amino)propane and ADMA, the reported values of activation energy are 14-23,[13] 11-13,[31] and 11 kJ/mol,[32] respectively. Therefore, the present value of 15-18 kJ/mol corresponds to the activation energy of twisting motion in the propyl group.

On the other hand, differentiating eq 8 with respect to pressure yields eq 11, where ΔV^*_0 is the intrinsic activation volume for IE

$$\frac{\partial \ln (\phi_{IE}/\phi_{LE})}{\partial P} = -\frac{\Delta V^*_0}{RT} - \frac{\eta}{(\eta^2 + B)^{1/2}} \frac{\partial \ln \eta}{\partial P} \quad (11)$$

formation. Further, eq 11 is rewritten as eq 12, where $\Delta V^*_\eta =$

$$\Delta V^*_{obsd} = \Delta V^*_0 + \frac{\eta}{(\eta^2 + B)^{1/2}} \Delta V^*_\eta \quad (12)$$

$RT\partial \ln \eta/\partial P$ is the activation volume of viscous flow. Using the value of B obtained above, we estimated the value of ΔV^*_0. At pressures of more than about 1.5 kbar, it showed a constant value of -2.5 cm^{-3}/mol for TMPD.

It is concluded that the intrinsic volume of activation for the IE formation with DPP is -2.5 cm^3/mol. As for the activation volumes of the IE formation, there are few data to be referred, However, we can say that its value is almost as comparable as the intrinsic volume change of rotational isomerization[33] and twisting isomerization.[34] And also we found that it is a few times smaller than that of intermolecular excimer formation.[35]

Concluding Remarks

We have shown that the rate of the IE formation in DPP deduced from the yield ratio is well correlated with solvent viscosity when it is studied by the high-pressure method over a wide range of viscosities (0,1-100 cP) in a continuous way with minimum specific interaction with solvent. This viscosity dependence is well described by the hindered molecular rotation model based on Kramers' expression. In terms of this model, the shape of the potential barrier was made clear. The frequency for the top of the barrier (7.6×10^{13} to 15×10^{13} s^{-1}) implies a considerably sharp barrier. The intrinsic activation parameters independent of solvent viscous flow were also determined. The intrinsic activation energy and the intrinsic activation volume of the IE formation are 15-18 kJ/mol and -2.5 cm^3/mol, respectively. These values correspond to the potential barrier of twisting motion in the propyl chain.

Registry No. 1,3-Di-1-pyrenylpropane, 61549-24-4.

(31) Van der Auweraer, M.; Gilbert, A.; De Schryver, F. C. *J. Am. Chem. Soc.* **1980**, *102*, 4007.
(32) Syage, J. A.; Felker, P. M.; Zewail, A. H. *J. Chem. Phys.* **1984**, *81*, 2233.
(33) Weale, K. E. *Chemical Reactions at High Pressures*; E. & F. N. Spon Ltd, London, 1967.
(34) Fanselow, D. L.; Drickamer, H. G. *J. Chem. Phys.* **1974**, *61*, 4567. Clark, F. T.; Drickamer, H. G. *Chem. Phys. Lett.* **1985**, *115*, 173.
(35) Förster, Th.; Leiber, C. O.; Seidel, H. P.; Weller, A. *Z. Phys. Chem. (Munich)* **1963**, *39*, 265.

Chemically Mediated Fluorescence Yield Switching in Nitroxide-Fluorophore Adducts: Optical Sensors of Radical/Redox Reactions

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Abstract: The absorption and fluorescence emission spectra and quantum yields of a series of paramagnetic nitroxide-naphthalene adducts are compared with those of diamagnetic analogues. While the absorption and emission energies of these compounds are unaffected by the presence of the nitroxyl radical substituent(s), the fluorescence quantum yields of the paramagnetic derivatives are 2.9- to 60-fold lower than the corresponding diamagnetic derivatives. Additionally, chemical reduction of the nitroxide moiety to a diamagnetic hydroxylamine produces a fluorescence yield increase that parallels nitroxyl radical loss. On the basis of this chemically mediated optical switching, compounds of this class may prove to be broadly applicable as sensitive optical probes for radicals and redox-active species in biological and chemical systems.

