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Synthesis of Novel Aromatic Nitroxides as Potential DNA Intercalators. An EPR Spectroscopical and DFT Computational Study

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The synthesis and electron paramagnetic resonance (EPR) spectroscopic properties of three novel aromatic nitroxides and potential DNA intercalators, the carbazole-based 3,6-dimethylcarbazole-9-oxyl, as well as the acridane-based 9-acridanylidenemalonitrile-10-oxyl and 9-ethylacridanylidenecyanoacetate-10-oxyl, are described. The two acridane-based nitroxides can be isolated and are stable in solution as well as in the solid state for several days. Continuous wave X-band EPR measurements and density functional theory (DFT) calculations demonstrated that the spin density is delocalized over the whole molecule in all three cases. Furthermore, the DFT calculations provided insight into the molecular and electronic structures of these nitroxides and yielded hyperfine coupling constants which are in very good agreement with the experimental data allowing therefore an unambiguous assignment of the hyperfine couplings.

Introduction

Spin labeling of DNA[1] or RNA[2] with nitroxides is commonly used to gain insight into the structure[3] and mobility[4] of these biopolymers. Nevertheless, spin labeling on the periphery of DNA or RNA may yield erroneous results if motions or electronic properties of bases within oligonucleotides, e.g., spin-exchange coupling through DNA bases, are studied. One way to overcome this problem is to use nitroxides that intercalate between two base pairs. These intercalating nitroxides need to be stable in liquid solution and to possess extended planar and heterocyclic π-systems. They should also be polarizable and polar or charged, as the well-known diamagnetic intercalators ethidium[5] or acridine.[6] Furthermore, they ought to intercalate with the N−O group between the bases or the spin density should be delocalized into the intercalating part of the nitroxide. Aromatic nitroxides[7] may be a class of substances, which could match these criteria. However, they are rare and mostly characterized as unstable,[7] because no β-methyl groups protect the N−O center as in TEMPO. Two aromatic nitroxides potentially capable of intercalating into DNA, carbazole-9-oxyl **1**• and 9,9-diphenylacridane-10-oxyl **2**• (Figure 1), were described earlier in the literature. **1**• was synthesized by Aurich et al. in a photochemical way[8] and showed a delocalization of the N−O spin density over the whole aromatic ring system, but was short-lived and could not be isolated. **2**• was synthesized by Greci et al. via oxidation of 9,9-Diphenylacridane-10-oxyl, isolated and described as stable.[9] We synthesized these two nitroxides again to study their stability and EPR properties in more detail. Additionally, we synthesized the three novel aromatic nitroxides **3**•, **4**•, and **5**• depicted in Figure 1 and also studied their stability and EPR properties. Finally, we performed DFT-based geometry optimizations and EPR property calculations on all five compounds to get more insight into their molecular and electronic structure as well as to assign the experimental hyperfine couplings.

Results and Discussion

Synthesis. The carbazole-based aromatic nitroxides **1**• and **3**• as well as the acridane-based nitroxides **4**• and

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(1) (a) Berliner, L. J., Ed. *Biological Magnetic Resonance. Spin Labeling the Next Millenium*; Plenum Press: New York, 1998; Vol. 14. (b) Strube, T.; Schiemann, O.; MacMillan, F.; Prisner, T. F.; Engels, J. W. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 1271.

(2) (a) Edwards, T. E.; Okonogi, T. M.; Robinson, B. H.; Sigurdsson, S. T. *J. Am. Chem. Soc.* **2001**, *123*, 1527. (b) Verma, S.; Eckstein, F. *Annu. Rev. Biochem.* **1998**, *67*, 99.

(3) (a) Rabenstein, M. D.; Shin, Y.-K. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 8239. (b) Macosko, J. C.; Pio, M. S.; Tinoco, J. R.; Shin, Y.-K. *RNA* **1999**, *5*, 1158.

(4) (a) Edwards, T. E.; Okonogi, T. M.; Sigurdsson, S. T. *Chem. Biol.* **2002**, *9*, 1. (b) Miller, T. R.; Alley, S. C.; Reese, A. W.; Solomon, M. S.; MacCallister, W. V.; Mailer, C.; Robinson, B. H.; Hopkins, P. B. *J. Am. Chem. Soc.* **1995**, *117*, 9377.

(5) Wan, C.; Fiebig, T.; Kelley, S. O.; Treadway, C. R.; Barton, J. K.; Zewail, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6014.

(6) Davis, W. B.; Hess, S.; Naydenova, I.; Haselsberger, R.; Ogrodnik, A.; Newton, M. D.; Michel-Beyerle, M.-E. *J. Am. Chem. Soc.* **2002**, *124*, 2422.

(7) (a) Volodarsky, L. B.; Reznikov, V. A.; Ovcharenko, V. I. *Synthetic Chemistry of Stable Nitroxides*; CRC Press: Boca Raton, New York, 1994. (b) Kochi, J. K. *Free Radicals*; Wiley-Interscience: New York, 1973; Vols. 1 and 2.

