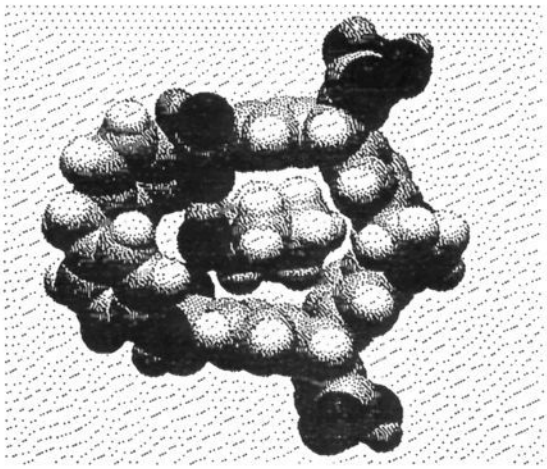
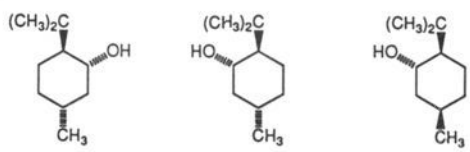


Figure 1. Representation of a molecular complex between synthetic receptor **1** (shown as the tetraacid) and cyclohexanol. The atomic radii used here are not van der Waals type radii, but instead approximate the radii used in CPK models. The profile of the cyclic alkane is well matched by the hexagonal lipophilic pocket enclosed by the receptor.

The terpenes offer a readily available library of defined alkane shapes.[14] Binding of this new receptor to (−)-menthol (**12**), (+)-menthol (**13**), and (+)-isomenthol (**14**) in a $ND_4^+Cl^-/ND_3$ buffer at pD 9.0 in D_2O at 20 °C was evaluated by analysis of NMR titration data.[15] It was found that (−)-menthol and (+)-menthol have association constants of 2500 ± 200 M^{-1} and 2000 ± 200 M^{-1}, respectively. There are no previous reports of binding of such small, partially water soluble alkanes to a synthetic receptor, but this result compares favorably with association constants obtained for much larger and less water soluble aliphatic guests.[7c]



12 **13** **14**

The axial methyl group of (+)-isomenthol was predicted to inhibit binding of this molecule within this carefully shaped cleft. Indeed, the association constant with (+)-isomenthol (1000 ± 200 M^{-1}) is half that observed for (+)-menthol. The limiting chemical shifts for the three methyl groups in each of these substrates indicate differences in the time-averaged binding position of the three isomeric menthols. Chemical shifts of the isopropyl methyl groups in (+)-isomenthol (+1.45/+1.20 ppm) are much larger than those observed for corresponding groups in (−)- and (+)-menthol (+0.66/+0.55 ppm and +0.43/+0.68 ppm, respectively).[16] This supports the idea that the axial methyl group in isomenthol causes the time-averaged position of the receptor to be shifted toward the isopropyl group.

Receptor **1** was designed to be a conformationally rigid molecule that would make intimate contact with an enclosed cyclohexanoid substrate: it was not designed to recognize the two enantiomers of menthol. The observed differences in binding of (+)- and (−)-menthol, though small, are unprecedented in the context of small alicyclic molecular recognition in aqueous media. This success suggests that the receptor–substrate contacts are quite intimate (Figure 1).

The synthesis and initial binding studies that are reported here illustrate that stereoselective binding can be achieved in water by a synthetic receptor without multiple receptor–substrate hydrogen bonds. The results are modest, but in these initial studies, partially water soluble guests were tested, and they were chosen because they were easily available and the association constants could be accurately measured. Less water soluble targets will have higher affinities for this receptor, and other guests will be bound more enantioselectively. Because the synthesis is convergent and capping structures more elaborate and interactive than 4,4′-methylenebis(aniline) can easily be utilized, other, more shape-selective synthetic receptors can be based on this general system.