The ability of nitroxides to scavenge efficiently a broad array of organic and inorganic radicals has long been recognized[1,2] and employed for the detection of radicals (and some redox-active centers) in biological[3] and chemical[4] systems. The paramagnetic nitroxides are also known to be efficient quenchers of excited singlet states of aromatic hydrocarbons,[5] presumably through an intermolecular electron-exchange interaction between the ground-state nitroxide and excited-state compound within a collision complex.[5,6]

(1) Ingold, K. U. In Landolt-Börnstein *Numerical Data and Functional Relationships in Science and Technology*, Subvolume C, *Radical Reaction Rates in Liquids*; Fischer, H., Ed.; Springer-Verlag: New York, 1983; Vol. 13, pp 166-270.
(2) (a) Willson, R. L. *Trans. Faraday Soc.* **1971**, *67*, 3008-3019. (b) Nigam, S.; Asmus, K.-D.; Willson, R. L. *J. Chem. Soc., Faraday Trans. 1* **1976**, 2324-2340.
(3) (a) Melhorn, R. J.; Packer, L. *Methods Enzymol.* **1984**, *105*, 215-220. (b) Melhorn, R. J.; Packer, L. *Can. J. Chem.* **1982**, *60*, 1452-1462. (c) Quintanilha, A. T.; Packer, L. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 570-574. (d) Sarna, T.; Korytowski, W.; Sealy, R. C. *Arch. Biochem. Biophys.* **1985**, *239*, 226-233. (e) Giangrande, M.; Kevan, L. *Photochem. Photobiol.* **1981**, *33*, 721-726. (f) Leterrier, F.; Mendyk, A.; Viret, J. *Biochem. Pharmacol.* **1976**, *25*, 2469-2474. (g) Gascoyne, P. R. C.; Pethig, R.; Szent-Györgyi, A. S. *Biochim. Biophys. Acta* **1987**, *923*, 257-262. (h) Stier, A.; Sackmann, E. *Biochim. Biophys. Acta* **1973**, *311*, 400-408. (i) Rosen, G. M.; Rauckman, E. J. *Biochem. Pharmacol.* **1977**, *26*, 675-678. (j) Yamaguchi, T.; Nagatoshi, A.; Kimoto, E. *FEBS Lett.* **1985**, *192*, 259-262.
(4) (a) Blough, N. V. *Environ. Sci. Technol.* **1988**, *22*, 77-82. (b) Brownlie, I. T.; Ingold, K. U. *Can. J. Chem.* **1967**, *45*, 2427-2432. (c) Robbins, W. K.; Eastman, R. H. *J. Am. Chem. Soc.* **1970**, *92*, 6077-6079. (d) Gerlock, J. L.; Bauer, D. R. *J. Polym. Sci., Polym. Lett. Ed.* **1984**, *22*, 447-455. (e) Bosfield, W. K.; Jenkins, I. D.; Thang, S. H.; Rizzardo, E.; Soloman, D. H. *Aust. J. Chem.* **1985**, *38*, 689-698. (f) Bales, B. L.; Kevan, L. *J. Phys. Chem.* **1982**, *86*, 3836-3839. (g) Reszka, K.; Sealy, R. C. *Photochem. Photobiol.* **1984**, *39*, 293-299. (h) Hiromitsu, I.; Kevan, L. *J. Phys. Chem.* **1986**, *90*, 3088-3091. (i) Mathew, L.; Warkentin, J. *J. Am. Chem. Soc.* **1986**, *108*, 7981-7984.
(5) (a) Green, J. A.; Singer, L. A.; Parks, J. H. *J. Chem. Phys.* **1973**, *58*, 2690-2695. (b) Green, J. A.; Singer, L. A. *J. Am. Chem. Soc.* **1974**, *96*, 2730-2733. (c) Watkins, A. R. *Chem Phys. Lett.* **1974**, *29*, 526-528. (d) Kuzmin, V. A.; Tatikolov, A. S. *Chem. Phys. Lett.* **1977**, *51*, 45-47. (e) Chattopadhyay, S. K.; Das, P. K.; Hug, G. L. *J. Am. Chem. Soc.* **1983**, *105*, 6205-6210. (f) London, E. *Mol. Cell. Biochem.* **1982**, *45*, 181-188. (g) Atik, S. S.; Singer, L. A. *J. Am. Chem. Soc.* **1978**, *100*, 3234-3235. (h) Scaiano, J. C.; Paraskevopoulos, C. I. *Can. J. Chem.* **1984**, *62*, 2351-2354.