(8) (a) Aurich, H. G.; Bach, G.; Hahn, K.; Küttner, G.; Weiss, W. J. *Chem. Res., Miniprint* **1977**, 1541. (b) Falle, H. R.; Luckhurst, G. R. *Mol. Phys.* **1967**, *12*, 493. (c) Aurich, H. G., Weiss, W. *Chem. Ber.* **1973**, *106*, 2408.

(9) (a) Carloni, P.; Greci, L.; Mar'in, A.; Stipa, P. *Polym. Degrad. Stab.* **1994**, *44*, 201. (b) Berti, C.; Colonna, M.; Greci, L.; Marchetti, L. *Gaz. Chim. Ital.* **1978**, *108*, 659.

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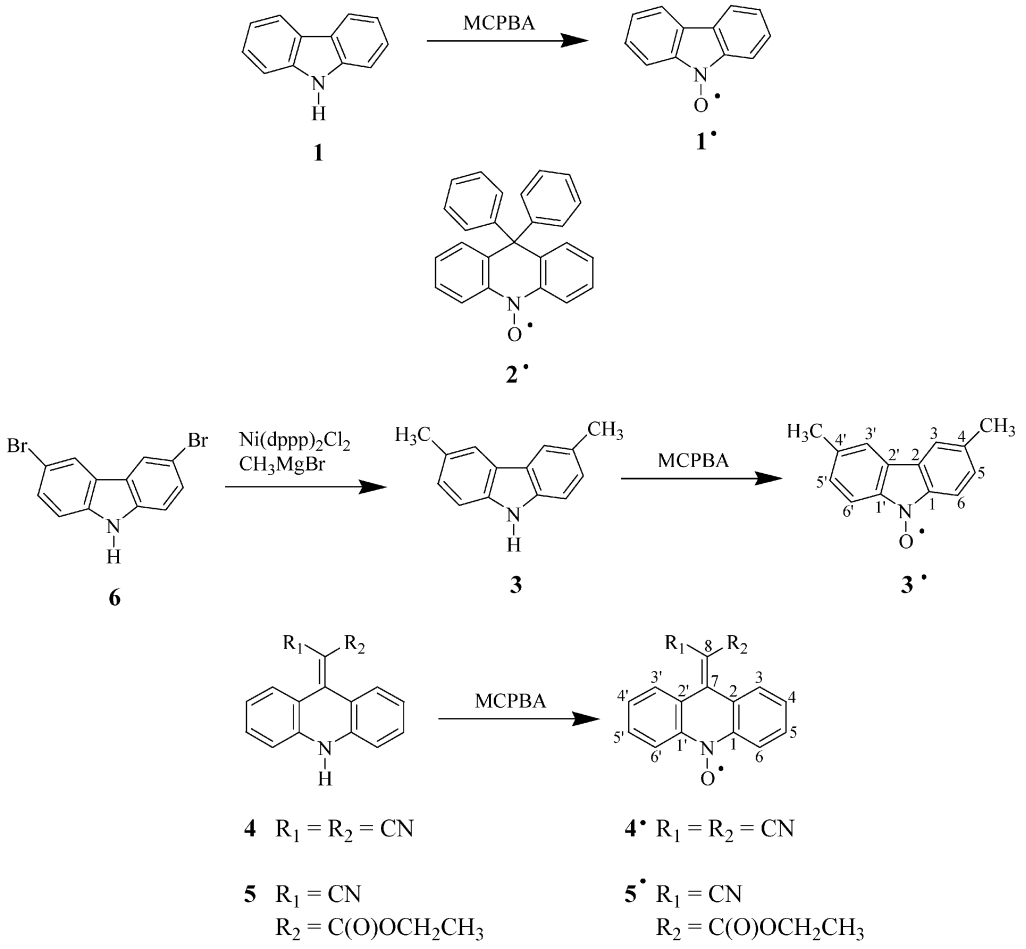

FIGURE 1. Structures of compounds **1**•−**5**• and reaction schemes.

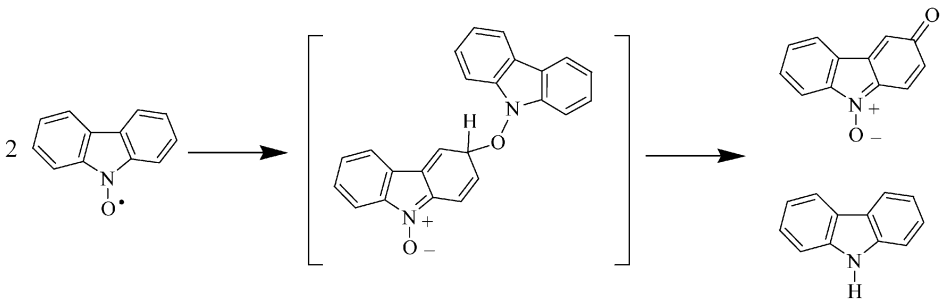

FIGURE 2. Proposed decomposition of **1**• via dimerization.

