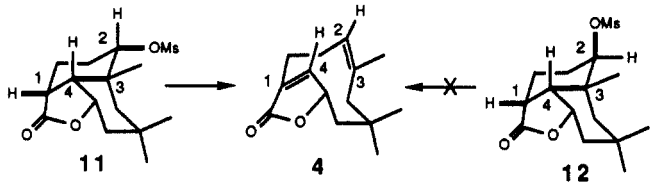
of undergoing an internal redox (substitution) reaction. With little nuclear motion, transfer of an electron (in the limiting case)[12] from the enolate HOMO to the C-I σ^* would produce diyl **10** directly or indirectly through its expectedly short-lived (10^{-8}-10^{-10} s)[13] C-I radical anion precursor. Alternatively, enolate-accelerated heterolysis of the CI bond could produce a zwitterion, differing from **10** only in electron distribution.[12] In either case, the stereochemical information stored in the original C-I bond would be lost at this point as the resulting C2 center in **10** would rapidly invert or be planar.[14] As generated in this least motion path, **10** is in a conformation suitable for direct orbital overlap controlled fragmentation[2-4] to the (*Z*,*Z*)-cyclodecadiene **4**. The conversion of **6** to diyl **10** finds analogy in substitution reactions proceeding by SET. For such reactions, diradical formation is followed by cage combination, while in the current case, the analogous diyl combination is frustrated by the energetic cost of closure to the strained bicyclo[2.2.0]hexane subunit.[15]

As a further test of the above analysis, the fragmentation of mesylates **11** and **12** was examined. Since the mesylate group retains the superior leaving-group ability of an iodide but is less easily reduced, an SET based fragmentation would be unlikely for these compounds. In accord with this expectation, mesylate **11**, under the above conditions, gave only (*Z*,*Z*)-diene **4** (84%) and starting material, while the epimeric mesylate (**12**) proved unreactive, even at 25 °C for 1.5 h.



11 **4** **12**

In summary, a fragmentation reaction is reported that proceeds in a stereochemical sense completely opposite that expected from the conventional mechanism. Preliminary evidence is consistent with this process occurring by a novel SET pathway or an anion-accelerated heterolysis. Either pathway represents a novel example of a frustrated substitution in which the nucleophile is positioned suitably close to an electrophilic center to transfer an electron or to induce heterolysis but closure of the resulting diyl or zwitterion is frustrated by a faster fragmentation. As it relates to synthesis, this novel fragmentation creates new opportunities for regulating the stereochemistry and mode selectivity of fragmentation reactions as evidenced by the efficient and selective formation of cyclodecadienes from readily available lactones.

Acknowledgment. This work was supported by a grant (CA31845) from the National Institutes of Health. The assistance of Dr. Gil Shoham with the X-ray structure determination of iodide **6** is gratefully acknowledged.

Supplementary Material Available: Spectroscopic data (NMR, IR, and MS) for compounds **6**, **9**, and **4** and tables of X-ray crystallographic data for compound **6** and the diol derivative of **4** (5 pages); listing of observed and calculated structure factors for compounds **6** and **4** (14 pages). Ordering information is given on any current masthead page.

(12) This zwitterion could undergo direct fragmentation or internal return to give **9** and thence fragmentation. For a relevant theoretical analysis, see: Hwang, J.-K.; King, G.; Creighton, S.; Warshel, A. *J. Am. Chem. Soc.* **1988**, *110*, 5297. A chain process involving SET can also be involved in the formation of **10** or **4**.

(13) For lead references, see: Infelta, J. J.; Schuler, R. H. *J. Phys. Chem.* **1972**, *76*, 987. Garst, J. F.; Roberts, R. D.; Pacifici, J. A. *J. Am. Chem. Soc.* **1977**, *99*, 3528. Andrieux, C. P.; Gallardo, I.; Saveant, J. M. *J. Am. Chem. Soc.* **1989**, *111*, 1620. Hawley, M. D. In *Encyclopedia of Electrochemistry of the Elements*; Bard, A. J., Lund, H., Eds.; Marcel Dekker: New York, **1980**; Vol. XIV.

