Scheme I

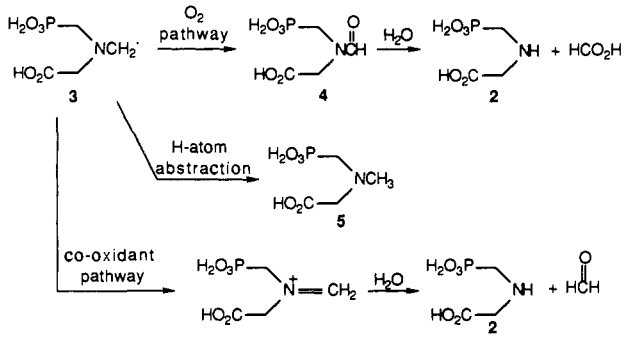
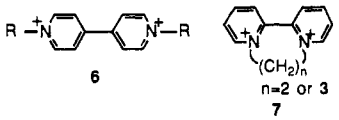


Table I

additive[a]	catalyst	selectivity to 2, %
none	Co[b]	59
none	V[c]	50
1-sulfo-9,10-anthraquinone sodium salt	Co[b]	72
2-sulfo-9,10-anthraquinone sodium salt	Co[b]	77.5
2,6-disulfo-9,10-anthraquinone disodium salt	Co[b]	73
1,5-disulfo-9,10-anthraquinone disodium salt	Co[b]	74
1-sulfo-9,10-anthraquinone sodium salt	V[c]	74
methylviologen[d] (6, R = Me)	Co[b]	78
methylviologen[d] (6, R = Me)	V[c]	96
1,1′-ethylene-2,2′-bipyridinium[d] (7, n = 2)	Co[b]	78
1,1′-ethylene-2,2′-bipyridinium[d] (7, n = 2)	V[c]	85
1,1′-trimethylene-2,2′-bipyridinium[d] (7, n = 3)	Co[b]	79
1,1′-trimethylene-2,2′-bipyridinium[d] (7, n = 3)	V[c]	88

[a] [Additive]/[MSO_4] = 0.5, where M = Co^{2+} or VO^{2+}. [b] Reaction conditions as described in text: T = 90 °C, 200 psig of O_2, reaction time = 200 min. [c] Reaction conditions as described in the text: T = 75 °C, 200 psig of O_2, reaction time = 200 min. [d] As the chloride salt.

organic oxidants were screened, and we found that two classes of oxidants, quinones and diquaternary bipyridinium salts, **6** and **7**, were effective agents for increasing the selectivity to product **2**. In addition, many different redox-active metal salts were tested



as cocatalysts under these conditions, with the result that either no effect is observed or the metal completely inhibits the reaction, as in the cases with Cu(II) and Fe(II and III) salts.

In Table I are shown some representative examples with several water-soluble quinones and bisquats. All of the additives shown in Table I have one-electron reduction potentials in the range −0.5 to −0.8 V (H_2O)[5] and are known to be efficient electron-transfer agents,[6-9] and their one-electron-reduction products react rapidly with O_2 to yield hydrogen peroxide via superoxide disproportionation.[10-12] The water-soluble quinones and bisquats shown in Table I exhibit marked selectivity-enhancing effects.[13,14] Since the V system is much more active than Co, extensive optimization studies were performed on the V system. For example, under the conditions employed in the screening study (75 °C, 200 psig of O_2), the ideal ratio of the [**6**(R=Me)] to [V] is about 4:1. With this level of added **6**, the selectivity to **2** is ~96% with only traces of the methylated product **5**. Studies with Co revealed that under a broad range of conditions the optimum ratio for the concentration of either the bisquat or quinone to [Co] is ~1.4:1. Enhanced selectivities (~93%) to **2** at low O_2 pressures (100–200 psig) result in little effect on the rate.