Acknowledgment. This work was funded by the National Institute of General Medical Sciences (GM-34846) and the Alfred P. Sloan Foundation. Dr. Fu-Tyan Lin provided support and advice in our NMR experiments. Molecular modeling software was obtained through the generosity of Professor W. C. Still (Department of Chemistry, Columbia University; MacroModel software) and Dr. B. Hesp, Ph.D. (ICI Pharmaceuticals; Enigma and Aesop software).

Supplementary Material Available: Preparative procedures and characteristic data (IR, ^1H NMR, ^{13}C NMR, MS) for **4**, **5**, **6a**, and **8–10**, complete binding data for association constants, and plots of calculated and observed chemical shifts for each binding experiment (8 pages). Ordering information is given on any current masthead page.

(11) (a) Wilcox, C. S.; Cowart, M. D. *Tetrahedron Lett.* **1986**, *27*, 5563–5566. (b) Sucholeiki, I.; Lynch, V.; Phan, L.; Wilcox, C. S. *J. Org. Chem.* **1988**, *53*, 98–104. (c) Cowart, M. D.; Sucholeiki, I.; Bukownik, R. R.; Wilcox, C. S. *J. Am. Chem. Soc.* **1988**, *110*, 6204–6210.

(12) Johnson, R. A. U.S. Patent No. 4,447,607, 1984.

(13) The first example of such a macrocyclization: Masafumi, F.; Inazu, T. *J. Inclusion Phenom.* **1984**, *2*, 223–229.

(14) Simonsen, J. L. *The Terpenes*; University Press: Cambridge, 1947.

(15) For a review of these methods, see: Wilcox, C. S. In *Frontiers of Supramolecular Organic Chemistry and Photochemistry*; Schneider, H.-J., Dürr, H., Eds.; VCH: Weinheim, 1991.

(16) The differences in chemical shift induced for the two enantiomers of menthol suggest that host **1** could be a valuable new type of chiral shift reagent. In preliminary experiments, optically impure samples of menthol have been analyzed. Further work is underway.

Oxidative Ligand Transfer to Alkanes: A Model for Iron-Mediated C–X Bond Formation in β-Lactam Antibiotic Biosynthesis

Randolph A. Leising, Yan Zang, and Lawrence Que, Jr.*

Department of Chemistry, University of Minnesota Minneapolis, Minnesota 55455

Received June 3, 1991

Oxidative C–X bond formation reactions are proposed in the biosynthesis of antibiotic β-lactams such as isopenicillin N, cephalosporin, and clavaminate. The respective enzymes, isopenicillin N synthase,[1] deacetoxycephalosporin C synthase,[2] and clavaminate synthase,[3] all require Fe(II) in a non-heme active site and utilize O_2 to effect these transformations; the latter two also require the cofactor, α-ketoglutarate. It has been speculated that the C–X bond forms via an iron–oxo intermediate such as A which abstracts hydrogen from RH and transfers the X group to the caged alkyl radical (see Scheme I).[4] However, examples for such oxidative ligand transfers in simple model systems are lacking. Funtionalized alkanes have been obtained from $Mn^{III}TPP/PhIO$[5] and $Fe(PA)_2/H_2O_2$[6] systems;[7] however, these transformations result from the interception by Mn(TPP)X and

(1) (a) Baldwin, J. E. In *Recent Advances in the Chemistry of β-Lactam Antibiotics*; Bentley, P. H., Southgate, R., Eds.; Royal Society of Chemistry: London, 1989; Chapter 1. (b) Robinson, J. A. *Chem. Soc. Rev.* **1988**, *17*, 383–452.

(2) Baldwin, J. E.; Adlington, R. M.; Schofield, C. J.; Sobey, W. J.; Wood, M. E. *J. Chem. Soc., Chem. Commun.* **1989**, 1012–1015.

