*-butyl-phenylnitrone extends the life span of mice. Biosci Biotech Biochem 1998;62:792–794.
[14] Floyd RA, Hensley K, Forster MJ, Kelleher-Andersson JA, Wood PL. Nitrones, their value as therapeutics and probes to understand aging. Mech Ageing Dev 2002;123:1021–1031.
[15] Green AR, Ashwood T, Odergren T, Jackson DM. Nitrones as neuroprotective agents in cerebral ischemia, with particular reference to NXY-059. Pharmacol Ther 2003;100:195–214.
[16] Durand G, Polidori A, Ouari O, Tordo P, Geromel V, Rustin P, Pucci B. Synthesis and preliminary biological evaluations of ionic and nonionic amphiphilic phenyl-*t*-butylnitrone derivatives. J Med Chem 2003;46:5230–5237.
[17] Allouch A, Roubaud V, Lauricella R, Bouteiller JC, Tuccio A. Preparation and use as spin trapping agents of new ester-nitrones. Org Biomol Chem 2003;1:593–598.
[18] Chen GM, Bray TM, Janzen EG, Mccay PB. Excretion, metabolism and tissue distribution of a spin trapping agent, phenyl-*t*-butyl-nitrone (PBN) in rats. Free Radic Res Commun 1990;9:317–323.
[19] Lapchak PA, Araujo DM. Development of the nitrone-based spin trap agent NXY-059 to treat acute ischemic stroke. CNS Drug Rev 2003;9:253–262.
[20] Ionita P. Is DPPH stable free radical a good scavenger for oxygen active species? Chem Paper 2005;59:11–16.
[21] Damiani E, Castagna R, Astolfi P, Greci L. Aromatic and aliphatic mono- and bis-nitroxides: A study on their radical scavenging abilities. Free Radic Res 2005;39:325–336.
[22] Borisenko GG, Martin I, Zhao Q, Amoscato A, Kagan VE. Nitroxides scavenge myeloperoxidase-catalyzed thiyl radicals in model systems and in cells. J Am Chem Soc 2004; 126:9221–9232.
[23] Yukowa O, Nakajima T, Yukawa M, Ozawa T, Yamada T. Induction of radical scavenging ability and protection against radiation-induced damage to microsomal membranes following low-dose irradiation. Int J Radiat Biol 1999; 75:1189–1199.
[24] Goldschmidt S, Renn K. Amine oxidation IV. Diphenyl-trinitrophenylhydrazyl. Chem Ber 1922;55:628–643.
[25] Fargere T, Abdennadher M, Delmas M, Boutevin B. Determination of peroxides and hydroperoxides with 2,2-diphenyl-1-picrylhydrazyl (DPPH)—application to ozonized ethylene-vinyl acetate copolymers (EVA). Eur Polym J 1995;31:489–497.
[26] Emaru K, Askal H, Saleh G. Spectrophotometric determination of tetracycline and oxytetracycline in pharmaceutical preparations. Talanta 1991;38:1219–1221.
[27] Rigobello MP, Stevanato R, Momo F, Fabris S, Scutari G, Boscolo R, Folda A, Bindoli A. Evaluation of the antioxidant properties of propofol and its nitrosoderivative. Comparison with homologue substituted phenols. Free Radic Res 2004;38:315–321.
[28] Ionita P, Caproiu MT, Meghea A, Maior O, Rovinaru M, Ionita G. Synthesis based on 9-amino-*N*-picrylcarbazyl. Pol J Chem 1999;73:1177–1183.
[29] Damiani E, Belaid C, Carloni P, Greci L. Comparison of antioxidant activity between aromatic indolinonic nitroxides and natural and synthetic antioxidants. Free Radic Res 2003;37:731–741.
[30] Gille L, Prosch U, Stoesser R. Reactions of 1,1-diphenyl-2-picryl-hydrazyl with NO_2, Cl_2 and F_2. Radiat Phys Chem 1992;40:461–468.
[31] Caproiu MT, Covaci IC, Ionita P, Luca C, Constantinescu T, Balaban AT. Regioselectively [(NO_2)-N-^{15}]-labeled *N*-methoxypicramide and DPPH prepared by using crown ether and solid sodium [N-^{15}]nitrite. J Radioanal Nucl Chem 1999; 242:773–776.
[32] Ionita P, Gilbert BC, Whitwood AC. Synthesis and characterisation of some novel hetero-diradicals containing linked hydrazyl and aminoxyl (nitroxide) moieties. J Chem Soc Perkin Trans 2 2001;9:1453–1462.
[33] Ionita P, Gilbert BC, Whitwood AC. Authentic versus alternative mechanisms in spin trapping. Formation of azide spin-adducts in biphasic and non-aqueous systems by the oxidation of azide anion with a variety of hydrazyl radicals. J Chem Soc Perkin Trans 2 2000;12: 2436–2440.
[34] Ionita P, Gilbert BC, Whitwood AC. Generation of oxygen-, sulfur, carbon-, nitrogen- and phosphorus-centred short-lived radicals via one-electron oxidation with stable hydrazyl radical. Lett Org Chem 2004;1:70–74.
[35] Deby C, Benmilou M, Magottea G. Effect of a stable free-radical on survival of X-irradiated mice. Eur J Clin Biol Res 1970;15:678–680.
[36] Chalier F, Ouari O, Tordo P. ESR study of spin-trapping with two glycosylated analogues of PBN able to target cell membrane lectins. Org Biomol Chem 2004;2:927–934.
[37] Buettner GR. Spin trapping—electron-spin-resonance parameters of spin adducts. Free Radic Biol Med 1987;3: 259–303.