5• were synthesized by oxidation of the respective secondary amines using meta-chloroperbenzoic acid as the oxidant (Figure 1). **2**• was synthesized according to Greci.[9a]

As already stated in the literature,[8] **1**• could not be isolated due to decomposition. Yet, mass spectroscopy and EPR measurements of **1**• in diluted solutions proved its identity. One reason for the instability of **1**• is thought to be a dimerization reaction between two nitroxides according to Figure 2.[7] Therefore, **3**• was synthesized with methyl groups protecting the 4,4′-positions. Its precursor **3** was synthesized via a nickel(0)-catalyzed cross-coupling reaction of 3,6-dibromocarbazole **6** with methylmagnesium bromide. Various attempts to synthesize **3** by methylation of **6** without this catalyst but using different methylation reagents and conditions failed. Although **3**• could not be isolated as a solid, its stability in solution is increased compared to **1**• (see EPR part). Finally, the acridane derivatives **4**• and **5**• could be isolated as yellow solids and are stable up to a week, like derivative **2**•, previously reported in the literature.[9b] **5**• was synthesized because its carboxy group provides the possibility to tether it to DNA oligonucleotides,[10] allowing to restrict the site of intercalation and to overcome the weak water solubility of the compounds.

(10) (a) Gloss, G. L.; Johnson, M. D.; Miller, R. J.; Piotroviak, P. *J. Am. Chem. Soc.* **1989**, *111*, 3751. (b) Gloss, G. L.; Miller, R. J. *Science* **1988**, *240*, 440. (c) Wachter, L.; Jablonski, J.-A.; Ramachandran, K. L. *Nucleic Acids Res.* **1984**, *14*, 7985.

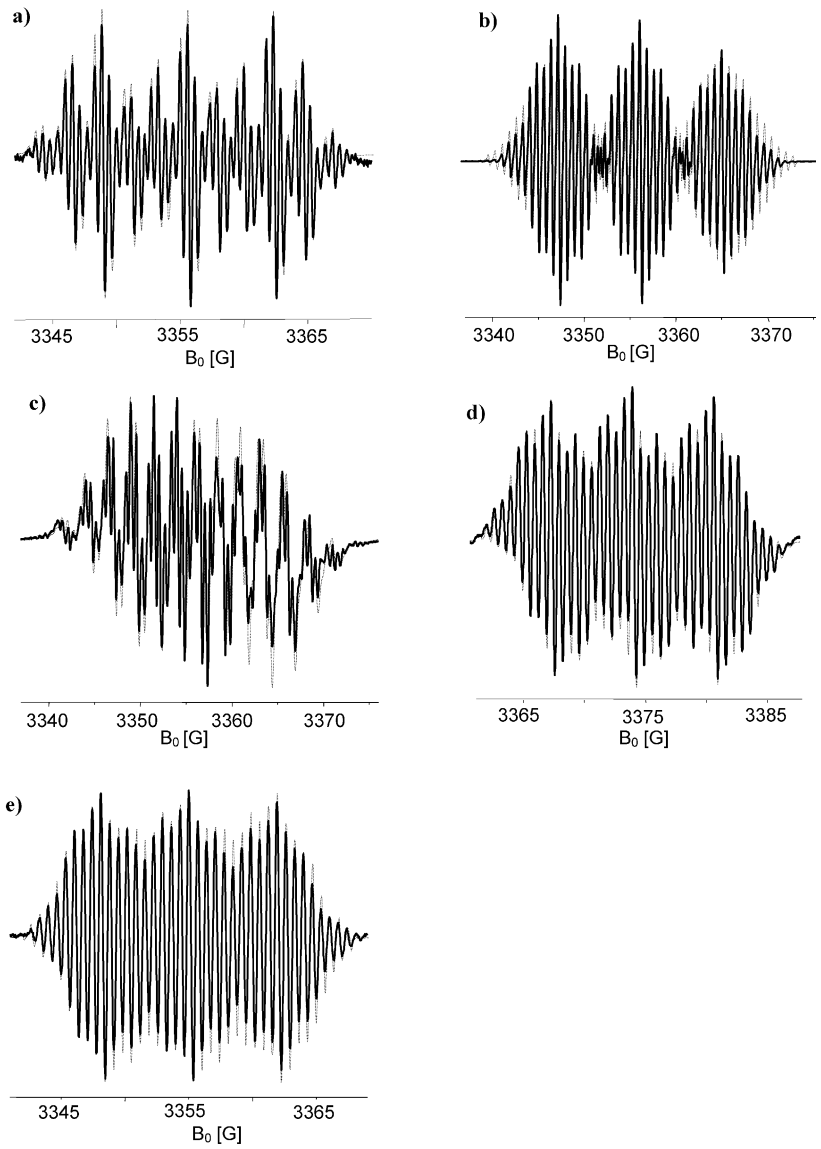

FIGURE 3. CW X-band EPR spectra of (a) **1**• (f = 9.4270 GHz), (b) **2**• (f = 9.4270 GHz), (c) **3**• (f = 9.4270 GHz), (d) **4**• (f = 9.4777 GHz), and (e) **5**• (f = 9.4270 GHz) in benzene-d_6. All spectra were recorded at room temperature. The dotted curves represent the simulated spectra.