Studies at low O_2 pressure (50 psig, 75 °C) with V under optimized conditions ([MV]/[V] = 6) show that the level of formic acid is reduced (from 50% in the absence of electron-transfer agent, [HCO_2H] = [**2**], to ~12%) and the level of formaldehyde (from hydrolysis of the iminium cation) increases from less than 5% to ~82%. This supports our proposed mode of action for the electron-transfer cocatalysts; namely, that O_2 trapping of radical **3** is no longer required to prevent H-atom transfer to **3**. Oxidation of **3** with either the quinone or bisquat electron-transfer agent allows one to reduce O_2 pressure to much lower levels and still achieve high selectivity. Since O_2 is an efficient oxidant of the one-electron-reduction product of the additives,[10,11] O_2 remains as the ultimate oxidant in these cocatalyst systems. Each of the additives listed in Table I possesses sufficient oxidizing power to oxidize the one-electron-reduction product of an iminium cation, such as **3**.[15] Importantly, the additives show good stability in these systems; e.g., methylviologen (**6**, R=Me) is able to survive repeated recycles (10) with no loss in integrity.

The use of electron-transfer cocatalysts to intercept an intermediate in an oxygen-driven oxidation is an important concept and should have great potential for lowering the pressures required for molecular oxygen oxidations. We are continuing to pursue the mechanistic implications of these dual-component catalyst systems and are investigating their use in other O_2-catalyzed oxidations.

(5) *CRC Handbook Series in Organic Electrochemistry*; Meites, L., Zuman, P., Rupp, E., Eds.; CRC Press Inc.: West Palm Beach, FL, 1982.
(6) Methyl viologen is an electron-transfer agent in solar energy storage systems: (a) Graetzel, A. *Acc. Chem. Res.* **1981**, *14*, 376. (b) Whitten, D. G. *Acc. Chem. Res.* **1980**, *13*, 83. (c) Willner, E.; Laane, C.; Otros, J.; Calvin, M. *Adv. Chem. Ser.* **1982**, *177*, 71.
(7) Rieger, A.; Edwards, J. O. *J. Org. Chem.* **1988**, *53*, 1481.
(8) Bard, A. J.; Ledwith, A.; Shine, J. J. *Adv. Phys. Org. Chem.* **1976**, *13*, 55.
(9) Denisov, E. T.; Khudyakov, I. V. *Chem. Rev.* **1987**, *87*, 1313.
(10) The rate constant for the O_2 oxidation of the MV radical cation is ~1.2×10^7 M^{-1} s^{-1} at 12 °C: Liu, P.; Zha, Q.; Xie, C.; Li, C.; Wang, H. *Cuihua Xuebao* **1983**, *4*(2), 131.
(11) The rate constant for the O_2 oxidation of the 2,6-disulfo-9,10-anthraquinone radical anion in H_2O is 5×10^8 M^{-1} s^{-1}: Wilson, R. L. *Trans. Faraday Soc.* **1971**, *67*, 3020.
(12) (a) Evans, A. G.; Dodson, N. K.; Rees, N. H. *J. Chem. Soc., Perkin Trans. 2* **1976**, 859. (b) Evans, A. G.; Alford, R. E.; Rees, N. H. *J. Chem. Soc., Perkin Trans. 2* **1977**, 445.
(13) Fields, D. L.; Riley, D. P.; Grabiak, R. C. U.S. Patent 4952723, 1990.
(14) Fields, D. L.; Riley, D. P.; Grabiak, R. C. U.S. Patent 4937376, 1990.
(15) The $E_{1/2}$ of a representative iminium cation, 1-(4-methylbenzylidene)pyrrolidinium, is −1.36 V (NHE, H_2O). *CRC Handbook Series in Organic Electrochemistry*; Meites, L., Zuman, P., Rupp, E., Eds.; CRC Press Inc.: West Palm Beach, FL; Vol. III, p 214.

Inversion of Enzyme Enantioselectivity Mediated by the Solvent

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Recent evidence that enzymes can catalyze reactions in neat organic solvents has also led to the realization that enzymatic properties can be markedly altered simply by switching from one such solvent to another.[1] In particular, following our discovery[2] that enzyme enantioselectivity in nonaqueous media greatly depends on the solvent, this phenomenon has been observed, by us[3a,b] and others,[3c-g] for various asymmetric enzymatic processes. In