0002-7863/88/1510-1915$01.50/0

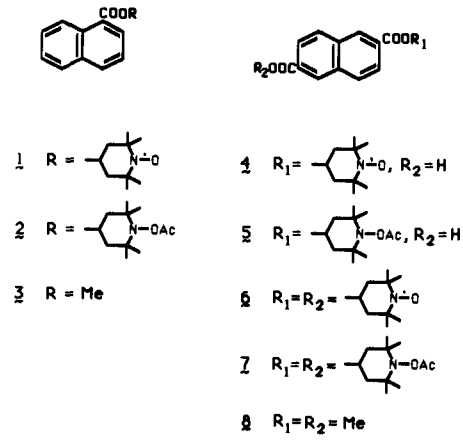
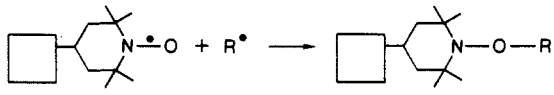


Figure 1. Compounds examined in this study.

In an effort to construct highly sensitive optical probes of radical/redox species, we sought to exploit both these properties by constraining the nitroxide and fluorophore to reside in a permanent or easily accessible "collision complex" through covalent linkage. This idea is illustrated below;



Here the square represents a fluorophore to which a nitroxide is covalently attached. Because of the proximity of the paramagnetic group,[7] fluorescence emission from the fluorophore should be quenched. Preferential reaction of the nitroxide with a radical leads to the formation of a diamagnetic product,[1-4] thereby eliminating the intramolecular quenching pathway and resulting in an increased fluorescence yield that reflects radical/redox scavenging.

In this report we demonstrate the feasibility of this approach and show that intramolecular paramagnetic quenching of singlet states by nitroxides is highly efficient. Nitroxide–naphthalene adducts (Figure 1) exhibit a 2.9- to 60-fold reduction in fluorescence quantum yield as compared with diamagnetic analogues. Additionally, chemical reduction of the paramagnetic nitroxide moiety to a diamagnetic hydroxylamine is shown to produce a fluorescence yield increase that parallels radical loss. To our knowledge, these measurements provide the first data on the efficiency of short-range, intramolecular quenching of excited singlet states by a stable radical species.

Room-temperature absorption and emission spectra of **1–8**[8] in spectral-grade methanol (Aldrich) or pH 8.0, 50 mM phosphate buffer were obtained with a Hewlett-Packard 8451A diode array spectrophotometer (resolution, 2 nm) and a SLM-Aminco SP-F-500 spectrofluorometer (2-nm band-pass, emission), respectively. Relative quantum yields of fluorescence were measured[9] with respect to 1 μg/mL of quinine sulfate in 0.1 N H_2SO_4 (Regis Chemical Co.), $\phi_{350} = 0.55$.[10] For **2**, **3**, **7**, and **8** in methanol, deaeration with prepurified N_2 (LINDE) was required to eliminate partial oxygen quenching. An IBM 200D EPR spectrometer was employed to monitor spin concentration.