EPR. The cw X-band EPR spectra of substances **1**•−**5**• measured at room temperature are depicted in Figure 3 together with the simulated spectra. The simulations were performed with a home-written program and fit to the experimental spectra utilizing a simplex fit routine. The obtained ^{1}H and ^{14}N hyperfine coupling constants are summarized in Table 1 together with the g_{iso} values.

Generally, cw X-band EPR spectra of alkyl nitroxides in fluid solutions are characterized by three lines due to the isotropic ^{14}N hyperfine coupling of ∼14−15 G as well as small and usually not resolved hyperfine couplings to nearby ^{1}H nuclei.[7] The reason for this is the localization of spin density to over 95% on the N−O group. The aromatic nitroxides studied here showed, in contrast to alkyl nitroxides, resolved hyperfine couplings for all the ring protons and for **3**•, **4**•, and **5**• even to the methyl and nitrile substituents, respectively. This delocalization of spin density from the N−O group into the ring system decreased the ^{14}N hyperfine coupling to about 7 G for **1**•, **3**•, **4**•, and **5**• and to 9 G for **2**•, leaving only roughly 50% of the spin density on the N−O nitrogen. The hyperfine coupling constants for **2**• found here differ from the ones reported in the literature.[8,9] The line broadening with increasing field observable for **3**• was accounted for by incorporating an m_I-dependence of the line width for the ^{14}N hyperfine couplings.

The stability of the nitroxides in diluted solution and at room temperature was studied by monitoring the EPR signal intensity as a function of time. In this way, **1**• was found to be fully decomposed after 12 h. The introduction of methyl groups at the 4 and 4′ positions of **1**• increased the stability slightly in such a way that the EPR signal of **3**• was detectable for 48 h. The reason for the instability of **3**• may be the high amount of spin density at the methyl groups, indicated by the relatively large hyperfine coupling constant for the methyl hydrogens, which makes a decomposition by dimerization involving the methyl groups possible. The acridane-based nitroxides **2**•, **4**•, and

TABLE 1. EPR Parameters of 1•, 2•, 3•, 4•, and 5• As Obtained from Fits of the Experimental Spectra[a]

	g_{iso}	$A_{iso}(^{14}N)$ (G)	$A_{iso}(^{1}H)$ (G)	ΔH_{pp} (G)
1•	2.0072	6.73/+5.37 (NO)	2.36/−2.86 (H6,6′)	0.17
			2.27/−2.76 (H4,4′)	
			0.59/+1.05 (H5,5′)	
			0.50/+0.98(H3,3′)	
2•	2.0066	8.92/+7.57 (NO)	3.03, 3.10/−3.09 (H6,6′)	0.17
			2.39, 2.28/−2.99 (H4,4′)	
			1.58, 1.52/+1.50 (H5,5′)	
			0.80, 0.71/+1.42 (H3,3′)	
3•	2.0061	6.98/+5.48 (NO)	2.52/−2.86 (H6,6′)	$a = 0.19^c$
			2.47/+2.50 $(CH_3)^b$	$b = -0.04^c$
			0.61/+1.12 (H5,5′)	$c = 0.01^c$
			0.61/+0.93 (H3,3′)	
4•	2.0067	6.91/+5.56 (NO)	2.10/−2.89 (H6,6′)	0.19
		0.61/+0.76 (2xCN)	2.06/−2.64 (H4,4′)	
			0.71/+1.60 (H5,5′)	
			0.17/+1.37 (H3,3′)	
5•	2.0075	6.90/+6.11 (NO)	2.07/−2.98 (H6,6′)	0.14
		0.70/+0.79 (CN)	2.00/−2.80 (H4,4′)	
			0.81/+1.69 (H5,5′)	
			0.66/+1.57 (H3,3′)	

[a] The hyperfine coupling constants following the slash are the ones obtained from the unrestricted PBE0/6-31G(d) DFT calculations. The numbering of the protons follows the numbering scheme given in Figure 1. [b] The isotropic ^{1}H hyperfine coupling constants of CH_3 in **3•** were calculated by averaging over the three methyl hydrogens: $A_{iso} = (A_{iso,1} + A_{iso,2} + A_{iso,3})/3$. [c] m_I-dependence of the line width of the ^{14}N hyperfine couplings according to $\Delta H_{pp} = a + bm_I + cm_I^2$.

5• were more stable in solution than the carbazole-derived nitroxides, yielding unchanged EPR signals even after 6 days, which may originate from the steric protection of the 4,4′ positions by the phenyl, nitrile, or ester substituents. The stability of **1•** and **3•** is too low to use them as DNA intercalators, whereas **2•**, **4•**, and **5•** are suitable candidates from a stability point of view.