† On leave from Mitsui Toatsu Chemicals Co., Togo Mobara City, Japan.
(1) Klibanov, A. M. *Trends Biochem. Sci.* **1989**, *14*, 141. Dordick, J. S. *Enzyme Microb. Technol.* **1989**, *11*, 194.
(2) Sakurai, T.; Margolin, A. L.; Russell, A. J.; Klibanov, A. M. *J. Am. Chem. Soc.* **1988**, *110*, 7236.
(3) (a) Kitaguchi, H.; Fitzpatrick, P. A.; Huber, J. E.; Klibanov, A. M. *J. Am. Chem. Soc.* **1989**, *111*, 3094. (b) Fitzpatrick, P. A.; Klibanov, A. M. *J. Am. Chem. Soc.* **1991**, *113*, 3166. (c) Kanerva, L. T.; et al. *Acta Chem. Scand.* **1990**, *44*, 1032. (d) Hirata, H.; Higuchi, K.; Yamashina, T. *J. Biotechnol.* **1990**, *14*, 157. (e) Parida, S.; Dordick, J. S. *J. Am. Chem. Soc.* **1991**, *113*, 2253. (f) Nakamura, K.; Takebe, Y.; Kitayama, T.; Ohno, A. *Tetrahedron Lett.* **1991**, *32*, 4941. (g) Bovara, R.; Carrea, G.; Ferrara, L.; Riva, S. *Tetrahedron: Asymmetry* **1991**, *2*, 931.

0002-7863/92/1514-1882$03.00/0

Table I. Initial Rates and Enantioselectivities of the Transesterification between L or D *N*-Ac-Phe-OEtCl and Propanol Catalyzed by *Aspergillus oryzae* Protease in Anhydrous Solvents[a]

entry	solvent	initial rate v, μM h^{-1} (mg of protein)$^{-1}$ L enantiomer	initial rate v, μM h^{-1} (mg of protein)$^{-1}$ D enantiomer	enantioselectivity (v_L/v_D)
1	acetonitrile	0.85	0.12	7.1
2	dimethylformamide	0.017	0.003	5.7
3	pyridine	0.65	0.15	4.3
4	*tert*-butyl alcohol	1.4	0.83	1.7
5	dioxane	0.70	0.55	1.3
6	acetone	0.54	0.41	1.3
7	tetrahydrofuran	1.2	0.93	1.3
8	cyclohexanone	0.46	0.41	1.1
9	dichloromethane	0.29	0.33	0.88
10	3-octanone	0.16	0.22	0.73
11	nitrobenzene	0.57	0.95	0.60
12	*tert*-butyl acetate	1.4	3.2	0.44
13	triethylamine	2.7	8.0	0.34
14	methyl *tert*-butyl ether	2.2	6.4	0.34
15	cyclohexane	3.2	12	0.27
16	toluene	0.82	3.2	0.26
17	octane	2.9	12	0.24
18	tetrachloromethane	1.7	8.9	0.19

[a] The initial rates for the individual enantiomers of **1** were measured at 100 mg/mL enzyme[4] (Sigma), 10 mM ester (2 mM for entries 15 and 17), and 100 mM propanol. One-milliliter reaction mixtures were shaken at 45 °C and 300 rpm; periodically, 0.5-μL aliquots were analyzed by capillary gas chromatography.[3b] No reaction was observed without enzyme. Organic solvents, dehydrated by shaking with 3-Å molecular sieves, had the water content below 0.01%.[3b]

these studies, however, a given enzyme always favored the same enantiomer, although the extent of this preference was quite different depending on the solvent. Herein we report, for the first time, a *complete reversal* of enzyme enantioselectivity upon a change in the solvent.

Table I depicts the results of a kinetic investigation of the transesterification between *N*-acetyl-(L or D)-phenylalanine 2-chloroethyl ester (**1**) and 1-propanol catalyzed by *Aspergillus oryzae* protease[4] in 18 anhydrous solvents. One can see from the table's last column that enantioselectivity of the enzyme dramatically depends on the solvent (under otherwise identical conditions). Moreover, while in hydrophilic acetonitrile, dimethylformamide, and pyridine the L enantiomer of **1** is much more reactive than its D counterpart, the opposite holds for hydrophobic toluene, octane, and tetrachloromethane. This striking inversion of enantioselectivity was confirmed when the ratios of the specificity constants, $(k_{cat}/K_M)_L/(k_{cat}/K_M)_D$, were determined to be 6.6 in acetonitrile and 0.24 in toluene.

