Herrmann, *Tetrahedron Lett.* **1997**, *38*, 2073–2074; f) N. P. Reddy, M. Tanaka, *Tetrahedron Lett.* **1997**, *38*, 4807–4810.

[4] a) C. Desmarets, R. Schneider, Y. Fort, *Tetrahedron Lett.* **2000**, *41*, 2875–2879; b) E. Brenner, R. Schneider, Y. Fort, *Tetrahedron Lett.* **2000**, *41*, 2881–2884; c) J. P. Wolfe, S. L. Buchwald, *J. Am. Chem. Soc.* **1997**, *119*, 6054–6058; d) R. Cramer, D. R. Coulson, *J. Org. Chem.* **1975**, *40*, 2267–2273.

[5] K. Hori, M. Mori, *J. Am. Chem. Soc.* **1998**, *120*, 7651–7652.

[6] For aromatic aminations a) in the absence of solvent, see: L. Schio, G. Lemoine, M. Klich, *Synlett* **1999**, 1559–1562; b) under high pressure, see: H. Kotski, H. Sakai, T. Shinohara, *Synlett* **2000**, 116–118; c) by nucleophilic substitution, see: A. J. Belfield, G. R. Brown, A. J. Foubister, P. D. Ratcliffe, *Tetrahedron* **1999**, *55*, 13285–13300.

[7] G. V. Smith, F. Notheisz in *Heterogeneous Catalysis in Organic Chemistry*, Academic Press, San Diego, **1999**.

[8] a) J. S. Bradley, B. Tesche, W. Busser, M. Maase, M. T. Reetz, *J. Am. Chem. Soc.* **2000**, *122*, 4631–4636; b) M. T. Reetz, E. Westermann, *Angew. Chem.* **2000**, *112*, 170–173; *Angew. Chem. Int. Ed.* **2000**, *39*, 165–168; c) M. T. Reetz, R. Breinbauer, P. Wedemann, P. Binger, *Tetrahedron* **1998**, *54*, 1233–1240.

[9] a) B. H. Lipshutz, P. A. Blomgren, *J. Am. Chem. Soc.* **1999**, *121*, 5819–5820; b) B. H. Lipshutz, T. Tomioka, P. A. Blomgren, J. Sclafani, *Inorg. Chim. Acta* **1999**, *296*, 164–169; c) B. H. Lipshutz, J. A. Sclafani, P. A. Blomgren, *Tetrahedron* **2000**, *56*, 2139–2144.

[10] It was important to maintain a strong reflux, which was accomplished by setting the oil bath temperature to 130°C.

[11] D. Guillaneux, H. B. Kagan, *J. Org. Chem.* **1995**, *60*, 2502–2505.

[12] A similar observation has been made in the case of 2,2′-bis(diphenylphosphanyl)-1,1′-binaphthyl (BINAP) and its monodentate analogue.[2a] By contrast, in refluxing dioxane (5% Ni/C, LiO*t*Bu, 18 h), use of 2.5% DPPF led to 95% conversion.

[13] M. Prashad, B. Hu, Y. Lu, R. Draper, D. Har, O. Repic, T. J. Blacklock, *J. Org. Chem.* **2000**, *65*, 2612–2614.

[14] B. Witulski, *Synlett* **1999**, 1223–1226.

[15] S. Djuric, J. Venit, P. Magnus, *Tetrahedron Lett.* **1981**, *22*, 1787–1790. Commercially available from Gelest.

[16] Representative procedure for the amination of aryl chlorides (Table 1, entry 1): Ni^{II}/C (50.5 mg, 0.038 mmol, 0.74 mmol g^{-1}), DPPF (10.7 mg, 0.019 mmol), and lithium *tert*-butoxide (74.3 mg, 0.90 mmol) were added to a flame-dried 5 mL round-bottomed flask under a blanket of argon at room temperature. Dry toluene (0.5 mL) was added by syringe and the slurry allowed to stir for 90 min. *n*-Butyllithium (31 μL, 2.42 M in hexanes, 0.075 mmol) was added dropwise with swirling. After 30 min, 4-chlorobenzonitrile (104.2 mg, 0.75 mmol), which was dissolved in dry toluene (0.5 mL), and morpholine (132 μL, 1.50 mmol) were added, followed by heating to reflux for 2.5 h (the oil bath temperature was set to 130°C). After cooling to room temperature, the crude reaction mixture was then filtered through a glass filter and the filter cake further washed with methanol and dichloromethane. The filtrate was collected, solvents were removed on a rotary evaporator and the crude product was then purified by flash chromatography with cyclohexane/EtOH (7/3) to give 123.8 mg (0.66 mmol; 88%) *N*-(4-cyanophenyl)-morpholine as a pale yellow solid.[4c]