The absorption and fluorescence emission energies of naphthalenes **1**, **4**, and **6** in methanol did not differ significantly from those of the corresponding diamagnetic *O*-acetyl (**2**, **5**, **7**) or methyl ester (**3**, **8**) derivatives (Table I), indicating that no new absorbtive or emissive states are formed as a result of the presence of the nitroxide substituent(s). However, the quantum yields of the paramagnetic naphthalene compounds were substantially smaller, ca. 0.01 (Table II). For **1** and **6**, the yields were ~50- to 60-fold lower than the diamagnetic *O*-acetyl and methyl ester derivatives. The *O*-acetyl and methyl ester derivatives of a given substitution exhibited similar high yields, suggesting that, unlike aliphatic and aromatic amines,[11] intramolecular quenching by the *O*-substituted hydroxylamine(s) (**2**, **7**) is unimportant. Primarily as a result of the low quantum yield of the *O*-acetyl derivative **5**, the reduction in quantum yield of **4** was considerably smaller, ~3-fold. However, in an aqueous pH 8.0, 50 mM phosphate buffer the yields of **4** and **5** increased by factors of 2.7 and 9.3, respectively, leading to a diamagnetic to paramagnetic quantum yield ratio of 10 (Table II).

Complete ascorbate reduction of **4** to the hydroxylamine[12] in deaerated pH 8.0 phosphate buffer resulted in a 10-fold increase in fluorescence yield, indicating that the *O*-acetyl (**5**) and the hydroxylamine derivatives have similar yields (Figure 2, Table II). Under a large excess of ascorbate over **4**, the rise of fluorescence and loss of spin followed identical pseudo-first-order kinetics, illustrating the direct relationship between the loss of

Table I. Absorption and Fluorescence Emission Maxima of the Paramagnetic and Diamagnetic Substituted Naphthalenes in Methanol[a]

compd	abs max,[b] cm^{-1}	extincn coeff,[c] M^{-1} cm^{-1}	emissn max,[d] cm^{-1}
1–3[e]	45 000	4.2×10^4	27 100
	33 000	7.1×10^3	
4–5[e]	40 300 (40 300)	7.0×10^4 (6.4×10^4)	27 800 (~27 000, sh)
	34 700 (34 500)	1.2×10^4 (1.2×10^4)	26 700 (26 200)
	33 600 (33 300)	1.1×10^4 (1.1×10^4)	
	29 900 (29 600)	1.8×10^3 (1.9×10^3)	
	28 700 (28 600)	2.0×10^3 (2.0×10^3)	
6–7[e]	40 300	8.3×10^4	27 500
	34 700	1.4×10^4	26 300
	33 600	1.4×10^4	
	29 600	2.1×10^3	
	28 200	2.4×10^3	
8	40 600	8.4×10^4	27 600
	35 000	1.4×10^4	26 400
	33 800	1.3×10^4	
	29 600	2.3×10^3	
	28 400	2.8×10^3	

[a] See Figure 1. Values in parentheses obtained in aqueous 50 mM phosphate buffer, pH 8.0. For **1**, **4**, and **6**, a weak nitroxide absorption band was also observed at ~460 nm (ε ~12, ~24 for **6**). Because of the low exctinction coefficient of the higher energy nitroxide band (λ ~246 nm, ε ~1.9 × 10^3), as compared to the naphthalene absorption, it could not be detected. [b] Spectrophotometer resolution, 2 nm. [c] Estimated uncertainty, ±8%. [d] Spectrofluorometer emission band-pass, 2 nm. For **1–3**: excitation wavelength, 300 nm; band-pass, 4 nm. For **4–5**: excitation wavelength, 298 nm; band-pass, 2 nm. For **6–8**: excitation wavelength, 296 nm; band-pass, 2 nm. [e] The values for these compounds were identical within the stated uncertainties.