The observed phenomenon can be rationalized as illustrated in Figure 1. We hypothesize that **1** can bind to the active center of *A. oryzae* protease in two distinct modes, productive (conducive to the enzymatic conversion) and nonproductive. For the L enantiomer of the substrate, the latter binding mode exposes **1**'s side chain to the solvent, whereas in the productive mode the benzyl group is hidden in the hydrophobic pocket of the enzyme; for the D enantiomer, the situation is reversed. Consequently, in hydrophilic solvents the hydrophobic side chain should partition into the hydrophobic binding pocket, thus leading to the productive mode for the L enantiomer and the nonproductive one for the D.

The model in Figure 1 affords several predictions that can be tested experimentally. First, hydrophilic solvents should favor the enzymatic reaction with the L enantiomer, while hydrophobic solvents should favor that with the D. As seen in Figure 2A, there is indeed generally an inverse relationship between v_L/v_D and solvent hydrophobicity. This correlation became even more convincing when various binary mixtures of two extreme (in terms

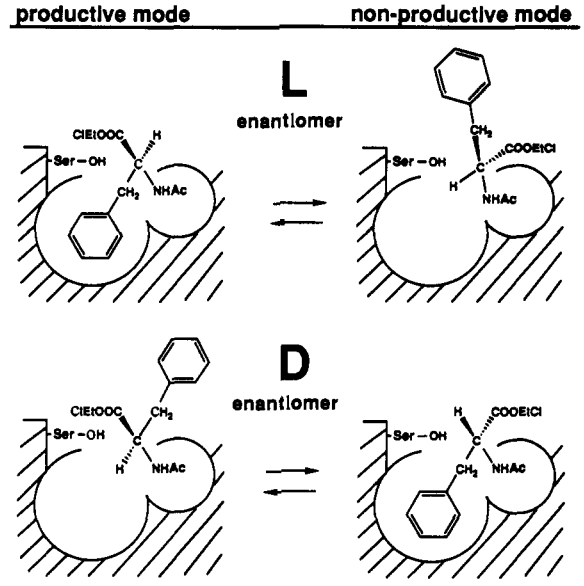

Figure 1. Schematic representation of binding of L (top) and D (bottom) enantiomers of **1** to the active center of *A. oryzae* protease. The bigger unfinished circle depicts the hydrophobic binding pocket. The serine residue shown is the head nucleophile of the enzyme;[4] its hydroxyl is properly aligned to attack the ester carbonyl only in the productive binding mode.

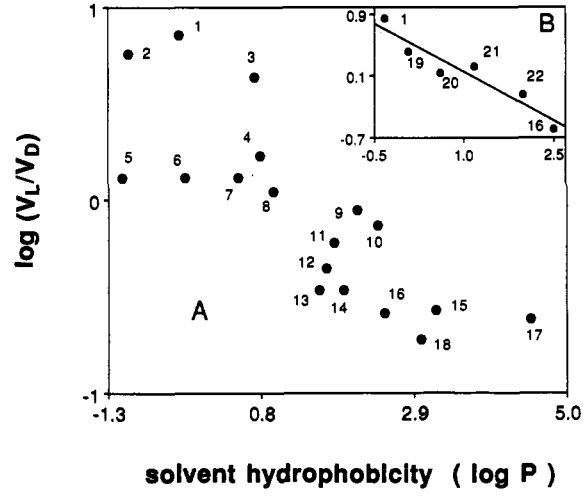

Figure 2. Dependence of enantioselectivity of *A. oryzae* protease on the solvent hydrophobicity for (A) 18 anhydrous solvents and (B) binary mixtures of acetonitrile and toluene. Enantioselectivity, expressed as log (v_L/v_D), was measured in the same enzymatic reaction and under the same conditions as in Table I. Hydrophobicity is defined as log P where P is a partition coefficient for a given solvent between 1-octanol and water; the values are taken or calculated from the following: Rekker, R. F. *The Hydrophobic Fragmental Constant*; Elsevier, Amsterdam, 1977. Solvents: from 1 to 18 are the corresponding entries in Table I; 19–22 are mixtures of 1 and 16 (75, 50, 30, and 10% (v/v) of the former, respectively).

of hydrophobicity) solvents, acetonitrile and toluene, were used as reaction media: a satisfactory linear dependence was observed between the protease's enantioselectivity and log P of the solvent mixture (Figure 2B).

