

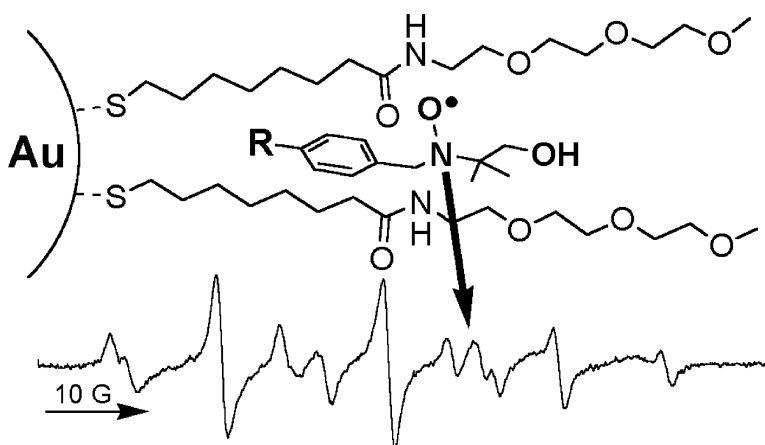
Article

EPR Study of Dialkyl Nitroxides as Probes to Investigate the Exchange of Solutes between the Ligand Shell of Monolayers of Protected Gold Nanoparticles and Aqueous Solutions

Marco Lucarini, Paola Franchi, Gian Franco Pedulli, Paolo Pengo, Paolo Scrimin, and Lucia Pasquato

J. Am. Chem. Soc., **2004**, 126 (30), 9326-9329 • DOI: 10.1021/ja048554f • Publication Date (Web): 08 July 2004

Downloaded from <http://pubs.acs.org> on April 1, 2009



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 4 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

[View the Full Text HTML](#)

EPR Study of Dialkyl Nitroxides as Probes to Investigate the Exchange of Solutes between the Ligand Shell of Monolayers of Protected Gold Nanoparticles and Aqueous Solutions

Marco Lucarini,^{*,†} Paola Franchi,[†] Gian Franco Pedulli,[†] Paolo Pengo,[‡]
Paolo Scrimin,[‡] and Lucia Pasquato^{*,§}

Contribution from the Department of Organic Chemistry "A. Mangini", University of Bologna, Via San Donato 15, 40127, Bologna, Italy; Department of Chemical Sciences and ITM-CNR, Padova Section, University of Padova, via Marzolo 1, 35131 Padova, Italy; and Department of Chemical Sciences University of Trieste, via L. Giorgieri 1, 34127 Trieste, Italy

Received March 12, 2004; E-mail: lucarini@alma.unibo.it; pasquato@dsch.univ.trieste.it

Abstract: EPR spectroscopy has been used to study the interaction of *para*-substituted benzyl hydroxyalkyl nitroxides with the monolayer of water-soluble protected gold cluster made by a short alkyl chain and a triethylene glycol monomethyl ether unit. The inclusion of nitroxide probes in the more hydrophobic environment of the monolayer gave rise to a reduction of the value of both nitrogen and β -proton hyperfine splittings. The spectra also showed selective line broadening attributed to modulation of the spectroscopic parameters as the result of exchange between free and complexed nitroxide. The rate constants were obtained by analyzing the EPR line shape variations as functions of nanoparticle concentration and temperature. This represents, to the best of our knowledge, the first determination of rate constants for the solubilization of organic substrates in a monolayer-protected cluster.

Introduction

Hydrophobic interactions dominate many important processes such as aggregation of surfactants and partition of biomolecules in biological membranes.¹ Interest in membrane mimetic systems stems from their ability to provide relevant features pertinent to natural membranes but also from the possibility to introduce unnatural functional groups thus expanding the range of their applications.²

Monolayer-protected nanoparticles (like gold nanoparticles, Au-MPC) constitute one additional example of membrane mimetic systems.³ They present the peculiarity of an extremely slow exchange of the monomers and of a limited mobility in the monolayer, thus allowing us to define precisely radial subregions of different polarity.^{3,4} This limited mobility is at the basis of their behavior as multivalent systems showing cooperativity in the recognition of substrates^{5a} or in performing catalytic processes.^{5b} The recent accessibility of water-soluble

systems⁶ has expanded their field of application also to this solvent where hydrophobic interactions are dominant.