[17] The extent of nickel bleed from the charcoal was quantitatively assessed to be 2.3–2.9% of the 5% Ni/C (i.e., 0.15% relative to substrate) using inductively coupled plasma (ICP) atomic emission spectrometry. For an overview of this technique, see: *Inductively Coupled Plasma Mass Spectrometry* (Ed.: A. Montaser), Wiley-VCH, New York, **1998**.

Application of a Spin-Labeled Spin-Trap to the Detection of Nitric Oxide (NO)**

Lucien Marx and André Rassat*

When a dilute monoradical reacts to give diamagnetic products, its EPR signal decays without major changes, while when a diradical is transformed into a monoradical, a new EPR spectrum is generally observed. In the case of a diradical with strong dipolar interaction,[1] the single broad line is replaced by three narrow lines, and there is a very large increase of the monoradical signal. A narrow-line monoradical in very low concentration and a broad-line diradical in much higher concentration display peaks of similar height,[2] as shown in Figure 1 where the EPR spectra of synthetic mixtures of tempone (2,2,6,6-tetramethyl-4-oxo-1-oxypiperidine (radical); line width $\Delta H = 0.18$ mT) and of diradical **1**[3] ($\Delta H = 4.2$ mT) are presented: even when the monoradical constitutes only 0.1% of the total paramagnetic species, its signal can be detected.

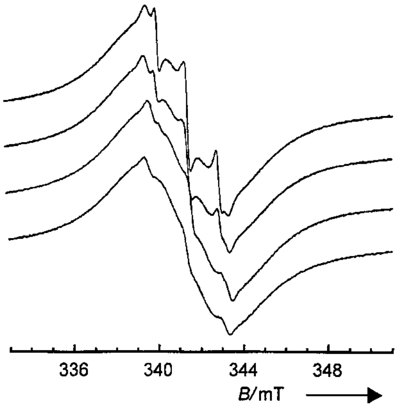


Figure 1. EPR spectra of ethanol solutions of synthetic mixtures containing (from top to bottom) 0, 0.1, 0.5, and 1 mole% of tempone relative to 10^{-3} M diradical **1**.

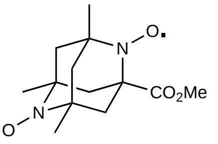
This suggests a new use of diradicals as *spin-labeled spin-traps.* These reagents would combine a stable radical (R_T), designed to trap selectively the radical to be detected and to yield diamagnetic products, and another radical (R_L), unreactive during this reaction, at a distance such that the diradical EPR spectrum would be a single line.[4] In this way, as R_T reacts, the signal of the R_L moiety would be detected at the very beginning of the reaction, with both the concentration of spin trap and of spin adduct being monitored on the same spectrum. Furthermore, the rate of the trapping reaction (usually bimolecular) could easily be increased by increasing the biradical concentration.

---

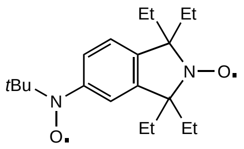
[*] Prof. A. Rassat, Dr. L. Marx
UMR CNRS 8640, Département de Chimie
Ecole Normale Supérieure
24 rue Lhomond, 75231 Paris CEDEX 05 (France)
Fax: (+33) 144323325
E-mail: andre.rassat@ens.fr

[**] This work was supported by the French Ministry of Education (ENS), the French Ministry of Defense, and CNRS.