(3) (a) Elson, S. W.; Baggaley, K. H.; Gillett, J.; Holland, S.; Nicholson, N. H.; Sime, J. T.; Woroniecki, S. R. *J. Chem. Soc., Chem. Commun.* **1987**, 1736–1738. (b) Krol, W. J.; Basak, A.; Salowe, S. P.; Townsend, C. A. *J. Am. Chem. Soc.* **1989**, *111*, 7625–7627. (c) Salowe, S. P.; Krol, W. J.; Iwata-Reuyl, D.; Townsend, C. A. *Biochemistry* **1991**, *30*, 2281–2292.

(4) (a) Baldwin, J. E.; Abraham, E. *Nat. Prod. Rep.* **1988**, *5*, 129–145. (b) Townsend, C. A.; Basak, A. *Tetrahedron* **1991**, *47*, 2591–2602 and references therein.

(5) Brown, R. B.; Hill, C. L. *J. Org. Chem.* **1988**, *53*, 5762–5768 and references therein.

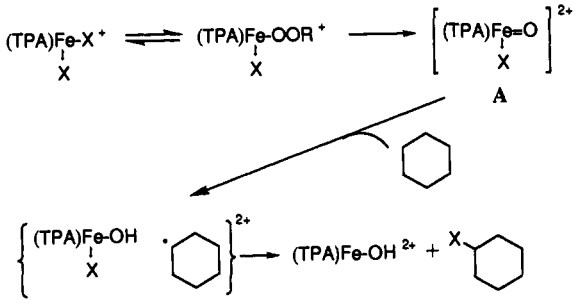
0002-7863/91/1513-8555$02.50/0

Table I. Product Distributions for the Iron-Catalyzed (3.0 mM) TBHP Oxidation of Cyclohexane (1.15 M) in Acetonitrile under 1 atm of Argon at 25 °C

catalyst	equiv[a] of TBHP	reactn time, h	products[b] Cl	Br	N_3	OOtBu
$[Fe(TPA)Cl_2](ClO_4)$	1	1	0.7	0	0	0
$[Fe(TPA)Cl_2](ClO_4)$	5	1	1.0	0	0	0.4
$[Fe(TPA)Br_2](ClO_4)$	1	0.5	0	0.7	0	0
$[Fe(TPA)Br_2](ClO_4)$	5	0.5	0	1.0	0	0.8
$[Fe(TPA)Br_2](ClO_4)$/BHT[c]	5	0.5	0	1.0	0	0
$[Fe(TPA)(N_3)_2](ClO_4)$	1	4	0	0	0.8	0
$[Fe(TPA)(N_3)_2](ClO_4)$	5	4	0	0	1.0	1.2[d]
$Fe(BPA)Br_3$	20	20	0	2.0	0	3.0[e]

[a] Relative to catalyst. [b] Amount = moles of product/moles of catalyst. [c] BHT concentration = 15 mM. [d] Also observed were 0.2 equiv of cyclohexanone and 0.1 equiv of cyclohexanol. [e] Also observed were 1.0 equiv of cyclohexanone and 1.0 equiv of cyclohexanol.

Scheme I



PhSeSePh of alkyl radicals generated from the oxidizing systems to afford RX and RSePh, respectively. Substoichiometric amounts of chlorocyclohexane form together with cyclohexanol and cyclohexanone from cyclohexane in a $Fe^{III}Cl_3/H_2O_2/CH_3CN$ system, but no mechanistic insight is provided for the formation of the haloalkane.[8,9] In our alkane functionalization studies mediated by ROOH/$[Fe^{III}(TPA)Cl_2]^+$, we uncovered a stoichiometric halide transfer from the metal center to the organic substrate. These results represent the first well-characterized example of oxidative ligand transfer mediated by a non-heme iron center and provide a model for the C–X bond formation reactions observed in β-lactam biosynthesis.