EPR spectroscopy has been recently utilized to obtain useful information on the interaction of nitroxides with gold nanoparticles⁷ and to investigate the mechanism of the place-exchange reaction of thiols on the covering monolayer.^{8,9}

In recent studies on complexation of radical species in cyclodextrins,^{10a-c} calixarenes,^{10d} and micelles^{10e} in aqueous solutions, we have found benzyl *tert*-butyl nitroxide and related dialkyl nitroxides to be very suitable probes to investigate host-guest interactions. Evidence for the formation of paramagnetic complexes between these radicals and the host systems was provided by large spectral changes ($a(N)$ and $a(2H\beta)$) due to the less polar environment experienced by the radical guest and to conformational changes occurring upon complexation. The EPR spectra also showed a strong line width dependence on

[†] University of Bologna.

[‡] University of Padova.

[§] University of Trieste.

- (1) (a) Ben-Naim, A. In *Hydrophobic Interactions*; Plenum Press: New York, 1980. (b) Tanford, C. In *The Hydrophobic Effect*, 2nd ed.; Wiley: New York, 1980.
- (2) (a) Hoffmann, H.; Ebert, G. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 902–912. (b) Fuhrhop, J.-H.; Koenig, J. *Membranes and Molecular Assemblies, The Synergetic Approach*; Royal Society of Chemistry: Cambridge, 1994.
- (3) Badia, A.; Lennox, B. R.; Reven, L. *Acc. Chem. Res.* **2000**, *33*, 475–481.
- (4) (a) Hostetler, M. J.; Green, S. J.; Stokes, J. J.; Murray, R. W. *J. Am. Chem. Soc.* **1996**, *118*, 4212–4213. (b) Ingram, R. S.; Hostetler, M. J.; Murray, R. W. *J. Am. Chem. Soc.* **1997**, *119*, 9175–9178. (c) Hasan, M.; Bethell, D.; Brust, M. *J. Am. Chem. Soc.* **2002**, *124*, 1132–1133.
- (5) (a) Fantuzzi, G.; Pengo, P.; Gomila, R.; Hunter, C. A.; Pasquato, L.; Scrimin, P. *Chem. Commun.* **2003**, 1004–1005. (b) Pasquato, L.; Rancan, F.; Scrimin, P.; Mancin, F.; Frigeri, C. *Chem. Commun.* **2000**, 2253–2254.

- (6) (a) Kanaras, A. G.; Kamounah, F. S.; Schaumburg, K.; Kiely, C. J.; Brust, M. *Chem. Commun.* **2002**, 2294–2295 and references therein. (b) Zheng, M.; Davidson, F.; Huang, X. *J. Am. Chem. Soc.* **2003**, *125*, 7790–7791. (c) Pengo, P.; Polizzi, S.; Battagliarin, M.; Pasquato, L.; Scrimin, P. *J. Mater. Chem.* **2003**, *13*, 2471–2478.
- (7) (a) Templeton, A. C.; Hoestler, M. J.; Warmoth, E. K.; Chen, S.; Hartshorn, C. M.; Krishnamurthy, V. M.; Forbes, M. D. E.; Murray, R. W. *J. Am. Chem. Soc.* **1998**, *120*, 4845–4849. (b) Zhang, Z.; Berg, A.; Levanon, H.; Fessenden, R. W.; Meisel, D. *J. Am. Chem. Soc.* **2003**, *125*, 7959–7963.
- (8) Chechik, V.; Wellsted, H. J.; Korte, A.; Gilbert, B. C.; Caldaru, H.; Ionita, P.; Caragheorghopol, A. *Faraday Discuss.* **2004**, *125*, 279–291.
- (9) Ionita, P.; Caragheorghopol, A.; Gilbert, B. C.; Chechik, V. *J. Am. Chem. Soc.* **2002**, *124*, 9048–9049.
- (10) (a) M. Lucarini, M.; Luppi, B.; Pedulli, G. F.; Roberts, B. P. *Chem.—Eur. J.* **1999**, *5*, 2048–2054. (b) Franchi, P.; Lucarini, M.; Pedulli, G. F. *Angew. Chem., Int. Ed.* **2003**, *42*, 1842–1845. (c) Franchi, P.; Lucarini, M.; Mezzina, E.; Pedulli, G. F. *J. Am. Chem. Soc.* **2004**, *126*, 4343–4354. (d) Franchi, P.; Lucarini, M.; Pedulli, G. F.; Sciotto, D. *Angew. Chem., Int. Ed.* **2000**, *39*, 263–266. (e) Brigati, G.; Franchi, P.; Lucarini, M.; Pedulli, G. F.; Valgimigli, L. *Res. Chem. Intermed.* **2002**, *28*, 131–141.