Second, Figure 1 implies that the reversal of enantioselectivity upon transition from hydrophilic to hydrophobic solvents will diminish when **1**'s benzyl group is replaced with a less hydrophobic side chain. Indeed, for the enzymatic transesterification of *N*-Ac-Ala-OEtCl,[5] v_L/v_D was 4.1 in acetonitrile and 0.57 in toluene (vs 7.1 and 0.26, respectively, for **1**; Table I), thus lowering the inversion effect almost 4-fold.

(4) Alkaline serine protease (aspergillopeptidase B), EC 3.4.21.14. Subramanian, A. R.; Kalnitski, G. *Biochemistry* **1964**, *3*, 1861, 1868. Matsubara, H.; Feder, J. In *The Enzymes*; Boyer, P. D., Ed.; Academic: New York, 1971; Vol. 3, Chapter 20.

(5) Otherwise, experimental conditions were the same as in Table I.

Third, solvent-mediated inversion (as opposed to a mere change[2,3]) of enantioselectivity via our model would not be expected for most enzymes. This prediction was verified by the ν_L/ν_D values in acetonitrile and toluene, respectively, for the transesterification of **1** catalyzed by six unrelated lipases:[5] 4.7 and 32 for porcine pancreatic, 5.1 and 1.7 for *Chromobacterium viscosum*, 13 and 4.5 for *Pseudomonas cepacia*, 20 and 74 for *Mucor meheii*, 16 and 4.7 for *Aspergillus niger*, and 10 and 3.8 for *Pseudomonas* sp. lipoprotein lipase. Thus while the dependence of the enantioselectivity on the solvent was different for all enzymes, its inversion was observed only for *A. oryzae* protease.

The data in Table I indicate that the stereochemical outcome of the protease-catalyzed transesterification can be fundamentally altered simply by replacing the solvent. Note that this phenomenon can be explained without invoking solvent-induced conformational changes of the enzyme (no evidence for those has been observed for other serine proteases in anhydrous media[6]). Therefore, enzyme enantioselectivity in such systems can be predicted solely on the basis of physicochemical properties of the solvent.

(6) Burke, P. A.; Smith, S. O.; Bachovchin, W. W.; Klibanov, A. M. *J. Am. Chem. Soc.* **1989**, *111*, 8290. Kanerva, L. T.; Klibanov, A. M. *J. Am. Chem. Soc.* **1989**, *111*, 6864. Adams, K. A. H.; Chung, S.-H.; Klibanov, A. M. *J. Am. Chem. Soc.* **1990**, *112*, 9418.

(7) This work was supported by NIH Grant GM 39794.

Poly(arylmethyl) Octet ($S = {}^7/_2$) Heptaradical and Undecet ($S = 5$) Decaradical

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The rational design of large molecules and clusters with a significant preference for high-spin ordering is important for a recent topic of organic magnetism.[1-5] Our previous studies suggested that, in the series of 1,3-connected poly(arylmethyls), extension of conjugation does not affect their electronic structure;[6]

