this difference is 186 kcal mol^{-1} while the present experiments show that, in solution, the value is ca. 50 kcal mol^{-1} [12] and point to a dramatic and preferential stabilization of one of the ions by ca. 136 kcal mol^{-1}.

In summary, modulated photochemical generation of free radicals, with phase-sensitive detection, allowed measurements of their oxidation and reduction potentials. The method was applicable to radicals which normally undergo diffusion controlled self-reaction. Under our experimental conditions, radical concentrations were typically 10^{-7}–10^{-8} M and lifetimes only ca. 10^{-3} s. As in most phase-sensitive techniques, the system may be used to measure the lifetimes of the transients under investigation and could thus provide a method for kinetic studies of carbonium and carbanion reactions.

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(11) Drzaic, P. S.; Brauman, J. I. *J. Phys. Chem.* **1984**, *88*, 5285–5290.
(12) The electrochemical oxidation of the benzyl radical is irreversible. However, the $\Delta\Delta H_f$ calculated will represent an *upper limit* since any associated overpotential will be in the anodic direction.
(13) Arnold, D. R., unpublished results.

An Efficient, Site-Specific DNA Target for Bleomycin

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The bleomycins (BLM's) are a group of structurally related antitumor antibiotics that are believed to mediate their therapeutic effect primarily via oxidative DNA strand scission.[2] By the use of supercoiled covalently closed circular DNA's (cccDNA's) and DNA fragments obtained by restriction endonuclease digestion of cccDNA's, it has been possible to demonstrate that bleomycin produces both single- and double-strand nicks in double-strand DNA, and does so with considerable sequence selectivity.[3] Also established convincingly by the use of such substrates is the requirement for an appropriate metal ion and O_2.[4,5] The use of large DNA fragments of defined structure for analysis of the chemistry of DNA strand scission has proven more difficult.[6] An alternative approach has involved extensive degradation of bulk DNA by high concentrations of bleomycin; subsequent chemical or enzymatic workup of the oligomeric product mixtures has permitted identification of some of the chemical products of BLM-mediated DNA strand scission.[7] However, this approach is poorly suited to the analysis of unstable reaction products, makes the assumption that reaction products obtained following extensive DNA degradation involve the same chemistry mediated at high

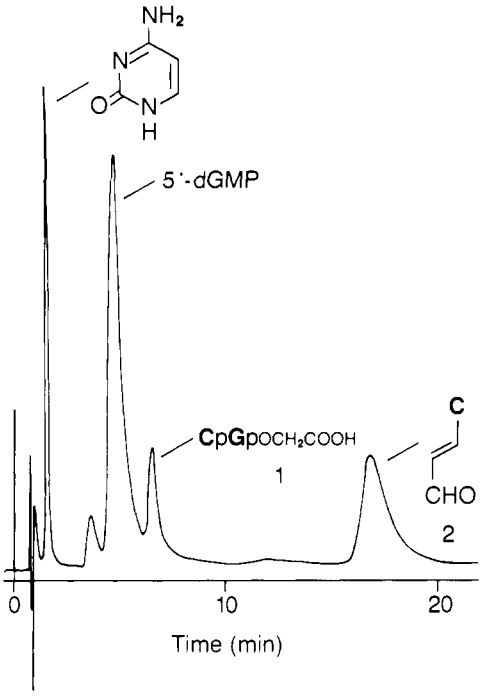
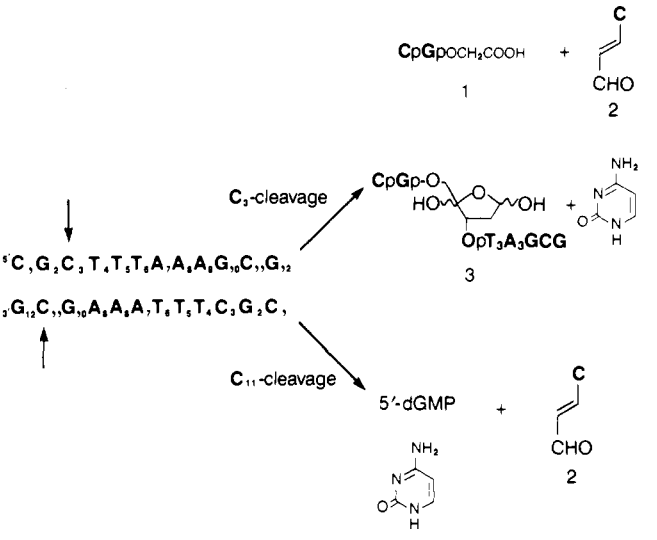