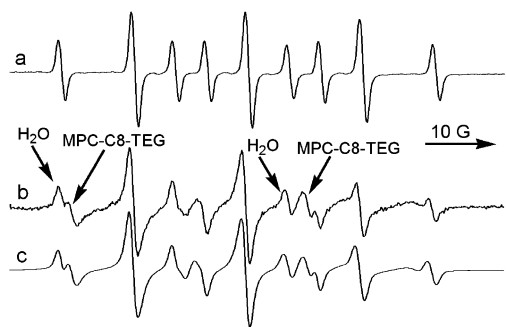
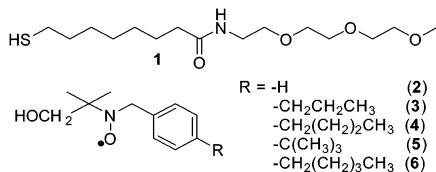


Figure 1. EPR spectra of **5** recorded in water (a) and in the presence of MPC-C8-TEG 0.3 mM (b) and computer simulation of the latter one (c).

Scheme 1



temperature, indicating that the lifetime of nitroxides in the associated and free form is comparable to the EPR time scale; this enabled us to measure the rate constants for the association and dissociation processes. In this paper, we describe the use of EPR to address the specific question of the interaction of an hydrophobic probe with the monolayer of water-soluble Au-MPC (MPC-C8-TEG) and how it compares with that of micelles. The monolayer of these nanoparticles was made by thiol **1** (see Scheme 1) comprising a short alkyl chain (C7) close to the gold surface and a triethylene glycol monomethyl ether unit (TEG).^{6c}

Results and Discussion

The radical probes we have used in this study belong to a family of *para*-substituted benzyl hydroxyalkyl nitroxides (**2–6**, Scheme 1). They were generated directly inside an EPR tube by oxidizing the parent amine (1 mM) with Oxone (1 mM) in the presence of variable amounts of MPC-C8-TEG. The amine precursors were prepared by reacting 2-methylalanine with the appropriately substituted benzoyl chlorides followed by reduction with 2 equiv of LiAlH₄ (see Supporting Information). The stability, under oxidative conditions, of MPC-C8-TEG nanoparticles was checked spectrophotometrically after the addition to a water solution of nanoparticles of a larger excess of Oxone. After 5 min, no significative shifting of the surface-plasmon band of the MPC-C8-TEG in the UV spectrum was observed, indicating that MPC is stable for the time required for recording an EPR spectrum.

Good EPR spectra of nitroxides **2–6** were obtained by oxidation of the corresponding amines (1 mM) with Oxone (1 mM) in water at 298 K. As an example, in Figure 1a is reported the spectrum obtained with nitroxide **5**. All the spectra were straightforwardly interpreted on the basis of the coupling of the unpaired electron with nitrogen and with the two equivalent benzylic protons, the spectroscopic parameters being reported in Table 1. When the EPR spectrum of nitroxide **2** is recorded in the presence of MPC-C8-TEG, no additional lines beside those due to the nitroxide radical in water solution were observed, this suggesting negligible affinity of **2** for the monolayer of MPC-C8-TEG.

Table 1. EPR Spectral Parameters in Water and in MPC (Bold) for Nitroxides **2–6**; Equilibrium (K_{eq}) and Rate (k^+ , k^-) Constants for the Complexation Process

probe	$a(N)^a$	$a(2H_\beta)^a$	g -factor	K_{eq}^a (M ⁻¹)	$k^+{}^b$ (M ⁻¹ s ⁻¹)	$k^-{}^b$ (s ⁻¹)
2	16.17	10.26	2.0056			
3	16.17	10.18	2.0056	4178	7.8×10^9	2.1×10^6
4	16.25	10.38	2.0056	11 250	8.0×10^9	7.4×10^5
5	16.14	10.16	2.0056	5683	7.7×10^9	1.9×10^6
6	16.32	10.35	2.0056	18 374	8.2×10^9	4.9×10^5

^a $T = 298$ K. ^b $T = 310$ K.

On the contrary, EPR spectra of nitroxides containing *para*-alkyl substituents in the phenyl ring (**3–6**) recorded in the presence of MPC-C8-TEG showed additional signals (see Figure 1b). When the concentration of MPC-C8-TEG nanoparticle was increased up to 1.3 mM, the spectrum of the new species became dominant, allowing the determination of its spectral parameters (see Table 1).