Table II. Fluorescence Quantum Yields of the Paramagnetic and Diamagnetic Substituted Naphthalenes in Methanol[a]

compd	ϕ_F	ϕ_F^D/ϕ_F^P[b]
1	0.0082	
2	0.47	57
3	0.42	51
4	0.015 (0.040)	
5	0.043 (0.40)	2.9 (10)
6	0.011	
7	0.66	60
8	0.60	55

[a] See Figure 1. Values in parentheses obtained in aqueous 50 mM phosphate buffer, pH 8.0. [b] Diamagnetic to paramagnetic quantum yield ratio.

(6) Quenching may result from an exchange-induced intersystem crossing to the triplet[5d] or internal conversion to the ground state.[5d,e] Electron-exchange-facilitated charge transfer (or energy transfer) may also be important in some cases.[5e]

(7) A study[5a] of diffusional quenching by nitroxides suggests effective interaction distances of 4–6 Å.

(8) Compound syntheses and characterizations are described in the supplementary material.

(9) Calvert, J. G.; Pitts, J. N. *Photochemistry*; Wiley: New York, 1966; pp 799–804.

(10) Melhuish, W. H. *J. Phys. Chem.* **1961**, *65*, 229.

(11) Davidson, R. S. *Adv. Phys. Org. Chem.* **1983**, *19*, 1–130.

(12) (a) Okazaki, M.; Kuwata, K. *J. Phys. Chem.* **1985**, *89*, 4437–4440. (b) Couet, W. R.; Brasch, R. C.; Sosnousky, G.; Lukszo, J.; Prakash, I.; Gnewuch, C. T.; Tozer, T. N. *Tetrahedron* **1985**, *41*, 1165–1172.

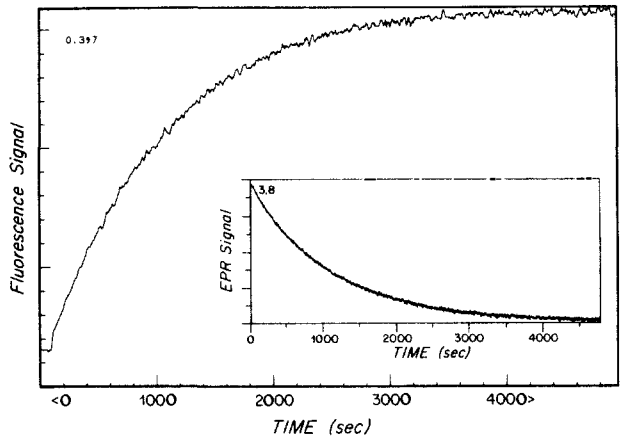


Figure 2. Time courses for the rise of fluorescence and loss of radical spin (inset) following addition of 50 μM ascorbate to a 3 μM solution of **4** in deaerated 50 mM phosphate buffer, pH 8.0; temperature, 24 ± 1 °C. Initially, the base-line fluorescence level of **4** was recorded for ~100 s (λ_{exc}, 330 nm; λ_{em}, 382 nm). Subsequently, a small volume of concentrated, degassed ascorbate solution was injected into the fluorescence cell containing **4**. After mixing, a 50-μL capillary was employed to quickly withdraw a sample for the concurrent measurement of spin loss by EPR. The time course of nitroxide loss was followed at a field position corresponding to the maximum of the low-field nitrogen hyperfine line. Other instrument settings were as follows: microwave frequency, 9.79 GHz; power, 10 mW; modulation frequency, 100 kHz; modulation amplitude, 1.6 G; gain, 3.2×10^6; time constant, 2.5 s. Approximate delay times between sample mixing and initial signal observation were 60 and 250 s for fluorescence and EPR detection, respectively.

paramagnetism and increased yield of fluorescence (Figure 2).[13] The second-order rate constant calculated from these time courses, 19 M^{-1} s^{-1}, agrees reasonably with earlier studies[12] of ascorbate reduction of piperidine nitroxyls.