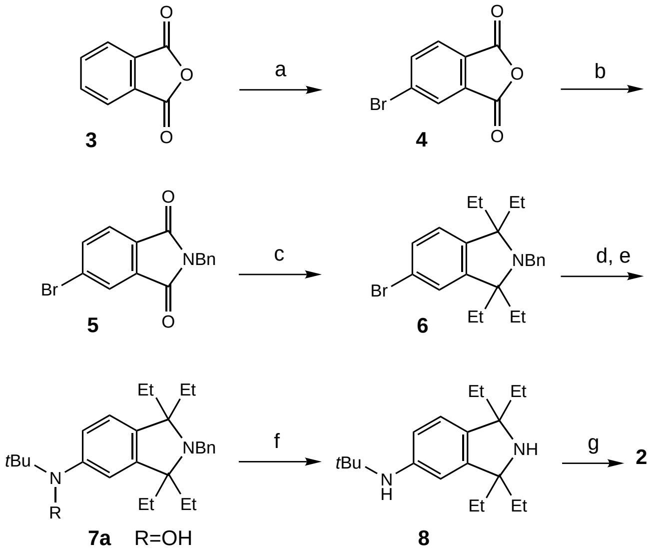
As an example of this method, we have studied the trapping of NO by a new diradical **2**. Aromatic nitroxides like diphenylnitroxide and derivatives[5] or some dihydroquinoline nitroxides[6] are known to react with NO, while the non-aromatic 3-carbamoyl-2,2,5,5-tetramethyl-3-pyrroline-1-yloxyl (radical) is reported to be unchanged in the presence of NO.[7] Thus **2** (see Table 1) was designed to combine these two aminoxyl groups on an isoindoline backbone (for the non-aromatic moiety ethyl rather than methyl substituents were chosen to decrease any eventual reactivity)[8] and was prepared in six steps from phthalic anhydride **3** (Scheme 1).[9]



1



2



Scheme 1. Synthesis of diradical **2**: a) Br_2, 51 %; b) $BnNH_2$, 95 %; c) EtMgBr, 25 %; d) *t*BuLi, (−78 °C, then 0 °C 30 min); e) *t*BuNO, (−78 °C), 51 %; f) H_2, Pd/C, 95 %; g) mCPBA, 60 %. Bn = benzyl, mCPBA = 3-chloro peroxybenzoic acid.

Figure 2 shows the EPR spectra of **2** in ethanol; in fluid solution (a), the broad line observed ($\Delta H = 2.5$ mT) is in accord with the large dipolar interaction ($D = 400$ MHz, $D/2\mu_B = 14$ mT)[10] measured on (b). Traces of monoradical (less than 1 %) are seen in (a) and are more visible on the central part of (b). After several hours at room temperature, these spectra were unchanged.

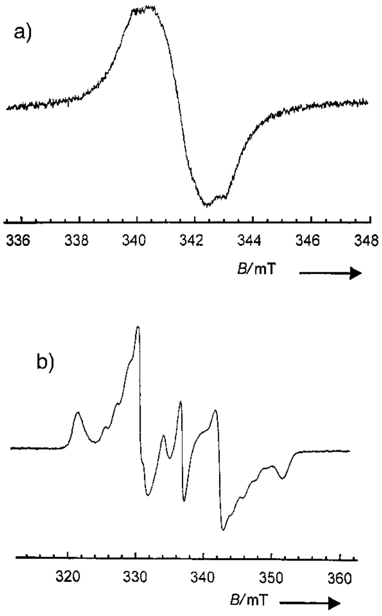


Figure 2. EPR spectrum of the diradical **2** (4×10^{-3} M in EtOH), a) at 20 °C, b) at 114 K.

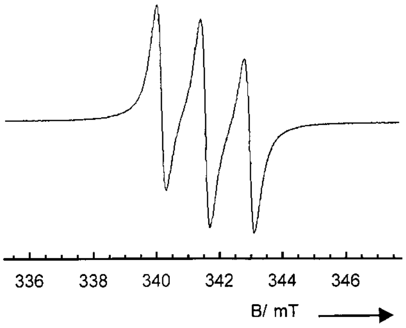
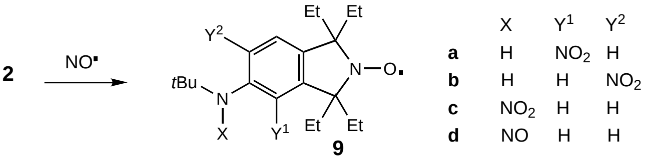


Figure 3. EPR spectrum obtained from a sample take after 30 minutes of the reaction of diradical **2** with NO.