(14) Cyclohexyl: Ogawa, S.; Fessenden, R. W. *J. Chem. Phys.* **1964**, *41*, 994. Bonnazola, L.; Leray, N.; Marx, R. *Chem. Phys. Lett.* **1974**, *24*, 88. Jensen, F. R.; Gale, L. H.; Rodgers, J. E. *J. Am. Chem. Soc.* **1968**, *90*, 5793. McConnell, H. M.; Fessenden, R. W. *J. Chem. Phys.* **1959**, *31*, 1866.

(15) For discussions bearing on this analysis, see refs 1 and 11. For earlier and more recent observations bearing on this analysis, see: Wiberg, K. B.; Pratt, W. E.; Bailey, W. F. *J. Am. Chem. Soc.* **1977**, *99*, 2298. Adcock, A.; Gangodawila, H. *J. Org. Chem.* **1989**, *54*, 6040. Hassenruck, K.; Radzisewski, G.; Balaji, V.; Murthy, G. S.; McKinley, A. J.; David, D. E.; Lynch, V. M.; Martin, H.-D.; Michl, J. *J. Am. Chem. Soc.* **1990**, *112*, 873. Eaton, P. E.; Tsanaktsidis, J. *J. Am. Chem. Soc.* **1990**, *112*, 876.

Observation of an Isotope Effect in the Chorismate Synthase Reaction

Shankar Balasubramanian,† Chris Abell,*,† and John R. Coggins‡

University Chemical Laboratory, University of Cambridge Lensfield Road, Cambridge CB2 1EW, England
Department of Biochemistry, University of Glasgow Glasgow G12 8QQ, Scotland
Received July 6, 1990

Chorismate synthase (EC 4.6.1.4) catalyzes the seventh step on the shikimate pathway, the conversion of 5-enolpyruvylshikimate 3-phosphate (**1**, EPSP) to chorismate (**2**) (Scheme I).[1] The reaction involves the removal of the *pro-R* hydrogen on C-6 and loss of phosphate in what is formally a 1,4-elimination to generate a diene.[2,3] The overall anti stereochemistry of the elimination has led to a reluctance to postulate a concerted mechanism, and consequently several other mechanisms of varying plausibility have been proposed. These include, inter alia, an X-group mechanism,[3] a 1,3-suprafacial shift of phosphate to C-1 followed by an E2 elimination,[1] and a carbonium ion mechanism where loss of phosphate precedes C-H bond breaking.[4] Each of these mechanisms avoids the problem that concerted anti 1,4-eliminations are historically disfavored. This conclusion comes from studies on model systems[5] and from molecular orbital considerations.[6] However, these objections to a concerted process are not soundly based in the context of an enzyme-catalyzed reaction where the orientation of catalytic groups may well play a decisive role.

The first step toward elucidating the actual mechanism of the enzyme-catalyzed reaction is to establish the timing of the two bond-breaking steps, at C-3 and C-6. This is experimentally accessible if bond breaking at either position is partly rate determining and proceeds with an associated isotope effect. In this communication we report the observation of a primary kinetic isotope effect for cleavage of the carbon-hydrogen bond at C-6. This result is surprising in the light of a preliminary study, which failed to detect an isotope effect.[7]

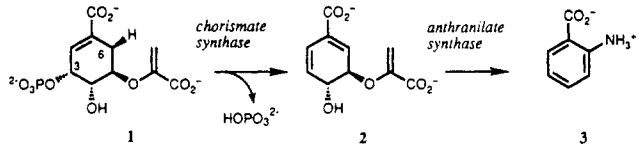
The synthesis of (6*R*)-[6-^{2}H]EPSP is outlined in Scheme II. (6*R*)-[6-^{2}H]shikimic acid (**5**) (and enantiomer) was synthesized from (*Z*)-[3-^{2}H]acrylic acid[8] and (*E*,*E*)-1,4-diacetoxybutadiene by the route of Raphael and Smissman.[9] The resulting (±)-shikimic acid had 94 ± 2% deuterium cis to the C-5 hydroxyl group and 5 ± 2% in the trans position.[10] The minor deuteriated

* Author to whom correspondence should be sent: Dr. C. Abell, University Chemical Laboratory, University of Cambridge, Lensfield Rd., Cambridge CB2 1EW, England. Telephone +44-223-336405; FAX +44-223-336362.
† University of Cambridge.
‡ University of Glasgow.