Calculations. Geometry optimizations of all five nitroxides revealed that the carbazole nitroxides **1•** and **3•** are planar (Figure 4a). On the other hand, the three acridane nitroxides **2•**, **4•**, and **5•** deviate from planarity by an angle of 22° for **2•** and of 17° in the case of **4•** and **5•** (Figure 4b). Moreover, the substituents at the double bond between the carbon atoms 7 and 8 are not in plane but bent above and below the double bond plane. These deviations from planarity are thought to be induced by a steric interaction of the hydrogens in the 3 and 3′ positions with the phenyl, nitrile, or ester substituents.

The geometry-optimized structures were then used to calculate the electronic and EPR properties as well as dipole moments and polarizabilities. These calculations showed that in contrast to the localized singly occupied molecular orbitals (SOMOs) of alkyl nitroxides[11] the SOMOs of all five aromatic nitroxides are extended over the whole π-system and even onto the substituents in the case of **3•**, **4•**, and **5•** as shown for **3•** and **5•** in Figure 5a and c. The positive spin density distribution follows the shape of the SOMOs (Figure 5b and d). Due to spin polarization, negative spin density is induced at those carbon atoms of the rings for which the SOMOs possess a node.[12] The spin density on the ring hydrogens is also caused by spin polarization, as indicated by the opposite

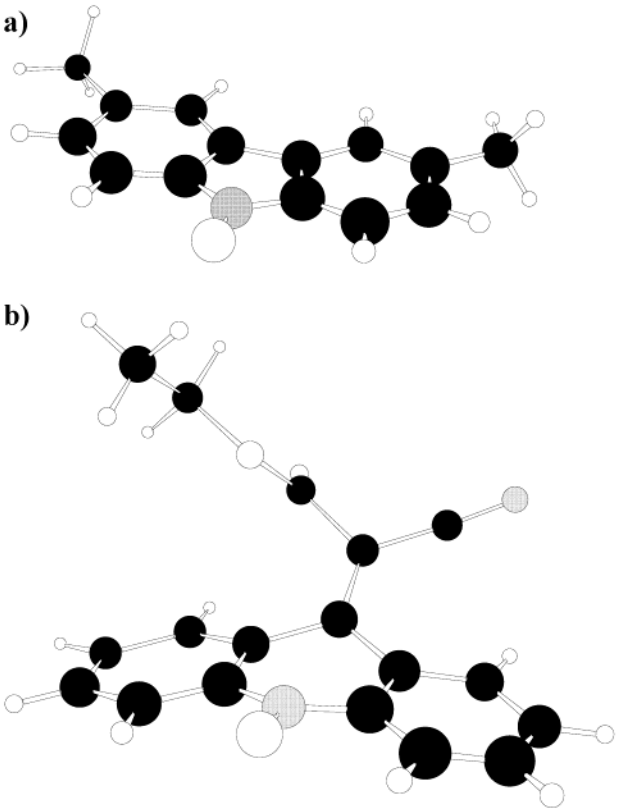

FIGURE 4. Geometry-optimized molecular structures of the (a) planar carbazole nitroxide **3•** and (b) nonplanar acridan nitroxide **5•** as obtained from unrestricted PBE0/6-31G(d) calculations.

sign of the spin density at the corresponding carbon atom (Figure 5b and d). On the other hand the spin density at the hydrogens of the methyl groups of **3•** is transferred by hyperconjugation, leading to the same sign for the spin density as for the methyl substituted ring carbon atoms. A considerable amount of spin density was also found on the nitrile groups in **4•** and **5•** (Figure 5d) as expected from the shape of the SOMOs and in agreement with the EPR measurements. Finally, it should be mentioned that the total atomic spin density of about +0.50 on the oxygen of the N−O group observed for the aromatic nitroxides is comparable to those found in alkyl nitroxides, whereas the spin density on the nitrogen (+0.25) is diminished by 50%.

With these spin density distributions, isotropic ^{14}N and ^{1}H hyperfine coupling constants were calculated that are in very good agreement with the experimentally obtained ones, allowing a reliable assignment of the experimental hyperfine couplings to the respective nuclei (Table 1). Yet, the calculated isotropic ^{14}N hyperfine coupling constants of the N−O group are slightly smaller than the experimental ones, as commonly found in DFT calculations of nitroxides,[13] whereas the calculated isotropic ^{1}H hyperfine couplings are slightly larger.

Additionally, the dipole moments and average polarizabilities of compounds **1•**−**5•** were calculated and are collected in Table 2. A comparison of the dipole moments

(11) (a) Improta, R.; Scalmani, G.; Barone, V. *Chem. Phys. Lett.* **2001**, *336*, 349. (b) Fritscher, J.; Beyer, M.; Schiemann, O. *Chem. Phys. Lett.* **2002**, *364*, 393.

(12) Weil, J. A.; Bolton, J. R.; Wertz, J. E. *Electron Paramagnetic Resonance. Elementary Theory and Practical Applications*; Wiley-Interscience: New York, 1994; pp 248−255.

(13) Barone, V. In *Recent Advances in Density Functional Methods*; Chong, D. P., Ed.; World Scientific: Singapore, 1995; p 287.