When nitric oxide was bubbled into a deoxygenated solution of **2** in ethanol, samples were taken after 10, 30, 60, and 90 minutes and analyzed by thin layer chromatography (tlc) and EPR, a three-line spectrum, not apparent in the spectrum of the sample taken after 10 min, appeared superimposed on the diradical spectrum of the sample taken after 30 min (Figure 3). Four new nitroxides were then detected: they all displayed the same typical isoindolidinyloxyl (radical) 3-line EPR spectrum (toluene, $a_N = 1.37$ mT), totally different from the 9-line spectrum ($a_N = 1.26$ mT, $a_H = 0.23$ mT, 2 H) of **7b** (R=O$^{\bullet}$, obtained by oxidation of **7a**; Scheme 1). Traces of **2** were no longer observed in the sample taken after 90 min, and after chromatography on alumina, four new compounds **9a–d** (Scheme 2) eluted in this order, were assigned the structures shown on the basis of their EPR, NMR,[11] and UV spectra[12] and mass spectrometry (Table 1). These compounds display narrow lines in the EPR spectrum, a characteristic particularly useful in dynamic nuclear polarization (DNP),[13] and have recently been observed to present a large DNP enhancement factor.[14]



	X	Y1	Y2
a	H	NO2	H
b	H	H	NO2
c	NO2	H	H
d	NO	H	H

Scheme 2. Reaction of diradical **2** with NO.

Table 1. Selected analytical data for **2** and **9a**–**d**.

2: red liquid; elemental analysis calcd(%)for $C_{20}H_{34}N_2O_2$: C 72.28, H 9.63, N 8.43; found: C 72.23, H 9.83, N 8.46. **9a**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.2$ (s, 1H; CH), 5.1 (s, 1H; CH), 1.5 (s, 9H; CH_3); UV/Vis (EtOH): λ_{max}[nm] (ε) = 443 (1800), 246 (16000); high resolution MS (FAB): *m/z* (%): 364.2589 (21) [M^++2H], 363.2536 (40) [M^++H], 362.2450 (27) [M^+]. **9b**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 10.4$ (s, 1H; CH), 8.5 (s, 1H; CH), 1.6 (s, 9H; CH_3); UV/Vis (EtOH): λ_{max}[nm] (ε) = 440 (4600), 285 (*shoulder*, 4000), 242 (21000); high resolution MS (FAB): *m/z* (%): 363.2528 (100) [M^++H], 334.2135 (82.6) [$M^+ - C_2H_4$]. **9c**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.43$ (s, 1H; CH), 8.5 (s, 1H; CH), 5.9 (s, 1H; CH), 1.8 (s, 9H; CH_3); high resolution MS (FAB): *m/z* (%): 364.2594 (63) [M^++2H], 363.2518 (60) [M^++H], 362.2451 (51) [M^+], 348.2657 (73). **9d**: ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 9.8$ (s, 1H; CH), 9.1 (s, 1H; CH), 5.6 (s, 1H; CH), 1.8 (s, 9H; CH_3); high resolution MS (FAB): *m/z* (%): 362.2804 (12) [M^++CH_4], 348.2655 (22) [M^++2H], 347.2577 (44) [M^++H]

The use of this spin-trap for the in vivo detection of NO is more problematic because of the presence of reducing agents: when **2** was treated with ascorbic acid and the reaction monitored by EPR spectrocopy, the typical spectrum of **9e** (Scheme 2 where $X = OH$, $Y^1 = Y^2 = H$) immediately appeared and then decayed, but, as expected, at a much lower rate.[8] However, it is possible to distinguish between the NO adducts and **9e** by combining different analytical methods such as mass spectrometry, ^{1}H NMR spectroscopy,[7] (for **9a** and **9b**) and probably by high-field EPR spectroscopy.[15]

Analogously other spin-labeled spin-traps may be used to study the reactions of a radical R_T with reactive radicals.[16]

Received: January 31, 2000
Revised: September 25, 2000 [Z14623]

---

[1] J. Michon, A. Rassat, *J. Am. Chem. Soc.* **1974**, *96*, 335–337.

[2] Because EPR spectra are presented as "derivative of absorption", the number of spins responsible for an EPR line of peak-height *h* and width ΔH is proportional to $h(\Delta H)^2$.

[3] R. Chiarelli, A. Rassat, *Bull. Soc. Chim. Fr.* **1993**, *130*, 299–303.

[4] Typically, a single broad line is observed in fluid solutions when the average distance between the unpaired spins is smaller than 500 pm. (See ref. [1].)

[5] H. Wieland, K. Roth, *Chem. Ber.* **1920**, *53*, 210–230.

[6] H. Weber, A. Grzesiok, *Pharmazie* **1995**, *50*, 365–366, and references therein.

[7] R. J. Singh, N. Hogg, H. S. Mchaourab, B. Kalyanaraman, *Biochem. Biophys. Acta* **1994**, *1201*, 437–444.