This species was identified as the radical hosted in the MPC monolayer, in equilibrium with the free nitroxide (Scheme 2), on the basis of the following experimental evidence.

(i) The EPR nitrogen hyperfine splitting $a(N)$ was significantly smaller than that of the free radical (see Table 1), this indicating that the nitroxide group is located in a less polar environment shielded from the aqueous solvent. The reduction of $a(N)$, remaining approximately constant by increasing the length of the linear alkyl chain, is due to the larger weight of the nitroxide mesomeric forms in which the unpaired electron is localized on the oxygen rather than on the nitrogen atom. This indicates that the polarity of the environment surrounding the nitroxide function is similar for all radicals **3–6** and that the N–O group is deeply inside the monolayer with the polar O–H group close to the hydrophilic region.

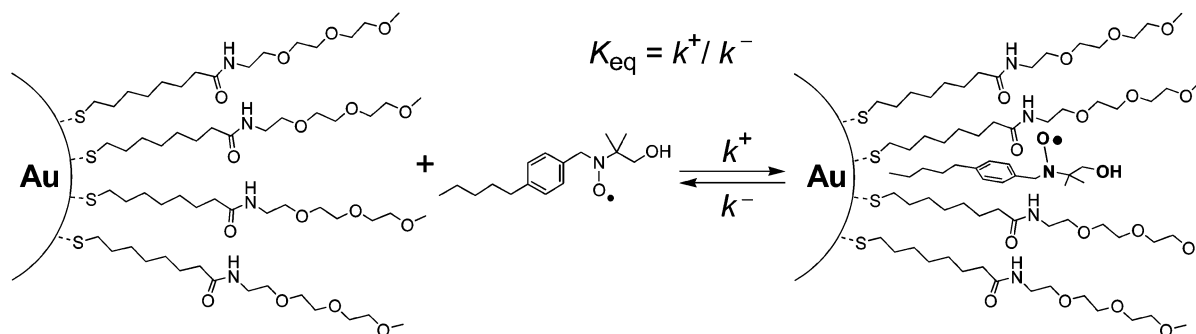
(ii) The high-field EPR lines of the new species were characterized by a lower height, due to the slower motion of the radical probe when hosted in the monolayer, resulting in incomplete averaging of the anisotropic components of the hyperfine and g -tensors.

(iii) The molar ratio between the complexed and free radical, obtained from the EPR spectra, increased linearly with increasing concentration of the dissolved MPC-C8-TEG. Addition of some water to the most concentrated solution in MPC-C8-TEG (1.3 mM) resulted in an EPR spectrum consisting only of the signals due to the unbound species, this providing experimental evidence that the EPR signals observed in the presence of MPC-C8-TEG are due to the radical hosted in the MPC monolayer in equilibrium with the free nitroxide.

It should be remarked that under the above experimental conditions the nitroxide is stable in the presence of Au-MPC during EPR measurements. In the literature, it has been reported^{7,11} that adsorption of nitroxide radicals onto a Au particle surface lead to the loss of its EPR signal. This behavior has been attributed to exchange interactions between the unpaired electron and conduction-band electrons of the metal. Our results clearly indicate that protection of a Au nanoparticle with organic monolayer avoids a direct contact between the

(11) Barkley, P. G.; Hornak, J. P.; Freed, J. H. *J. Phys. Chem.* **1986**, *84*, 1886–1900.

Scheme 2



nitroxide function and the metal surface due to the location of the spin probe inside the monolayer.

Since the MPC-C8-TEG nanoparticles were present in large excess with respect to the radical species,¹² whose concentration was in the range $(1-2) \times 10^{-5}$ M in all experiments, the association constant, K_{eq} , was obtained from the following equation: $K_{\text{eq}} = x_{\text{MPC}} / (x_{\text{water}} \times [\text{MPC}])$. Here [MPC] denotes the initial concentration of MPC-C8-TEG nanoparticles, and x_{MPC} and x_{water} are the mole fractions of the complexed and free radical species, respectively, determined by simulation of the EPR spectra (see Figure 1c). The values of K_{eq} (see Table 1) indicate that the solubilization of the probes by MPC-C8-TEG is clearly dependent on the hydrocarbon chain length: K_{eq} increases significantly by increasing the probe lipophilicity, with the exception of the bulky *tert*-butyl group.