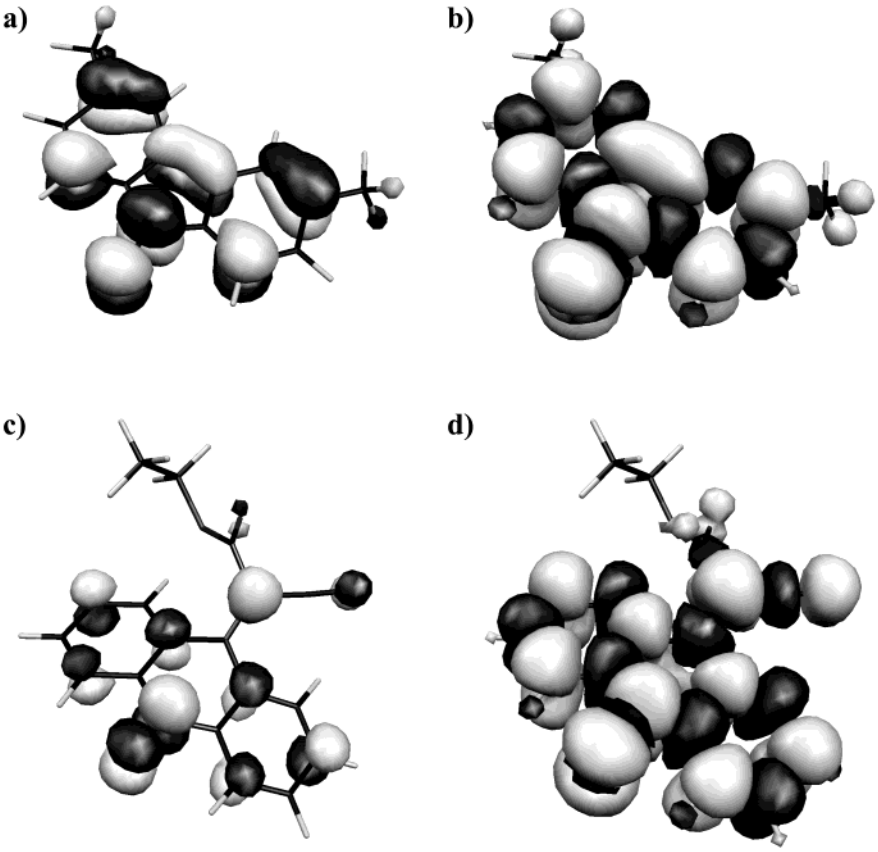

FIGURE 5. Singly occupied molecular orbitals (SOMOs; a and c) and spin density distributions (b and d) of **3**• and **5**•, respectively, showing the delocalization of the unpaired electron due to the heterocyclic π-system. Computations were performed on the unrestricted PBE0/6-31G(d) level of theory. Positive spin densities are shown in light gray and negative ones in dark gray.

TABLE 2. Dipole Moments in Debye and Average Polarizabilities in Bohr^3 of 1•−5• As Obtained from Unrestricted PBE0/6-31G(d) DFT Calculations

	dipole moments (D)	average polarizability $<\alpha>^a$ (B^3)
1•	2.67	135.7
2•	2.95	259.5
3•	3.27	165.4
4•	5.61	203.9
5•	4.42	221.8

^{a} $<\alpha> = {}^1/_3(\alpha_{xx} + \alpha_{yy} + \alpha_{zz})$.

showed that they are increasing from 2.67 to 5.61 D in the order **1**•, **2**•, **3**•, **5**•, and **4**•, which is attributed to the increasing number and strength of the electron-withdrawing groups. The values for **1**•, **2**•, and **3**• of roughly 3 D are typical for nitroxides as was found experimentally[14] and theoretically.[15] The dipolar vectors point from the nitriles to the N−O group for **4**• and **5**• and along the O−N bond axis from the oxygen to the nitrogen for **1**•, **2**•, and **3**•.

The polarizabilities were calculated because Hobza et al. showed that the intercalation between base pairs is mostly driven by dispersion forces caused by the polarizability.[16] The calculated average polarizabilities are relatively large with values up to 260 B^3. A comparison of the large dipole moments of 5.61, 4.42, and 2.95 D as well as the large polarizabilities of 203.9 B^3, 221.8 B^3, and 259.5 B^3 calculated for **4**•, **5**•, and **2**•, respectively, with the ones of ethidium (2.3 D, 236 B^3) and daunomycin (18.6 D, 297 B^3),[16] both well-known and strong DNA intercalators, makes all three aromatic nitroxides promising candidates as DNA intercalators.