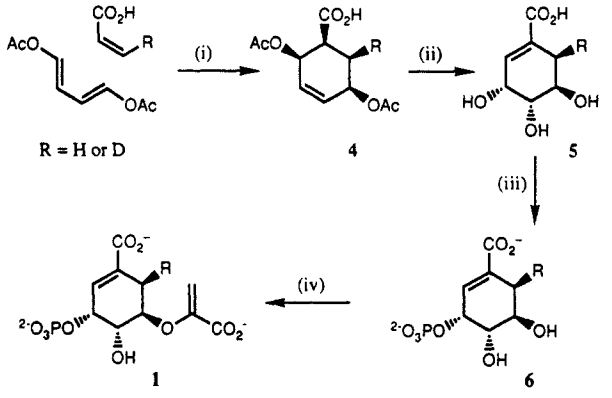
(1) Ganem, B. *Tetrahedron* **1978**, *34*, 3353-3383.
(2) Hill, R. K.; Newkome, G. R. *J. Am. Chem. Soc.* **1969**, *91*, 5893-5894.
(3) Onderka, D. K.; Floss, H. G. *J. Am. Chem. Soc.* **1969**, *91*, 5894-5896. Onderka, D. K.; Floss, H. G.; Carroll, M. *J. Biol. Chem.* **1972**, *247*, 736-744.
(4) Hawkes, T. R.; Lewis, T.; Coggins, J. R.; Mousdale, D. M.; Lowe, D. L.; Thorneley, R. N. F. *Biochem. J.* **1990**, *265*, 899-902.
(5) Hill, R. K.; Bock, M. G. *J. Am. Chem. Soc.* **1978**, *100*, 637-639. Toromanoff, E. *C. R. Seances Acad. Sci., Ser. C* **1980**, *290*, 81-84.
(6) Fukui, K. *Tetrahedron Lett.* **1965**, 2427-2432. Anh, N. T. *J. Chem. Soc., Chem. Commun.* **1968**, 1089-1090.
(7) Bartlett, P. A.; McLaren, K. L.; Alberg, D. G.; Fassler, A.; Nyfeler, R.; Lauhon, C. T.; Grissom, C. B. *Proceedings of the Society for Chemical Industry Pesticides Group Meeting*; BCPC Monograph 42; Lavenham Press: Lavenham, Suffolk, England, 1989; pp 155-170.
(8) Crout, D. H. G.; Corkill, J. A. *Tetrahedron Lett.* **1977**, 4355-4357.
(9) Mc.Crindle, R.; Overton, K. H.; Raphael, R. A. *J. Chem. Soc.* **1960**, 1560-1565. Smissman, E. E.; Suh, J. T.; Oxman, M.; Daniels, R. *J. Am. Chem. Soc.* **1959**, *81*, 2909-2910. Smissman, E. E.; Suh, J. T.; Oxman, M.; Daniels, R. *J. Am. Chem. Soc.* **1962**, *84*, 1040-1041.

0002-7863/90/1512-8581$02.50/0

Scheme I



Scheme II[a]



[a] (i) Temperature 90 °C, 3 h, 40% after recrystallization to remove unwanted exo diastereomer. (ii) Six steps as described in ref 9, 22%. (iii) Shikimate kinase, 1.8 units, 24 h. (iv) EPSP synthase, 0.08 unit, 8 h. Transformations iii and iv were carried out sequentially on 100 mM shikimic acid in 1.0 mL of D_2O, pD 7.1 (Tris-DCl, 50 mM) at 25 °C containing 50 mM $MgCl_2$, 50 mM ATP, and 50 mM PEP, and were followed by ^1H NMR spectroscopy. Compounds **4** and **5** are racemic, and compounds **6** and **1** are homochiral.

isomer comes from a small amount of (*E*)-[3-^{2}H]acrylic acid formed in the sodium amalgam reduction of (*Z*)-3-bromoacrylic acid to (*Z*)-[3-^{2}H]acrylic acid.[8] The Diels–Alder reaction gave an approximately 80/20 endo/exo mixture of diastereomers, from which the required endo isomer **4** was purified by repeated recrystallization. Failure to remove the exo diastereomer of **4** results in more deuterium in the *pro-S* position of (−)-shikimic acid (**5**).