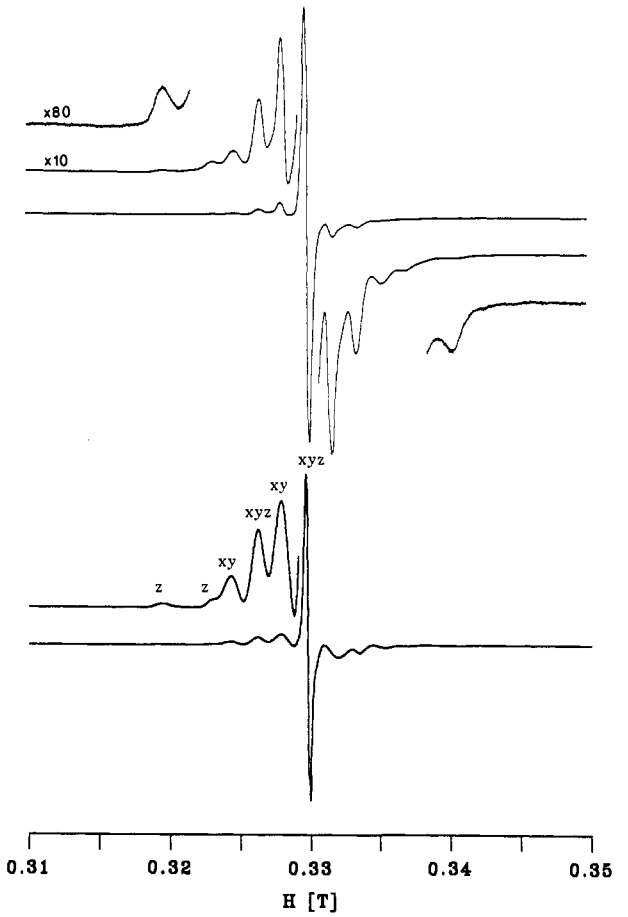
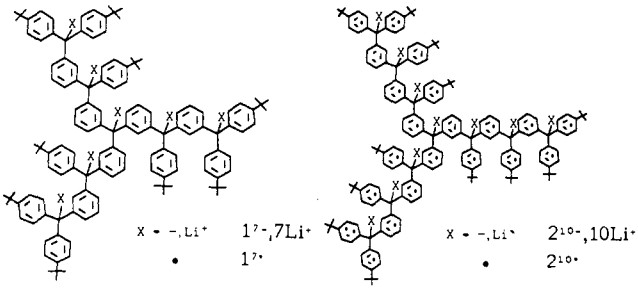


Figure 1. Top: X-band ESR spectrum for $\mathbf{1}^{7\bullet}$ in THF/2-MeTHF glass at 100 K. Bottom: Simulated $\Delta m_s = 1$ spectrum using m_s-dependent line widths and octet zfs, $|D/hc| = 0.001\,63\ cm^{-1}$ and $|E/hc| \approx 0\ cm^{-1}$.

that is, the known large preference for a triplet ground state for 1,3-connected diradicals may translate into the significant stabilization of a high-spin ground state in polyradicals.[7,8]

Now we report the preparation, magnetic resonance spectroscopy, and SQUID studies of the $S = {}^7/_2$ π-conjugated hydrocarbon heptaradical $\mathbf{1}^{7\bullet}$ and the homologous $S = 5$ decaradical $\mathbf{2}^{10\bullet}$. Both $\mathbf{1}^{7\bullet}$ and $\mathbf{2}^{10\bullet}$ are the highest spin π-conjugated polyradicals known to date,[6b,9] and the value $S = 5$ for $\mathbf{2}^{10\bullet}$ equals the S value of the highest spin organic molecule, the $S = 5$ pentacarbene.[4a]



(1) Proceedings of the Symposium on Ferromagnetic and High Spin Molecular Based Materials; 197th National Meeting of the American Chemical Society, Dallas, TX, April 1989. Miller, J. S.; Dougherty, D. A. *Mol. Cryst. Liq. Cryst.* **1989**, *176*, 1-562. Iwamura, H. *Adv. Phys. Org. Chem.* **1990**, *26*, 179. Dougherty, D. A. *Acc. Chem. Res.* **1991**, *24*, 88.

(2) Allemand, P.-M.; Khemani, K. C.; Koch, A.; Wudl, F.; Holczer, K.; Donovan, S.; Gruner, G.; Thompson, J. D. *Science* **1991**, *253*, 301. Kinoshita, M.; Turek, P.; Tamura, M.; Nozawa, K.; Shiomi, D.; Nakazawa, Y.; Ishikawa, M.; Takahashi, M.; Awaga, K.; Inabe, T.; Maruyama, Y. *Chem. Lett.* **1991**, 1225. Tamura, M.; Nakazawa, Y.; Shiomi, D.; Nozawa, K.; Hosokoshi, Y.; Ishikawa, M.; Takahashi, M.; Kinoshita, M. *Chem. Phys. Lett.* **1991**, *186*, 401.