The selective line-broadening, observed when recording the EPR spectra of **3–6** in the presence of protected gold nanoparticles at 310 K (see Figure 2), indicates rapid exchange of the nitroxide between the aqueous phase and the monolayer, which modulates the nitrogen and proton hyperfine splittings and, to a smaller extent, the Zeeman interaction. At 333 K, the observed splittings are completely averaged over the residence times in the two environments, in accordance with a fast exchange process in the EPR time scale. Simulation of the exchange-broadened EPR spectra, by using well-established

procedures based on the density matrix theory¹³ and assuming a two-jump model as illustrated in Scheme 2, led to the determination of the rate constants k^+ and k^- for the association and dissociation processes, respectively (see Table 1).

The dependence of these two rate constants on the nature of the *p*-alkyl group of the substrate was quite different, k^+ being almost independent of it, while k^- decreased significantly by increasing the lipophilicity of the probe, with the exception of the *tert*-butyl group. For the association process, both the independence on the R group and the high value of k^+ seem to indicate that rate of solubilization is controlled by diffusion. In the present case, where diffusion of a small molecule to a relatively larger sphere occurs, the process can be considered as a sequence of jumps over free-energy barriers between adjacent equilibrium positions in the liquid, so that the following equation can be derived: $k^+ = 4\pi NR_{\text{MPC}}D_{\text{probe}}$, where D is the diffusion coefficient of the probe in water, and R is the hydrodynamic radius of MPC-C8-TEG.¹⁴ Using $D_{\text{probe}} \approx 7 \times 10^{-6}$ cm²/s¹⁵ and $R_{\text{MPC}} = 41$ Å (see Experimental Section), one obtains $k^+ \approx 2.2 \times 10^{10}$ M⁻¹ s⁻¹, a value significantly higher than those experimentally measured for nitroxides **3–6** ($\approx 8.0 \times 10^9$ M⁻¹ s⁻¹). Actually, the energy barrier to the transfer of the probe from the aqueous phase to the interior of the monolayer can be imagined to reflect the search for a suitable spot on the surface among hydrophilic TEG chains and hydration water, a difficult process for nitroxides **3–6** containing a polar OH group.

The values of the dissociation rate constant, k^- , indicate that the escape parameters of a given probe are determined by the hydrophobicity of its alkyl chain, similarly to what was found in micelles.^{10e} Comparison of the values found when R = *n*- or *tert*-butyl indicates that for similar hydrophobicities an increased bulk reduces the residence time in the monolayer.

Conclusions

In conclusion, we have determined for the first time, using EPR spectroscopy, the partition isotherms and exchange rates of a radical probe between an aqueous solution and the monolayer of water-soluble Au-MPC. In comparison to micelles where it is impossible to know precisely how the monomers



Figure 2. EPR spectra of nitroxide **6** recorded in the presence of MPC-C8-TEG 0.3 mM at different temperatures.

- (12) Under these conditions, the nitroxides/MPC ratio was low enough to avoid possible anomalous effects due to multiple occupancy of MPC.
- (13) (a) Freed, J. H.; Fraenkel, G. K. *J. Chem. Phys.* **1963**, *39*, 326–348. (b) Hudson, A.; Luckhurst, G. R. *Chem. Rev.* **1969**, *69*, 191–225.
- (14) Rharbi, Y.; Li, M.; Winnik, M. A.; Hahn, K. G. *J. Am. Chem. Soc.* **2000**, *122*, 6242–6251.
- (15) Substituted aromatic compounds have D values in the range $5-9 \times 10^{-6}$ cm²/s. Burkey, T. J.; Griller, D.; Lindsay, D. A.; Scaiano, J. C. *J. Am. Chem. Soc.* **1984**, *106*, 1983–1985.

aggregate due to the disorder of the system, the monolayer of MPC-C8-TEG shows well-defined regions due to the specific orientation of the constituent thiols. This has allowed us to identify with confidence the locations of the probes in the monolayer at the boundary between the hydrocarbon and polyether regions, this being due to their amphiphilic character. Partition isotherms and rate of the exchange are nevertheless governed by the hydrophobic portions of the probes. Finally, EPR turned out to be a suitable technique to investigate the kinetic behavior of guest radicals in MPC, since the characteristic lifetime of the probes in the monolayer is comparable to the EPR time scale.

Experimental Section

General. NMR spectra involving nanoparticles were taken in CDCl_3 that had been stirred over K_2CO_3 for at least 24 h prior to use. ω -Bromooctanoic acid and thiolacetic acid were purchased from Aldrich, thiolacetic acid was distilled prior to use, tri(ethylene glycol) monomethyl ether was purchased from Fluka and used without further purification. Dry solvents were obtained from Fluka. All other solvents were reagent grade and used as received.