The (±)-shikimic acid was converted enzymatically to EPSP using purified *Escherichia coli* shikimate kinase[11] and EPSP synthase.[12] These conversions were carried out sequentially in an NMR tube, by monitoring the signal for the C-2 hydrogen of each compound. Addition of shikimate kinase to a mixture of the (±)-shikimic acid and cofactors in deuteriated buffer gave essentially complete conversion of one enantiomer of shikimic acid to shikimate 3-phosphate (**6**). EPSP synthase was then added and quantitatively converted the shikimate phosphate to EPSP (**1**). The enzyme reaction mixture was treated with apyrase (Sigma, grade VII) to degrade ATP and ADP in order to facilitate the purification of the EPSP by ion-exchange chromatography on Dowex 1-X8. Finally the EPSP was precipitated and stored as the barium salt.[13] Before use in an enzyme assay, the EPSP was converted to the potassium salt. Labeled and unlabeled samples were prepared in parallel experiments.

The undeuteriated and deuteriated EPSP were used as substrates for purified *Neurospora crassa* chorismate synthase[14] to determine any isotope effect on V_{max} and V_{max}/K_m. These are distinct experiments which required different experimental approaches. The first experiment was to measure V_{max} for the labeled and unlabeled EPSP using a direct UV assay following the appearance of the diene chromophore at 275 nm.[15] Figure 1 shows the trace obtained for protio, 94% deuterio, and a 1/1 mixture of the two. The initial concentration of each substrate was 50 μM, well above the reported K_m value of 2.7 μM.[16] The increase in absorbance is abruptly terminated on exhaustion of EPSP. These traces clearly demonstrate a pronounced isotope effect on V_{max}. Analysis of the linear portion of each curve gives $(V_{max})_H/(V_{max})_D = 2.7 \pm 0.2$.[17]

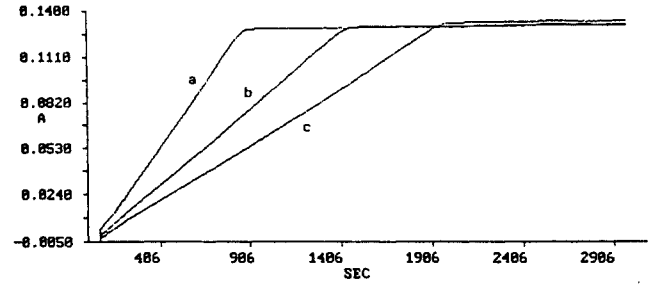


Figure 1. UV time course for the chorismate synthase reaction followed at 275 nm (ϵ_{275} = 2630 M^{-1} cm^{-1}). The assays were carried out at 25 °C, pH 7.0 (Bis-Tris-HCl, 50 mM), and included 2 milliunits of chorismate synthase, 20 μM NADPH, 10 μM FMN, 50 mM KCl, and 2.5 mM $MgCl_2$, in addition to 50 μM substrate, in a final volume of 1 mL. (a) EPSP; (b) (6*R*)-[6-^{2}H]EPSP (47% deuteriated); (c) (6*R*)-[6-^{2}H]-EPSP (94% deuteriated).