Conclusions

The novel aromatic nitroxides **3**•, **4**•, and **5**• were synthesized. Out of these, **4**• and **5**• were isolated and are stable in the solid state and in solution up to 1 week. All three compounds possess, as the literature-known aromatic nitroxides **1**• and **2**•, an extended heterocyclic and polar π-system and show the anticipated delocalisation of spin density into the π-system, as revealed by EPR spectroscopy and DFT calculations. Furthermore, it was found by geometry optimization studies that the π-systems of **1**• and **3**• are planar, whereas the annellated benzene systems of **4**• and **5**• deviate from planarity by 17° and the one of **2**• by 22°. Candidates for DNA-intercalating aromatic nitroxides are **2**•, **4**•, and **5**• since they are stable for several days, almost planar and possess large dipole moments and polarizabilities. Among these three, **5**• is especially promising since it can be tethered to DNA via the carboxy group, overcoming thereby the weak water solubility of these compounds and restricting the site of intercalation. Intercalation

(14) Rozantsev, E. G. *Free Nitroxyl Radicals*; Plenum Press: New York, 1970; p 126.
(15) Barone, V.; Cossi, M. *J. Phys. Chem. A* **1998**, *102*, 1995.
(16) Reha, D.; Kabelac, M.; Ryjacek, F.; Sponer, J.; Sponer, J.; Elstner, M.; Suhai, S.; Hobza, P. *J. Am. Chem. Soc.* **2002**, *124*, 3366.

studies of the aromatic nitroxides **2**•, **4**•, and **5**• will be reported in due course.

Experimental Section

Computational Details. Simulation and Fit Program. The simulation program is based on MATLAB. The isotropic spin Hamiltonian, describing the spin system under study, includes the electron Zeeman and electron−nuclear spin−spin interactions. Input data needed for describing the spectrum are fixed values as center field and microwave frequency, and variable parameters as g_{iso}, hyperfine coupling constants A_{iso}, and the line width ΔH_{pp}. The line width was added by means of a convolution procedure in the Fourier transform space. The variable parameters are optimized by a simplex fit routine minimizing the root-mean-square values.

DFT Calculations. All DFT calculations were carried out within the unrestricted Kohn−Sham formalism as implemented in the GAUSSIAN 98 program package.[17] For the geometry optimizations as well as for the calculations of the Fermi contact interactions, dipole moments and polarizabilities the PBE0 functional[18] together with the standard 6-31G(d) basis set was used. In addition, the isotropic hyperfine couplings were also computed with the EPR−II basis set[13] yielding, however, somewhat smaller ^{14}N but similar ^{1}H hyperfine couplings compared to the 6-31G(d) basis set, as found before.[13] Therefore, in this work only the data obtained with the 6-31G(d) basis set are shown.

EPR. The cw X-band EPR spectra were acquired on a cw X-band EPR spectrometer equipped with a TM_{102} rectangular resonator. For the EPR measurements, **2**•, **4**•, and **5**• were dissolved under argon in dry benzene-d_6 to yield final concentrations of 1 mM and transferred into quartz EPR tubes. The parameters of the signal channel were as follows: modulation amplitude 0.1 G, modulation frequency 100 kHz, conversion time 141 ms, time constant 141 ms. The microwave power was 1 mW.

Synthesis. Chemical synthesis under argon was carried out according to standard Schlenck techniques. The starting compound 3,6-dibromocarbazole **6** was synthesized by bromination of **1**,[19] and 9-acridanylidenemalonitrile **4** and 9-ethylacridanylidenecyanoacetate **5** were synthesized via 9-chloracridine **7** according to literature procedures.[20,21] 9,9-Diphenylacridane-10-oxyl **2**• was synthesized by a slightly modified procedure described by Greci.[9]

Carbazole-9-oxyl 1•. A 20.5 mg (0.123 mmol) portion of carbazole **1** was dissolved in 3 mL of CH_2Cl_2 and cooled to 0 °C. A solution of 31.8 mg (0.184 mmol) of *m*-CPBA in 1.5 mL of CH_2Cl_2 was also cooled to 0 °C and added during 20 min to the carbazole solution. The reaction mixture was stirred for 2 h and warmed during this time slowly to room temperature. Then the solution was extracted three times with 5% aqueous Na_2CO_3 solution, and the yellow organic layer was separated and dried over $MgSO_4$. It was not possible to isolate **1**• as a solid. For the EPR measurements, 3 mL of C_6D_6 was added and CH_2Cl_2 was evaporated off under reduced pressure, yielding a final concentration of 0.4 mM as quantified by EPR. MS (MALDI): 186.55 (52, M^+), 170.24 (100, $M^+ - O$).