Alkane functionalization reactions were conducted using 1.15 M cyclohexane and 3.0 mM $[Fe^{III}(TPA)X_2](ClO_4)$[10] (X = Cl, Br, N_3: **1–3**, respectively) with varying amounts of TBHP in acetonitrile at 25 °C under 1 atm of oxygen-free argon.[11] Reagents **1–3** display remarkable selectivity; with 1 equiv of TBHP, *only* RX is produced, and no oxygenated products are observed (Table I). The high efficiency of these reactions (70–80%) is also exceptional, especially when compared to the efficiency of Fe(TPP)Cl/PhIO hydroxylation of cyclohexane (8%).[9c,12] As the amount of TBHP is increased relative to **1–3**, the RX yield maximizes at 1 equiv/catalyst and oxygenated products form in amounts indicative of catalytic turnover. The appearance of RX prior to the formation of oxygenated products suggests that the catalyst for RX formation differs from the actual hydroxylation catalyst and that the latter is generated as a consequence of producing RX. The implications of these results for the Fe(TPA)-catalyzed alkane hydroxylations will be discussed separately.

These observations support the conclusion that the oxidative functionalization reactions of cyclohexane seen here are metal-centered reactions. First, the maximum amount of RX produced with **1–3** is stoichiometric to the amount of iron, not X, used in the reaction. Only 1 equiv of RX relative to $Fe(TPA)X_2^+$ is formed even in the presence of excess oxidant, though two X ligands are bound to the initial catalyst. Furthermore, this 1:1 stoichiometry is retained even in the presence of excess bromide (2–750 equiv) added to **2**/TBHP/cyclohexane, demonstrating that the mechanism of functionalization does not involve free halide in solution.

Second, the stoichiometry of RX formation can be modified by varying the number of X ligands bound to the iron center. The reagent $Fe^{III}BPABr_3$ (**4**), which contains three bromide ligands, reacts with TBHP/cyclohexane to give a maximum of 2 equiv of bromocyclohexane/iron catalyst. Taken together, the TPA and BPA results suggest the dissociation of one halide ligand from the metal coordination sphere to initiate the oxidation reactions and the transfer of the remaining coordinated halides to substrate (vide infra).

Third, the formation of the functionalized alkanes is unaffected by the radical scavenger BHT (Table I), implying the participation of a radical cage in this mechanism. This result contrasts those for (Por)MX/PhIO functionalization of alkanes reported by Hill, where radical traps intercept the incipient radical and quench the production of RX.[13] Additionally, the selectivity of **2**/TBHP for functionalizing adamantane C–H bonds serves to discount the possibility of a Br•-initiated reaction. Functionalization of adamantane by **2**/TBHP results in the formation of 1- and 2-bromoadamantane, with a tertiary to secondary ratio of 6, while the adamantane + Br• under similar conditions gives a tertiary to secondary ratio of 20.

The stoichiometric ligand transfer observed in this system leads us to propose a mechanism involving a high-valent iron–oxo intermediate[14] with a coordinated X ligand (A in Scheme I). Intermediate A would result from the displacement of an X ligand

(6) Sheu, C.; Sobkowiak, A.; Zhang, L.; Ozbalik, N.; Barton, D. H. R.; Sawyer, D. T. *J. Am. Chem. Soc.* **1989**, *111*, 8030–8032. For a related system see: Balavoine, G.; Barton, D. H. R.; Boivin, J.; Lecoupanec, P.; Lelandis, P. *New J. Chem.* **1989**, *13*, 691–700.

(7) Abbreviations used: BPA = bis(2-pyridylmethyl)amine; TPA = tris(2-pyridylmethyl)amine; TBHP = *tert*-butyl hydroperoxide; BHT = 2,6-di-*tert*-butyl-4-methylphenol; TPP = tetraphenylporphyrin; Por = porphyrin; PA = picolinic acid; DPAH = pyridine-2,6-dicarboxylic acid.

(8) Sugimoto, H.; Sawyer, D. T. *J. Org. Chem.* **1985**, *50*, 1784–1786.