(3) (a) Boyd, P. D. W.; Li, R.; Vincent, J. B.; Folting, K.; Chang, H. R.; Straib, W. E.; Huffmann, J. C.; Christou, G.; Hendrickson, D. N. *J. Am. Chem. Soc.* **1988**, *110*, 8537. (b) Caneschi, A.; Gatteschi, D.; Laugier, J.; Rey, P.; Sessoli, R.; Zanchini, C. *J. Am. Chem. Soc.* **1988**, *110*, 2795. (c) Caneschi, A.; Gatteschi, D.; Sessoli, R.; Barra, A. L.; Brunel, L. C.; Guillot, M. *J. Am. Chem. Soc.* **1991**, *113*, 5873. (d) McCusker, J. K.; Christmas, C. A.; Hagen, P. M.; Chadha, R. K.; Harvey, D. F.; Hendrickson, D. N. *J. Am. Chem. Soc.* **1991**, *113*, 6114.

(4) (a) Fujita, I.; Teki, Y.; Takui, T.; Kinoshita, T.; Itoh, K.; Miko, F.; Sawaki, Y.; Iwamura, H.; Izuoka, A.; Sugawara, T. *J. Am. Chem. Soc.* **1990**, *112*, 4074. (b) Sugawara, T.; Bandow, S.; Kimura, K.; Iwamura, H.; Itoh, K. *J. Am. Chem. Soc.* **1984**, *106*, 6449. Sugawara, T.; Bandow, S.; Kimura, K.; Iwamura, H.; Itoh, K. *J. Am. Chem. Soc.* **1986**, *108*, 368. (c) Takui, T.; Itoh, K. *Chem. Phys. Lett.* **1973**, *19*, 120. (d) Itoh, K. *Chem. Phys. Lett.* **1967**, *1*, 235. Wasserman, E.; Murray, R. W.; Yager, W. A.; Trozzolo, A. M.; Smolinsky, G. *J. Am. Chem. Soc.* **1967**, *89*, 5076.

(5) Mataga, N. *Theor. Chim. Acta* **1968**, *10*, 372. Tyutyulkov, N.; Polansky, O. E.; Schuster, P.; Karabunarliev, S.; Ivanov, C. I. *Theor. Chim. Acta* **1985**, *67*, 211.

(6) (a) Rajca, A. *J. Am. Chem. Soc.* **1990**, *112*, 5889. (b) Rajca, A. *J. Am. Chem. Soc.* **1990**, *112*, 5890. (c) Utamapanya, S.; Rajca, A. *J. Am. Chem. Soc.* **1991**, *113*, 9242.

(7) Kato, S.; Morokuma, K.; Feller, D.; Davidson, E. R.; Borden, W. T. *J. Am. Chem. Soc.* **1983**, *105*, 1791. Lahti, P. M.; Ichimura, A. S.; Berson, J. A. *J. Org. Chem.* **1989**, *54*, 958.

(8) Rajca, A.; Utamapanya, S.; Xu, J. *J. Am. Chem. Soc.* **1991**, *113*, 9235. Veciana, J.; Rovira, C.; Crespo, M. I.; Armet, O.; Domingo, V. M.; Palacio, F. *J. Am. Chem. Soc.* **1991**, *113*, 2552.

(9) (a) Seeger, D. E.; Berson, J. A. *J. Am. Chem. Soc.* **1983**, *105*, 5144, 5146. Seeger, D. E.; Lahti, P. M.; Rossi, A. R.; Berson, J. A. *J. Am. Chem. Soc.* **1986**, *108*, 1251. Berson, J. A. In *The Chemistry of Quinoid Compounds*; Patai, S., Rappoport, Z., Eds.; Wiley: New York, 1988; Vol. II, Chapter 10. (b) Novak, J. A.; Jain, R.; Dougherty, D. A. *J. Am. Chem. Soc.* **1989**, *111*, 7618. Dougherty, D. A. *Mol. Cryst. Liq. Cryst.* **1989**, *176*, 25. (c) Kirste, B.; Grimm, M.; Kurreck, H. *J. Am. Chem. Soc.* **1989**, *111*, 108. (d) Rajca, A. *J. Am. Chem. Soc.* **1990**, *112*, 5890.

0002-7863/92/1514-1884$03.00/0