Figure 1. Hplc analysis of Fe^{II}·BLM A_2-treated dodecanucleotide. The reaction mixture (total volume 50 μL) contained 1 mM d-($CGCT_3A_3GCG$) (final nucleotide concentration), 0.2 mM BLM A_2, and 0.2 mM $Fe(II)(NH_4)_2(SO_4)_2$ in 50 mM sodium cacodylate, pH 7.0. The reaction was initiated by addition of Fe(II), incubated at 0 °C for 15 min, and then analyzed promptly by HPLC on a Rainin Microsorb C_{18} column (3 μm), elution was with 0.1 M ammonium formate at a rate of 1.5 mL/min. In addition to the labeled peaks, small amounts of guanine (3.6 min) and adenine (~12 min) were observed.

Scheme I. Products Formed Concomitant with Fe^{II}·BLM-Mediated Degradation of d($CGCT_3A_3GCG$)



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(2) (a) Hecht, S. M. In "Bleomycin: Chemical, Biochemical and Biological Aspects"; Hecht, S. M., Ed.; Springer-Verlag: New York, 1979; p 1 ff. (b) Umezawa, H. In "Medicinal Chemistry Series: Anticancer Agents Based on Natural Product Models"; Cassady, J. M., Dourous, J. D., Eds.; Academic Press: New York, 1980; Vol. XVI, p 148 ff. (c) Povirk, L. F. In "Molecular Aspects of Anti-cancer Drug Action"; Neidle, S., Waring, M. J., Eds.; Macmillan: London, 1983; p 157 ff.
(3) Fe·BLM-mediated strand scission occurs preferentially at ...GC... and ...GT... sequences. (a) D'Andrea, A. D.; Haseltine, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 3608. (b) Takeshita, M.; Grollman, A.; Ohtsubo, E.; Ohtsubo, H. *Ibid.* **1978**, *75*, 5983. (c) Mirabelli, C. K.; Ting, A.; Huang, C. H.; Mong, S.; Crooke, S. T. *Cancer Res.* **1982**, *42*, 2779.
(4) (a) Ishida, R.; Takahashi, T. *Biochem. Biophys. Res. Commun.* **1975**, *66*, 1432. (b) Sausville, E. A.; Peisach, J.; Horwitz, S. B. *Biochemistry* **1978**, *17*, 2740. (c) Oppenheimer, N., J.; Chang, C.; Rodriquez, L. O.; Hecht, S. M. *J. Biol. Chem.* **1981**, *256*, 1514. (d) Ehrenfeld, G. M.; Rodriguez, L. O.; Hecht, S. M.; Chang, C.; Basus, V. J.; Oppenheimer, N. J. *Biochemistry* **1985**, *24*, 81. (e) Ehrenfeld, G. M.; Murugesan, N.; Hecht, S. M. *Inorg. Chem.* **1984**, *23*, 1496.
(5) O_2-independent DNA degradation by Co^{III}·BLM + *hν* has also been reported. See: Chang, C. H.; Meares, C. F. *Biochemistry* **1984**, *23*, 2268.
(6) These include difficulties in (i) preparation of such species in quantity for degradation, (ii) separation of the products resulting from modification at each of several loci within a given sequence, and (iii) analysis of the resulting oligomeric products by routine spectral techniques due both to the size of the formed products and the modest extent of product formation at any given site.
(7) See, e.g.: (a) Giloni, L.; Takeshita, M.; Johnson, F.; Iden, C.; Grollman, A. P. *J. Biol. Chem.* **1981**, *256*, 8608. (b) Murugesan, N.; Xu, C.; Ehrenfeld, G. M.; Sugiyama, H., Kilkuskie, R. E.; Rodriguez, L. O.; Chang, L.-H.; Hecht, S. M. *Biochemistry* **1985**, *24*, 5735. (c) Uesugi, S.; Shida, T., Ikehara, M.; Kobayashi, Y.; Kyogoku, Y. *Nucleic Acids Res.* **1984**, *12*, 1581.