Synthesis of MPC-C8-TEG. $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ (59.2 mg, 0.15 mmol) and 30 mL of milliQ water were introduced in a 250 mL round-bottom flask, and to the pale yellow solution thiol **1**^{6c} (32 mg, 0.1 mmol) dissolved in 30 mL of deoxygenated methanol was added. Upon addition of the thiol, the solution turned reddish-brown, and the mixture was allowed to stir 30 min at room temperature and for a further 30 min at 0 °C. To the solution NaBH_4 (66 mg, 1.7 mmol) in 15 mL of water was added in 3 min and 30 s. The reaction mixture turned immediately wine-red; it was stirred at 0 °C for 30 min and at room temperature for 2 h. The solvent was removed under reduced pressure without heating above 35 °C. The residue was dried in vacuo, then dissolved in dichloromethane, and centrifuged. The solution was concentrated to a small volume and treated with ether in order to precipitate the nanoparticles. The black solid was washed copiously with ether and purified by gel permeation chromatography (Sephadex LH-60, methanol). After this treatment, 35 mg of nanoparticles were obtained. UV-vis (water) λ_{max} (nm); a ($\text{l g}^{-1} \text{cm}^{-1}$) 510.4; 8.2. IR (KBr) ν (cm^{-1}) 3296, 2915, 2848, 1736, 1642, 1548, 1465, 1452, 1351, 1258, 1197, 1107, 1019, 1003, 798. ^1H NMR (250 MHz, CDCl_3) δ 3.63 (br), 3.54 (br), 3.38 (br), 2.19 (br), 1.61 (br), 1.33 (br). ^1H NMR (250 MHz, D_2O) δ 3.50 (br), 3.22 (br), 2.10 (br), 1.44 (br), 1.07.

TEM samples were prepared by placing a single drop of <1 mg/mL dichloromethane solution onto a 200-mesh copper grid coated with

amorphous carbon film. The grid was then dried in air for 24 h. Images were obtained with a JEOL 3010 high-resolution electron microscope (1.7 nm point-to-point) operating at 300 keV using a Gatan slow-scan CCD camera (mod.794). Diameters were measured manually using a Gatan software Digital Micrograph (ver. 3.4.1) on at least 100 particles.

The MPC used have an average core diameter of 3.4 ± 0.7 nm and a calculated MW of 324 700 Da. When a completely extended ligand is considered, the calculated MPC radius is about 4.1 nm.

EPR Measurements. Radicals **2–6** were generated by mixing 1 μL of a methanol solution containing the corresponding amine (0.1 M) and 1 μL of a water solution containing Oxone (0.1 M) with 100 μL of a water solution containing variable amounts of MPC-C8-TEG. Samples were transferred into capillary tubes (1 mm i.d.) and then placed inside the thermostated cavity of an EPR spectrometer. EPR spectra were obtained using a Bruker ESP300 spectrometer equipped with an NMR gaussmeter for field calibration and a Hewlett-Packard 5350B microwave frequency counter for the determination of the g -factors, which were referenced to that of the perylene radical cation in concentrated H_2SO_4 ($g = 2.00258$). The sample temperature was controlled with a standard variable temperature accessory and was monitored before and after each run using a copper-constantan thermocouple. The instrument settings were as follows: microwave power 5.0 mW, modulation amplitude 0.05 mT, modulation frequency 100 kHz, scan time 180 s. Digitized EPR spectra were transferred to a personal computer for analysis using digital simulations carried out with a program developed in our laboratory and based on a Monte Carlo procedure.^{10a} The input data for the program are the number of nonequivalent nuclei, the hyperfine splitting constants of the free and included radical, the intrinsic line width in the absence of exchange, and the rate constants for the exchange process.

Acknowledgment. Financial support from MIUR, Contract 2002038342 (P.F., M.L., and G.F.P.) and from the University of Padova (progetti di Ricerca di Ateneo 2000 and Progetti Giovani Ricercatori 2001) and MIUR, Contract 2003054199 (to L.P.) are gratefully acknowledged.

Supporting Information Available: General procedure for the preparation of probes **2–6**, some representative EPR spectra of **3–6**, detailed radical ratios determined from the EPR spectra, and stability experiments of MPC-C8-TEG in the presence of Oxone. This material is available free of charge via the Internet at <http://pubs.acs.org>.

JA048554F