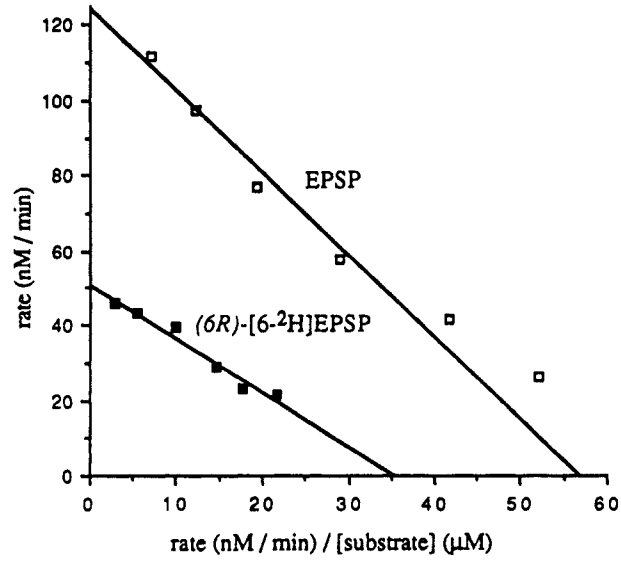


Figure 2. Eadie–Hofstee plot for EPSP (**1**) and (6*R*)-[6-^{2}H]EPSP. Fluorimetric assays (excitation at 313 nm, emission at 390 nm) were carried out at 25 °C, pH 7.0 (Bis-Tris-HCl, 50 mM), and included 0.12 milliunit of chorismate synthase, 6 milliunits of anthranilate synthase, 100 μM NADPH, 10 mM glutamine, 10 μM FMN, 50 mM KCl, and 2.5 mM $MgCl_2$, in addition to substrate, in a final volume of 1 mL. The fluorimeter was calibrated by using anthranilate standards. The data was analyzed by using Enzfitter.

The second experiment was to measure the isotope effect on V_{max}/K_m. This experiment involves assaying chorismate synthase at substrate concentrations close to K_m, a range that is below the sensitivity of the UV assay. Chorismate synthase was therefore coupled with anthranilate synthase[18] to convert chorismate (**2**) to anthranilate (**3**), which can be detected fluorimetrically.[19] This

(10) The site and extent of deuteriation were measured by ^1H and ^2H NMR spectroscopy, and mass spectrometry for intermediates in the reaction sequence. All intermediates have been characterized by NMR spectroscopy, HRMS, IR, and UV.

(11) Millar, G.; Lewendon, A.; Hunter, M. G.; Coggins, J. R. *Biochem. J.* **1986**, *237*, 427–437.

(12) Duncan, K.; Lewendon, A.; Coggins, J. R. *FEBS Lett.* **1984**, *165*, 121–127.

(13) Knowles, P. F.; Levin, J. G.; Sprinson, D. B. *Methods Enzymol.* **1970**, *17*, 360–362.

(14) White, P. J.; Millar, G.; Coggins, J. R. *Biochem. J.* **1988**, *251*, 313–322.

(15) Gibson, F. *Methods Enzymol.* **1970**, *17*, 362–364.

(16) Bartlett, P. A.; Maitra, U.; Chouinard, P. M. *J. Am. Chem. Soc.* **1986**, *108*, 8068–8071.

(17) This was confirmed by additional experiments to measure the isotope effect as a function of extent of deuteriation by diluting the 94% deuteriated sample with unlabeled EPSP, and also using a separately synthesized sample of EPSP which was only 80% deuteriated (not described).

(18) Coggins, J. R.; Boocock, M. R.; Chaudhuri, S.; Lambert, J. M.; Lumsden, J.; Nimmo, G. A.; Smith, D. D. S. *Methods Enzymol.* **1987**, *142*, 325–341.

(19) Welch, G. R.; Cole, K. W.; Gaertner, F. H. *Arch. Biochem. Biophys.* **1974**, *165*, 505–518.

assay is sensitive down to submicromolar concentrations of EPSP. Figure 2 is an Eadie–Hofstee plot of the data obtained by performing the coupled assay over a range of EPSP concentrations from 0.5 to 16 μM. The K_m of unlabeled EPSP was determined to be 2.2 ± 0.2 μM, in close agreement with the published value of 2.7 μM.[16] The line for the (6*R*)-[6-^{2}H]EPSP has a different gradient and intercepts on both axes, showing a change in both V_{max} and K_m. The ratio $(V_{max}/K_m)_H/(V_{max}/K_m)_D$ is 1.6 ± 0.1, with $(K_m)_D$ = 1.4 ± 0.1 μM. From this data the ratio $(V_{max})_H/(V_{max})_D$ is 2.5 ± 0.2, which agrees, within experimental error, with the value obtained directly from the UV assay.