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Table I. Quantitative Analysis of BLM-Mediated Product Formation from d($CGCT_3A_3GCG$)[a]

Fe^{II}·BLM A_2, μM	cytosine,[b] μM	cytosine propenal (**2**),[b] μM	total prod,[c] μM	cytosine + **2**/total product	total product/ Fe^{II}·BLM A_2
50	4.4	12	17	0.96	0.34
100	13	33	56	0.82	0.56
200	23	43	87	0.76	0.44
300	20	42	80	0.78	0.27
500	27	41	85	0.80	0.17
700	32	34	80	0.83	0.11

[a] Reaction mixtures (50 μL total volume) contained d(CGCTTTAAAGCG) (1 mM final nucleotide concentration), 50 mM sodium cacodylate (pH 7), and the indicated amount of Fe(II)·BLM A_2. Reaction was initiated by addition of Fe(II), incubated at 0 °C for 15 min, and then analyzed by HPLC. Values given for cytosine and cytosine propenal are an average of those obtained using two different HPLC conditions. [c] Total product is equal to the sum of all free bases and base propenals.[17] Quantitation of each was carried out by comparison with an authentic synthetic sample.

affinity sites, and precludes any direct correlation of site-specific events with the chemical basis of those events (e.g., reaction stoichiometry, or the chemical basis of sequence selectivity or double-strand cleavage).

Reported herein is a self-complementary dodecanucleotide (d($CGCT_3A_3GCG$)), accessible in quantity by solution and solid-phase synthesis according to known procedures.[8] This oligomer (i) constitutes the first synthetic DNA substrate for bleomycin, (ii) contains a single, preferred double-strand BLM recognition site, (iii) is cleaved with significantly higher efficiency by BLM than calf thymus DNA, (iv) affords products amenable to direct, facile analysis by comparison with authentic synthetic samples, and (v) acts as a BLM substrate following 5′-^{32}P labeling, thus providing the potential for product analysis at BLM concentrations comparable to those that must obtain in a therapeutic situation.

HPLC analysis of Fe^{II}·BLM-treated dodecamer was carried out by comparison with authentic synthetic samples (Figure 1);[7b,9] the four major peaks were found to correspond to cytosine, 5′-dGMP, 2′-deoxycytidylyl(3′→5′)(2′-deoxyguanosine-3′-(phosphoro-2″-*O*-glycolate)) (**1**) and *trans*-(3-(cytosin-1′-yl)propenal (cytosine propenal, **2**) (Scheme I).[10] The formation of **1** and **2** is consistent with cleavage of the ribose moiety in $cytidine_3$ via a C-4′ hydroperoxide intermediate;[7,11] analogous cleavage at $cytidine_{11}$ would produce the observed 5′-dGMP, as well as additional **2**.[12] Also formed as primary products of Fe^{II}·BLM-mediated oxidation at $cytidine_3$ (by a parallel mechanism, apparently involving C-4′ hydroxylation of ribose[13]) were free cytosine and an alkali labile lesion (**3**). Characterization of the latter has been effected following base treatment;[14] analogous oxidation at C_{11} would produce additional cytosine and 5′-dGMP.