(9) $Fe^{II}(DPAH)_2/O_2/BrCCl_3$/cyclohexane has also recently been reported to produce bromocyclohexane in substoichiometric amounts; see: Sheu, C.; Sawyer, D. T. *J. Am. Chem. Soc.* **1990**, *112*, 8212–8214. The formation of RX from reaction of R• with halogenated solvents (such as CCl_4 or $BrCCl_3$) has been observed in a number of other first row transition metal catalyzed oxidations. See: (a) Hill, C. L.; Schardt, B. C. *J. Am. Chem. Soc.* **1980**, *102*, 6374–6375. (b) Saussine, L.; Brazi, E.; Robine, A.; Mimoun, H.; Fischer, J.; Weiss, R. *J. Am. Chem. Soc.* **1985**, *107*, 3534–3540. (c) Groves, J. T.; Nemo, T. E. *J. Am. Chem. Soc.* **1983**, *105*, 6243–6248.

(10) Compounds **1–3** were synthesized following a previously described procedure (ref 11). CAUTION! Metal perchlorate complexes with organic ligands are potentially explosive.

(11) Leising, R. A.; Norman, R. E.; Que, L., Jr. *Inorg. Chem.* **1990**, *29*, 2553–2555.

(12) Dicken, C. M.; Lu, F.-L.; Nee, M. W.; Bruice, T. C. *J. Am. Chem. Soc.* **1985**, *107*, 5776–5789.

(13) Smegal, J. A.; Hill, C. L. *J. Am. Chem. Soc.* **1983**, *105*, 3515–3521. Interestingly, in our studies of Fe(TPA)-catalyzed oxygenation of cyclohexane, BHT does quench the formation of (*tert*-butyldioxy)cyclohexane (Table I), which has been proposed to derive from chain-terminating reactions of cyclohexyl and *tert*-butyldioxy radicals (see ref 11).

(14) The formation of a high-valent (TPA)iron–oxo intermediate in acetonitrile was recently demonstrated; see: Leising, R. A.; Brennan, B. A.; Que, L., Jr.; Münck, E.; Fox, B. G. *J. Am. Chem. Soc.* **1991**, *113*, 3988–3990.

by hydroperoxide on the iron center, followed by peroxide heterolysis. H abstraction from the alkane by the high-valent intermediate generates a caged radical species, which collapses to the observed product. Clearly this scheme resembles the well-known oxygen rebound mechanism proposed for (porphyrinato)metal-oxo oxidation of alkanes,[15] but with one critical difference. Unlike the planar porphyrin ligands which enforce a diaxial configuration for the oxo and X ligands, the tetradentate tripodal ligand provides a cis coordination geometry. Such a configuration allows the caged radical a choice of either OH or X transfer in the rebound step. Our present observations suggest that the X group is transferred *preferentially* over OH in this chemistry (perhaps because of the lower oxidation potential of the X group) and lend credence to the proposed C-X bond forming mechanisms involving high-valent iron intermediates in the biosynthesis of β-lactam antibiotics.[4]

Acknowledgment. This work was supported by grants from the National Institutes of Health (GM-33162). We acknowledge an NIH National Research Service Award to R.A.L. (GM-13343).

(15) For example, see: Groves, J. T. *J. Chem. Educ.* **1985**, *62*, 928-931.

Model Studies of DNA Photorepair: Radical Anion Cleavage of Thymine Dimers Probed by Nanosecond Laser Spectroscopy

Syun-Ru Yeh and Daniel E. Falvey*

Department of Chemistry and Biochemistry
University of Maryland
College Park, Maryland 20742

Received April 15, 1991
Revised Manuscript Received August 5, 1991

The major form of ultraviolet radiation (UV) damage to DNA results from [2 + 2] cycloaddition reactions between adjacent pyrimidines (eq 1).[1] DNA photolyase is an enzyme which mediates a net reversal of this damage.[2] This enzyme is somewhat unique[3] because the catalytic step is photochemical—absorption of a UV or visible photon by the enzyme-substrate complex is necessary for dimer cleavage. *Escherichia coli* photolyase possesses a 1,5-dihydroflavin cofactor which acts as a chromophore in the photochemical/catalytic step.[4] It has been proposed, based on indirect evidence, that dimer cleavage is initiated by single-electron transfer (SET) from the excited chromophore to the substrate.[5] The model studies described below were designed to determine if the anion radicals of thymine dimers cleave at a kinetically significant rate. The results support an SET mechanism for enzymatic photorepair.