The experimental data clearly show a primary kinetic isotope effect for carbon–hydrogen bond cleavage at C-6. Values of k_H/k_D of 2–3 are typical for enzymic reactions involving carbon–hydrogen cleavage where this step is partially rate limiting.[20] The reduced isotopic discrimination in the ratio $(V_{max}/K_m)_H/(V_{max}/K_m)_D$ of 1.6 indicates a high commitment to catalysis.[21]

This result is important as it opens up the way for a detailed kinetic analysis of the chorismate synthase reaction which can address the question of concertedness of bond breaking at C-6 and C-3. It has been reported that pre-steady-state experiments did not detect a burst of phosphate release, showing that loss of phosphate is not a fast step prior to the rate-determining step.[4] The absence of a burst of phosphate release and the isotope effect at C-6 together can best be accommodated by a concerted mechanism, and this mechanism must now receive serious consideration.

Acknowledgment. We thank S. E. Jackson, A. Matouschek, Dr. P. J. White, and Dr. T. R. Hawkes for their assistance with aspects of this project and Dr. F. J. Leeper and Professor D. E. Cane for their comments on the manuscript. We thank I.C.I. Agrochemicals and the SERC for a CASE Studentship for S.B.

(20) Northrop, D. B. *Biochemistry* **1975**, *14*, 2644–2651.
(21) Using the experimental values for V_{max} and K_m and assuming that the dissociation constants for protio and deuteriated EPSPs are equal, it is found that $(k_{cat})_D \approx k_{-1}$.

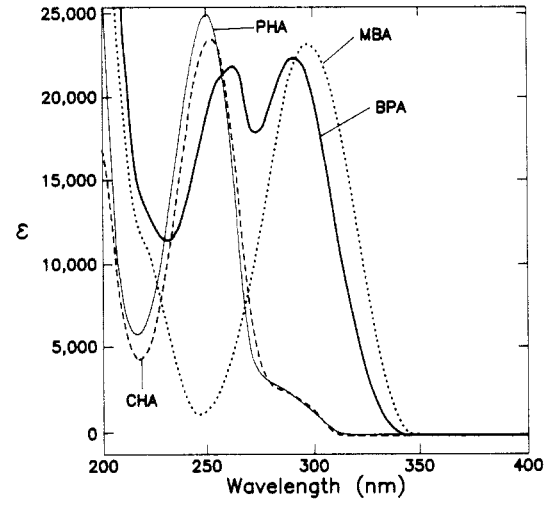
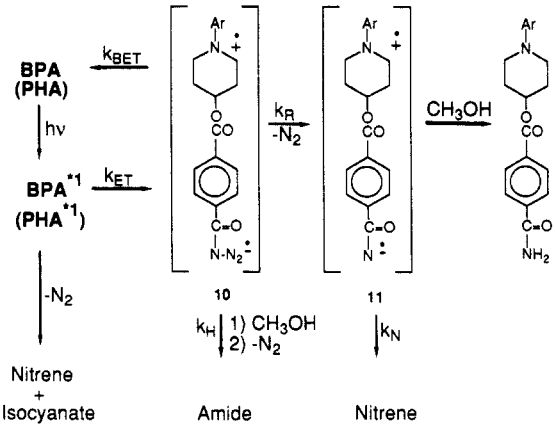


Figure 1. The absorption spectra of *N*,*N*-dimethyl-4-biphenylamine (MBA) and cyclohexyl-substituted (CHA), *N*-phenylpiperidinyl-substituted (PHA), and *N*-biphenylylpiperidinyl-substituted (BPA) benzoyl azides in CH_3CN solutions at room temperature.