The identification and quantitation of products formed concomitant with d($CGCT_3A_3GCG$) degradation was effected by HPLC analysis of the products formed following treatment with varying concentrations of Fe^{II}·BLM A_2;[15,16] a summary of product formation is given in Table I.[17] It is clear from this table that over a wide range of BLM concentrations, 76–96% of all products formed resulted from oxidative damage at C_3 or C_{11} (i.e., the preferred double-strand cleavage site[3]). The major "nonspecific" products observed during d($CGCT_3A_3GCG$) degradation were guanine and adenine propenal. Accordingly, distamycin A was added to the reaction mixtures to render the AT-rich region less accessible to Fe^{II}·BLM A_2.[18] As anticipated, in the presence of distamycin A, BLM-mediated strand scission occurred *exclusively* at GC.[19]

A number of observations suggest that activation of O_2 by Fe^{II}·BLM requires an additional electron.[20] In the absence of any added reducing agent, this electron has been proposed to derive from disproportionation of two Fe^{II}·BLM molecules,[20a] a suggestion consistent with the substantial diminution of total product formation from d($CGCT_3A_3GCG$) at 50 μM BLM A_2 concentration (Table I). Interestingly, at 100 μM Fe^{II}·BLM A_2 concentration, where product formation was maximal with respect to added Fe^{II}·BLM A_2, the ratio of products formed/Fe^{II}·BLM A_2 added was almost exactly 1:2. This result lends credence to the proposed[20] stoichiometry of O_2 activation by BLM. Also remarkable was the efficiency of Fe^{II}·BLM A_2-mediated d($CGCT_3A_3GCG$) degradation. All of the reactions in Table I employed d($CGCT_3A_3GCG$) at a final concentration of 83 μM; on the assumption that the stoichiometry of activation proposed above is correct, this implies very efficient utilization of the putative 50 μM "activated BLM" that would be produced at the BLM concentration optimal for product formation (i.e., at 100 μM added Fe^{II}·BLM A_2). Conversely, complete consumption of d(GCT_3A_3GCG)(83 μM) was obtained using only 200 μM added Fe^{II}·BLM A_2. Direct comparison of the BLM susceptibility of d($CGCT_3A_3GCG$) and calf thymus DNA (at 1 mM DNA nucleotide concentration) indicated that total product formation from d($CGCT_3A_3GCG$) was twice that of calf thymus DNA at 200 μM Fe^{II}·BLM A_2 and ~10-fold greater at 100 μM Fe^{II}·BLM A_2 *in spite of the smaller number of cleavage sites on the dodecanucleotide*.

Although the extent of cleavage at C_3 vs. C_{11} was invariant for Fe^{II}·BLM A_2 at all concentrations tested, the nature of the products formed was strongly dependent on the concentration of Fe^{II}·BLM A_2. At higher BLM concentrations the amount of cytosine produced increased, while that of cytosine propenal decreased. That this was due to depletion of available O_2 at higher

(8) (a) van Boom, J. H. *Heterocycles* **1977**, *7*, 1197. Matteucci, M. D.; Caruthers, M. H. *J. Am. Chem. Soc.* **1981**, *103*, 3185.

(9) Johnson, F.; Pillai, K. M. R.; Grollman, A. P.; Tseng, L.; Takeshita, M. *J. Med. Chem.* **1984**, *27*, 954.

(10) Reaction mixtures (total volume 50 μL) contained 1 mM d($CGCT_3A_3GCG$) (final nucleotide concentration), 0.2 mM BLM A_2, and 0.2 mM $Fe^{II}(NH_4)_2(SO_4)_2$ in 50 mM sodium cacodylate, pH 7.0. Reactions were initiated by addition of Fe(II), incubated at 0 °C for 15 min, and then analyzed promptly by HPLC on a Rainin Microsorb C_{18} column (3 μm); elution was with 0.1 M ammonium formate at a rate of 1.5 mL/min.

(11) Burger, R. M.; Berkowitz, A. R.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1980**, *255*, 11832.

(12) Also formed from each strand were larger oligomers whose abundance was inferred from the products and mechanisms outlined here.

(13) (a) Wu, J. C.; Kozarich, J. W.; Stubbe, J. *J. Biol. Chem.* **1983**, *258*, 4694. (b) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1983**, *257*, 8612.