Cycloreversion reactions initiated by reductive SET are relatively unknown.[6] To determine if reductive SET could initiate

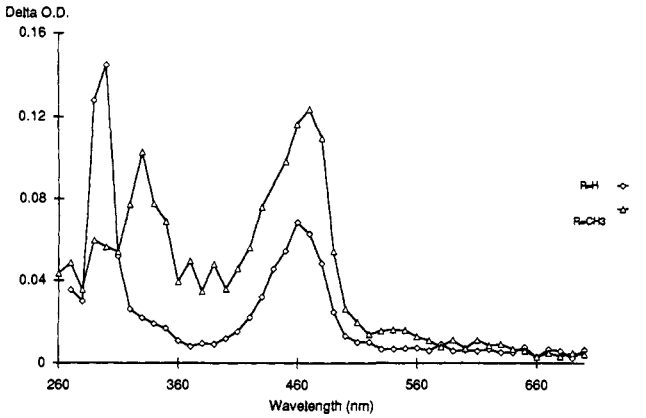
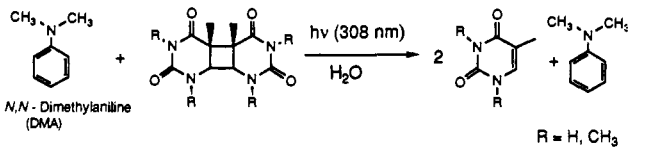


Figure 1. Transient spectra obtained after irradiating DMA with thymine dimer (diamonds) and dimethylthymine dimer (triangles) in pH 12 aqueous solution. Time is 3.0 ± 0.2 μs following the laser pulse.

dimer cleavage, we attempted to cleave simple pyrimidine dimers using *N,N*-dimethylaniline (DMA) as a sensitizer. This compound possesses an excited-state oxidation potential of −3.3 V.[7] The excited-state oxidation potential of the presumed enzymic sensitizer is calculated to be −2.5 V.[8] If simple SET is sufficient to cleave the thymine dimers,[9] then DMA should be an equally competent photosensitizer.



(1)

DMA is a photosensitizer for dimer cleavage. This was established by product studies and fluorescence quenching experiments. Irradiation of aqueous solutions (pH 12 or pH 7 0.05 M phosphate buffer, 308 nm) of DMA in the presence of either dimethylthymine dimers[10] or thymine dimers[11] results in efficient cleavage of the dimers. Only the corresponding monomers were detected by HPLC analysis of the reaction mixtures (80 ± 5% chemical yield). Dimethylthymine dimers quench the fluorescence of DMA in aqueous solutions. A Stern-Volmer[12] analysis gives a value for $k_q\tau$ of 38.3 M^{-1} (pH 12), where k_q is the bimolecular rate constant for quenching and τ is the singlet-state lifetime for DMA in the absence of dimer. The quantum yield for dimer splitting, Φ, depends on the concentration of dimers. A double-reciprocal plot of Φ^{-1} vs [dimer]$^{-1}$ gives a line with a slope of

(1) (a) Bruekers, R.; Berends, W. *Biochim. Biophys. Acta* **1960**, *41*, 550. (b) Wacker, A.; Dellweg, H.; Traeger, L.; Kornhauser, A.; Lodeman, E.; Tuerck, G.; Selzer, R.; Chandra, P.; Ishimoto, M. *Photochem. Photobiol.* **1964**, *3*, 369. (c) Blackburn, G. M.; Davis, R. J. *J. Am. Chem. Soc.* **1967**, *89*, 5941.