[8] L. Marx, R. Chiarelli, T. Guiberteau, A. Rassat, *J. Chem. Soc. Perkin Trans. 1* **2000**, 1181–1182.

[9] **4**: H. J. Waldmann, *J. Prakt. Chem.* **1930**, *126*, 65–68; **5**: A. M. El-Sharief, N. E. Hammad, *Indian J. Chem. Sect. B* **1981**, *12*, 1039–1042; **6**: adapted from K. Heidenbluth, R. Scheffler, *J. Prakt. Chem.* **1964**, *23*, 59–70.

[10] In the point-dipole approximation (N. Hirota, S. I. Weissman, *J. Am. Chem. Soc.* **1964**, *86*, 2538–2545), this interaction corresponds to a distance $d = 580$ pm between the two localized spins. It is also the distance found by molecular mechanics (Hyperchem software) between the two nitrogen atoms.

[11] D. G. Gilles, L. H. Sutcliffe, X. Wu, *J. Chem. Soc. Faraday Trans.* **1994**, *90*, 2345–2349.

[12] The structures of isomers **9a** and **9b** were assigned on the basis of the effect of steric hindrance on their order of elution and on their UV absorption (J. N. Murrell, *The Theory of the Electronic Spectra of Organic Molecules*, Methuen, London, **1963**, p. 242).

[13] D. Grucker, T. Guiberteau, B. Eclancher, J. Chambron, R. Chiarelli, A. Rassat, G. Subra, B. Gallez, *J. Magn. Reson. B* **1995**, *106*, 101–109.

[14] T. Guiberteau, D. Grucker, personal communication.

[15] Ya. S. Lebedev, O. Ya. Grinberg, A. A. Dubinsky, O. G. Poluektov in *Bioactive Spin Labels* (Ed.: R. I. Zhdanov), Springer, Berlin, **1992**, pp. 227–278.

[16] Spin-labeled spin-traps may be applied to living free radical polymerization: W. Huang, personal communication.

Novel NO Biosensor Based on the Surface Derivatization of GaAs by "Hinged" Iron porphyrins**

Deng Guo Wu,* Gonen Ashkenasy,* Dmitry Shvarts,
Rachel V. Ussyshkin, Ron Naaman,*
Abraham Shanzer,* and David Cahen*

Nitric oxide (NO) plays an important role in biology as a mediator, for example, of the endothelial-derived relaxing factor (EDRF)—an agent responsible for the regulation of blood vessel relaxation and for the maintenance of blood pressure.[1] Concentrations of 30 to 300 ppm of NO (1 ppm ≈ 3.3×10^{-5} M) are sufficient to activate the guanylyl cyclase signaling cascade.[2] Since NO is a gaseous and highly reactive species, its direct detection at low concentrations is difficult. The methods used to sense NO have generally been based on following changes in the UV/Vis spectra or electrochemical properties of the sensing elements.[3] Electron paramagnetic resonance (EPR) and NMR image visualization of the distributions of NO free radicals in vivo were also reported, but were found to be limited by spatial resolution and sample size.[4, 5] We report here a novel approach for the direct detection of low concentrations of NO free radicals in physiological aqueous solution (pH = 7.4).

The Molecular Controlled Semiconductor Resistor (MOCSER; see reference [6]) provides an excellent way to

---

[*] Prof. Dr. D. Cahen, Dr. D. G. Wu
Dept. of Materials and Interfaces
Weizmann Institute of Science
Rehovot 76100 (Israel)
Fax: (+972) 8-934-4239
E-mail: david.cahen@weizmann.ac.il

Prof. Dr. A. Shanzer, G. Ashkenasy
Dept. of Organic Chemistry
Weizmann Institute of Science
Rehovot 76100 (Israel)
Fax: (+972) 8-9342917
E-mail: coshanzr@wiccmail.weizmann.ac.il

Dr. D. Shvarts
AMOS Ltd., Rehovot 76100 (Israel)

Prof. Dr. R. Naaman, Dr. R. V. Ussyshkin
Dept. Of Chemical Physics
Weizmann Institute of Science
Rehovot 76100 (Israel)
Fax: (+972) 8-9344123
E-mail: ron.naaman@weizmann.ac.il

[**] We thank Rachel Lazar for her help in the synthesis. This work was supported by the Israel Ministry of Sciences Tashtyoth program (D.C., R.N., and A.S.), and by a grant from the Fussfeld foundation (D.C. and A.S.). A.S. holds the Siegfried and Irma Ullman professorial chair.