3,6-Dimethylcarbazole 3. An 800 mg (2.46 mmol) portion of dibromocarbazole **6** and 66.7 mg (0.12 mmol) of 1,3-bis-(diphenylphosphino)dichloronickel(II) ($Ni(dppp)_2Cl_2$) were suspended in 60 mL of absolute diethyl ether in a dried and argon-flushed 100 mL three-necked flask equipped with a reflux condenser. To the purple red suspension was added 2.5 mL of a 3 M CH_3MgBr solution in diethyl ether (7.38 mmol) over a period of 20 min, yielding a brown and clear solution. The reaction mixture was gently refluxed for 2 h, cooled to room temperature, and quenched with 8 mL of saturated aqueous NH_4Cl solution. The organic phase was treated three times with 7 mL of saturated aqueous Na_2CO_3 solution, three times with 7 mL of saturated aqueous NaCl solution, and finally three times with 7 mL of water. The organic phases were combined and dried over $MgSO_4$, and the solvent was removed under vacuo. The crude product was purified by HPLC using *n*-hexane/ethyl acetate 3:1 as the eluent, yielding 120 mg of **3** (0.61 mmol; 24%) as white needles. ^1H NMR (250 MHz; acetone-d_6): δ 2.48 (s, 6H, CH_3), 7.19 (dq, 2H, J = 1.65/8.23 Hz, H2/H7), 7.36 (d, 2H, J = 8.23 Hz, H1/H8), 7.86 (m, 2H, H4/H5), 10.01 (bs, 1H, NH). ^{13}C NMR (250 MHz; acetone-d_6): δ 20.93, 111.43 (2-fold), 120.74, 124.12, 127.64, 128.42. MS (MALDI): 196.79 (100, $M^+ + H$). IR (KBr): ν 3397, 2912, 2856, 1612, 1575, 1497, 1467, 1300, 1248, 880, 808 cm^{-1}. Anal. Calcd for $C_{14}H_{13}N$ (195.26): C, 86.12; H, 6.71; N, 7.17. Found: C, 86.26; H, 6.83; N, 6.98.

3,6-Dimethylcarbazole-9-oxyl 3•. A 19.3 mg (0.10 mmol) portion of **3** was dissolved in 3 mL of CH_2Cl_2 and cooled to 0 °C. A solution of 25.59 mg (0.15 mmol) of *m*-CPBA in 1.5 mL of CH_2Cl_2 was also cooled to 0 °C and added during 20 min to the carbazole solution. The reaction mixture was stirred for 2 h and warmed slowly to room temperature. The solution was extracted three times with 4 mL of a 5% aqueous Na_2CO_3 solution, and the yellow organic layer was separated and dried over $MgSO_4$. It was not possible to isolate **3**• as a solid. For the EPR measurements, 3 mL of C_6D_6 was added and CH_2Cl_2 was removed under reduced pressure, yielding a final concentration of 0.4 mM by means of EPR quantification. MS (MALDI): 214.45 (52, M^+), 198.34 (100, $M^+ - O$).

9-Acridanylidenmalonitrile-10-oxyl 4•. A 200 mg (0.82 mmol) portion of **4** was dissolved in 7 mL of CH_2Cl_2 and cooled to 0 °C. A solution of 213 mg (1.23 mmol) of *m*-CPBA in 5 mL of CH_2Cl_2 was also cooled to 0 °C and added during 20 min to the first solution. The reaction mixture was stirred for 4 h and warmed during this time to room temperature. The solution was treated with 20 mL of a 5% aqueous Na_2CO_3 solution, and the yellow organic layer was separated and dried over $MgSO_4$. The solvent was removed under vacuum yielding pure **4**• as a yellow solid. MS (MALDI): 258.45 (52, M^+), 242.43 (100, $M^+ - O$). Anal. Calcd for $C_{16}H_8N_3O$ (258.26): C, 74.41; H, 3.12; N, 16.27. Found C, 74.60; H, 3.03; N, 15.98.

9-Ethylacridanylidenecyanoacetate-10-oxyl 5•. To 58 mg (0.20 mmol) of **5** in 5 mL of benzene was added 128 μL of a 5 M solution of *tert*-butyl hydroperoxide in decane (0.64 mmol). A 58 mg (0.23 mmol) portion of PbO_2 was added under vigorous stirring at room temperature. After 12 h, 51.7 mg (0.30 mmol) of *m*-CPBA was added to the dark red solution resulting after 2 h in an orange red solution. The PbO_2 was filtered off, the solvent evaporated, and the crude product purified by column chromatography over silica gel using *n*-hexane/ethyl acetate (1:1) as the eluent (R_f 0.48). Pure **5**• was obtained after crystallization from toluene at 0 °C as a pale yellow solid. MS (MALDI): 327,0 (27, $M^+ + Na$), 289.0 (100, $M^+ - O$). Anal. Calcd for $C_{18}H_{13}N_2O_3$ (305.31): C, 70.81; H, 4.29; N, 9.18. Found C, 71.01; H, 3.98; N, 9.03.

(17) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.11; Gaussian, Inc.: Pittsburgh, PA, 1998.

(18) Adamo, C.; Barone, V. *J. Chem. Phys.* **1999**, *110*, 6158.

(19) Smith, K.; James, D. M.; Mistry, A. G.; Faulkner, D. J. *Tetrahedron* **1992**, *48*, 7479.

(20) Vinh Lap, B.; Boux, L. J.; Cheung, H. T. A.; Holder, G. M. *J. Heterocycl. Chem.* **1983**, *20*, 281.

(21) Tsuge, O.; Torii, A. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 283.

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Supporting Information Available: Optimized Cartesian coordinates and total energies from the theoretical studies carried out for the compounds **1**$^{\bullet}$, **2**$^{\bullet}$, **3**$^{\bullet}$, **4**$^{\bullet}$, and **5**$^{\bullet}$. This material is available free of charge via the Internet at http://pubs.acs.org.

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