(2) (a) Sancar, A.; Sancar, G. *Ann. Rev. Biochemistry* **1988**, *57*, 29. (b) Friedberg, E. C. *DNA Repair*; W. H. Freeman: New York, 1985. (c) Sancar, A.; Smith, F. W.; Sancar, G. B. *J. Biol. Chem.* **1984**, *259*, 6028. (d) Sutherland, B. M. *Nature* **1974**, *248*, 109.

(3) Protochlorophyllide reductase is another example of a photochemical enzyme. (a) Begley, T.; Young, H. *J. Am. Chem. Soc.* **1989**, *111*, 3095. (b) Nielsen, O. F.; Kahn, A. *Biochim. Biophys. Acta* **1973**, 117.

(4) (a) Jorns, M.; Sancar, G.; Sancar, A. *Biochemistry* **1984**, *23*, 2673. See, also: (b) Iwatsuki, N.; Joe, C. O.; Werbin, H. *Biochemistry* **1975**, *19*, 1172.

(5) (a) Hartman, D.; Van Camp, J.; Rose, S. *J. Org. Chem.* **1987**, *52*, 2685. (b) Jorns, M. S. *J. Am. Chem. Soc.* **1987**, *109*, 3133. (c) Sancar, G. B.; Jorns, M. S.; Payne, G.; Fluke, D.; Rupert, C. S.; Sancar, A. *J. Biol. Chem.* **1987**, *262*, 492. (d) Payne, G.; Sancar, A. *Biochemistry* **1990**, *29*, 7715.

(6) There are examples of pericyclic processes initiated by *oxidative* SET. See, for example: (a) Majima, T.; Pac, C.; Sakurai, H. *J. Am. Chem. Soc.* **1980**, *102*, 5265. (c) Lewis, F. D.; Kojima, M. *J. Am. Chem. Soc.* **1988**, *110*, 8664. (c) Bauld, N. L.; Bellville, D. J.; Harirchian, B.; Lorenz, K. T.; Pabon, R. A.; Reynolds, D. W.; Wirth, D. D.; Chiou, H.-S.; Marsh, B. K. *Acc. Chem. Res.* **1987**, *20*, 371. (d) Lamola, A. A. *Mol. Photochem.* **1972**, *4*, 107. Indole derivatives have been shown to sensitize the cleavage of thymine dimers by what is apparently a reductive SET mechanism: (e) Young, T.; Kim, S.-T.; van Camp, J. R.; Hartman, R. F.; Rose, S. D. *Photochem. Photobiol.* **1988**, *48*, 635. (f) Charlier, M.; Hélène, C. *Photochem. Photobiol.* **1975**, *21*, 31.

(7) Kavarnos, G. J.; Turro, N. J. *Chem. Rev.* **1986**, *86*, 401.

(8) This is based on the oxidation potential of dihydroflavin of −0.12 V (a) Anderson, R. F. *Biochim. Biophys. Acta* **1983**, *722*, 158 and a singlet-state energy of 56 kcal/mol from the fluorescence spectrum (b) Ghisla, S.; Massey, V.; Lhoste, J.-M.; Mayhew, S. G. *Biochemistry* **1974**, *13*, 589.

(9) In principle, it would be possible to determine the free energy change for reduction of the thymine dimer anion radical if its reduction potential were known. However, our attempts to measure the reduction potential for the thymine dimer (DMF solution with 1 M Bu_4NPF_6, Ag electrode) did not show a reduction wave >−2.1 V vs Ag/AgCl.

(10) Cis-syn [2 + 2] cycloadducts of 1,3-dimethylthymine: Kloepfer, R.; Morrison, H. *J. Am. Chem. Soc.* **1972**, *94*, 255.

(11) Wulff, D. L.; Fraenkel, G. *Biochim. Biophys. Acta* **1961**, *51*, 332.

(12) Turro, N. *J. Modern Molecular Photochemistry*; Benjamin/Cummings: Menlo Park, CA, 1978; Chapter 8.

0002-7863/91/1513-8557$02.50/0