These results show clearly that the fluorescence yield of a compound closely linked to a paramagnetic center can be substantially increased by reactions that lead to a loss of paramagnetism in the center. On this basis, compounds of this class represent a potentially more sensitive and versatile alternative to current methods for radical/redox detection in biological[3,14] and chemical[4] systems. For example, these types of compounds offer the possibility of examining localized radical/redox processes in large, organized assemblies such as cells[14] by fluorescence imaging.

One potential limitation of this approach is that highly reactive radicals such as OH may also react in part with the fluorophore, resulting in its alteration or destruction. We are currently examining this possibility, as well as extending this work to investigate the influence of solvent polarity and viscosity on the fluorescence yields and lifetimes[15] of **1-8**.

Acknowledgment. This work has been supported by the Office of Naval Research under ONR Contract N00014-87-K-0007 NR083-004 and Grant N00014-86-G-0164 issued under the Department of Defense University Research Instrumentation Program for the purchase of the EPR spectrometer. D.J.S. is a recipient of a Surdna Biomedical Postdoctoral Fellowship. We thank Dr. Nelson Frew and Carl G. Johnson for their assistance in obtaining the mass spectra. Helpful comments by Drs. D. Repeta, O. C. Zafiriou, and D. Mauzerall are acknowledged. This is Contribution No. 6532 from the Woods Hole Oceanographic Institution.

Supplementary Material Available: Compound syntheses and characterizations for **1**, **2**, and **4-7** (3 pages). Ordering information is given on any current masthead page.

(13) A quantitative measurement was not feasible for **1** because the ascorbate absorption band significantly attenuated the light at the available excitation wavelengths.

(14) (a) Swartz, H. M. *J. Chem. Soc., Faraday Trans. 1* **1987**, *83*, 191-202. (b) Stellmach, J. *Histochemistry* **1984**, *80*, 137-143.

(15) Consistent with a rapid intramolecular quenching process, the fluorescence decay of **1** in ethanol is cleanly first order with a lifetime of 190 ps: Mauzerall, D.; Simpson, D. J.; Blough, N. V., unpublished work.

Structure, Resolution, and Racemization of Decakis(dichloromethyl)biphenyl

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Abstract: Decakis(dichloromethyl)biphenyl (**1**) was obtained by photochlorination of decamethylbiphenyl. Crystals of the 1:1 THF solvate are monoclinic, space group $P2_1/n$, a = 17.296 (5) Å, b = 10.091 (3) Å, c = 21.731 (5) Å, β = 96.79 (2)°, Z = 4. The disposition of the dichloromethyl groups on both rings is all-geared, and the molecule has approximate C_2 symmetry. The ^{1}H NMR solution spectrum of **1** is consistent with a time-averaged C_2 conformation. The molecule assumes a curved shape that is well reproduced by empirical force field calculations. Homo- and heterodirectional relationships between subcycles of the molecular model are discussed. Enantiomers of **1** were separated by HPLC on a column of cellulose tris(3,5-dimethylphenylcarbamate). The biphenyl racemizes with a barrier ($\Delta G^{\ddagger}$) of 23.7 kcal mol^{-1}; the threshold mechanism for the enantiomerization most likely involves internal rotation of the dichloromethyl groups rather than rotation of the pentakis(dichloromethyl)phenyl groups about the central biphenyl bond.

Vicinal isopropyl or dichloromethyl groups (CHR_2, R = CH_3, Cl) attached to planar frames such as ethylene and benzene tend to assume gear-meshed conformations in which a methine hydrogen tooth is tucked into the notch created by the methyls or chlorines of a neighboring CHR_2 group.[2,3] A noteworthy feature

(1) (a) Princeton University. (b) Osaka University.

(2) Berg, U.; Liljefors, T.; Roussel, C.; Sandström, J. *Acc. Chem. Res.* **1985**, *18*, 80 and references therein.

0002-7863/88/1510-1917$01.50/0