Scheme I



Photoinduced Through-Bond Electron Transfer: Remote Activation of Unique Aroyl Azide Reactivity

Yong Zhu and Gary B. Schuster*

Department of Chemistry, Roger Adams Laboratory
University of Illinois, Urbana, Illinois 61801

Received July 13, 1990

Photoinduced electron transfer reactions play an important role in chemical and biochemical processes.[1] Experimental investigations have shown that this reaction may occur between species separated by distances far greater than the sum of their van der Waals radii.[2] Theoretical analyses are consistent with these findings.[3] Of particular relevance to the present investigation are the efficient intramolecular photoinduced electron transfer reactions reported for acceptor-substituted *N*-arylpiperidines.[4] In previous work in this field, through-bond electron transfer has been detected spectroscopically. The systems examined were selected for study particularly because electron transfer does not initiate an irreversible chemical change. Herein we report the first example[5] of unique photochemical reactivity associated with through-bond electron transfer in aroyl azide substituted *N*-arylpiperidines (see Chart I).[6] In methyl alcohol solution, light absorbed by the biphenylamine chromophore of BPA initiates reaction of an aroyl azide radical anion formed by through-bond electron transfer.

The absorption spectra of *N*,*N*-dimethyl-4-biphenylamine (MBA) and cyclohexyl-substituted (CHA), *N*-phenylpiperidinyl-substituted (PHA), and *N*-biphenylylpiperidinyl-substituted (BPA) benzoyl azides in CH_3CN solutions are shown

(1) Deisenhofer, J.; Epp, O.; Miki, K.; Huber, R.; Michel, H. *J. Mol. Biol.* **1984**, *180*, 385. Mattay, J. *J. Synth. Org. Chem., Jpn.* **1989**, 233. Winkler, J. R.; Nocera, D. G.; Yocom, K. M.; Bordignon, E.; Gary, H. B. *J. Am. Chem. Soc.* **1982**, *104*, 5798.
(2) Wasielewski, M. R.; Minsek, D. W.; Niemczyk, M. P.; Svec, W. A.; Yang, N. C. *J. Am. Chem. Soc.* **1990**, *112*, 2823. Schanze, K. S.; Cabana, L. A. *J. Phys. Chem.* **1990**, *94*, 2740. Brun, A. M.; Hubig, S. M.; Rodgers, M. A. J.; Wade, W. H. *J. Phys. Chem.* **1990**, *94*, 3869. Closs, G. L.; Miller, J. R. *Science* **1988**, *240*, 440. Heitele, H.; Finckh, P.; Michel-Beyerle, M. E. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 619. Heitele, H.; Michel-Beyerle, M. E. *J. Am. Chem. Soc.* **1985**, *107*, 8286.
(3) Marcus, R. A. *J. Chem. Phys.* **1965**, *43*, 679. Hush, N. S. *Trans. Faraday Soc.* **1961**, *57*, 557. Hoffmann, R.; Imamura, A.; Hehre, W. J. *J. Am. Chem. Soc.* **1968**, *90*, 1499. Closs, G. L.; Johnson, M. D.; Miller, J. R.; Piotrowiak, P. *J. Am. Chem. Soc.* **1989**, *111*, 3751.
(4) (a) Mes, G. F.; de Jong, B.; van Ramesdonk, H. J.; Verhoeven, J. W.; Warman, J. M.; de Hass, M. P.; Horsman-van den Dool, L. E. W. *J. Am. Chem. Soc.* **1984**, *106*, 6524. (b) Krijnen, B.; Beverloo, H. B.; Verhoeven, J. W.; Reiss, C. A.; Goubitz, K.; Heijdenrijk, D. *J. Am. Chem. Soc.* **1989**, *111*, 4433. (c) Wegewijs, B.; Hermant, R. M.; Verhoeven, J. W.; de Hass, M. P.; Warman, J. M. *Chem. Phys. Lett.* **1990**, *168*, 185. (d) Hermant, R. M.; Bakker, N. A. C.; Scherer, T.; Krinjen, B.; Verhoeven, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 1214. (e) Kroon, J.; Oliver, A. M.; Paddon-Row, M. N.; Verhoeven, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 4868. (f) Paddon-Row, M. N.; Wong, S. S. *Chem. Phys. Lett.* **1990**, *167*, 432.
(5) We earlier attempted a similar reaction with partial success. Shields, C. J.; Falvey, D. E.; Schuster, G. B.; Buchardt, O.; Nielsen, P. E. *J. Org. Chem.* **1988**, *53*, 3501.
(6) All new compounds gave acceptable elemental analyses and exhibited expected spectral properties.

0002-7863/90/1512-8583$02.50/0