(14) Sugiyama, H.; Xu, C.; Murugesan, N.; Hecht, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 4104.

(15) All products were identified by direct comparison with authentic synthetic samples. For the modified dinucleotides (CpGx), further verification was achieved by cleavage of 5′-[^{32}P]-d($CGCT_3A_3GCG$) to produce products identical with synthetic pCpGx's. The rapidity of the overall reaction and analysis (<40 min) facilitated the identification of unstable reaction products.[13]

(16) Quantitation of products was carried out by HPLC (cf. ref 10) after treatment of the dodecanucleotide with the indicated concentrations of Fe^{II}·BLM A_2. The HPLC response factor for each product was determined by the use of carefully purified, authentic synthetic materials.

(17) Because the amount of cytosine propenal (**2**) formed in each case was found to be equal to the sum of dinucleotide **1** + 5′-dGMP (cf. Scheme I), and the amount of cytosine was equal to that of lesion **3**,[12] the product quantitation can be expressed in terms of free base and base propenal formation.

(18) Distamycin A has been shown to bind to AT-rich regions of DNA. (a) Van Dyke, M. W.; Hertzberg, R. P.; Dervan, P. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 5470. (b) Sugiura, Y.; Suzuki, T. *J. Biol. Chem.* **1982**, *257*, 10544. (c) Kopka, M. L.; Yoon, C.; Goodsell, D.; Pjura, P.; Dickerson, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1376.

(19) Products formed from d($CGCT_3A_3GCG$) at sites other than C_3 or C_{11} in the presence of 42 μM distamycin A constituted <1% of the total reaction products, as judged by HPLC analysis.

(20) (a) Kuramochi, H.; Takahashi, K.; Takita, T.; Umezawa, H. *J. Antibiot.* **1981**, *34*, 576. (b) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1981**, *256*, 11636. (c) Murugesan, N.; Hecht, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 493.

BLM concentrations was suggested by the reversal of this trend upon aeration of the reaction mixtures.

The demonstration that a synthetic dodecanucleotide can act as a sequence-selective substrate for BLM provides a powerful new tool for the study of this antitumor antibiotic at its putative therapeutic locus and a novel approach for the study of naturally occurring and synthetic DNA interactive agents.

Acknowledgment. This study was supported at the University of Virginia by P.H.S. Grant CA38544, awarded by the National Cancer Institute, DHHS.

Photochemistry of [(Acyloxy)methyl]benzylsilanes. Evidence for the Primary Formation of a Benzyl–Silyl Radical Pair and Mechanism of Free Radical 1,2 (C → Si) Acyloxy Migration[1]

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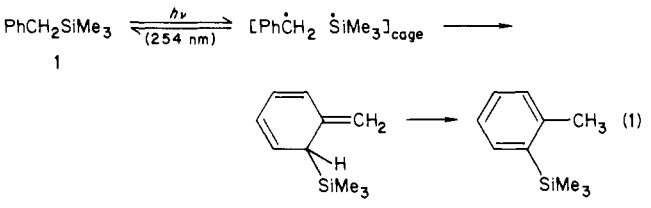
We report herein novel photochemistry of [(acyloxy)methyl]-benzyldimethylsilanes as well as benzyltrimethylsilane.[2] The results provide not only definitive evidence for the formation of a benzyl radical–silyl radical pair in the primary photochemical process but also an important insight into the detailed mechanism for free-radical 1,2 (C → Si) acyloxy migration found recently.[7]

Whereas benzyltrimethylsilane (**1**) has been described as photochemically inert,[3] the detailed study by our hands revealed that **1** actually isomerized to *o*-tolyltrimethylsilane (**2**) but with very low efficiency.[8] The results may suggest that the primary photochemical process is the homolysis of a benzyl carbon–silicon bond affording a benzyl–silyl radical pair in the solvent cage and that **2** is formed through recombination of the pair, while the major pathway of the reaction is the reformation of **1** (eq 1).



(1)

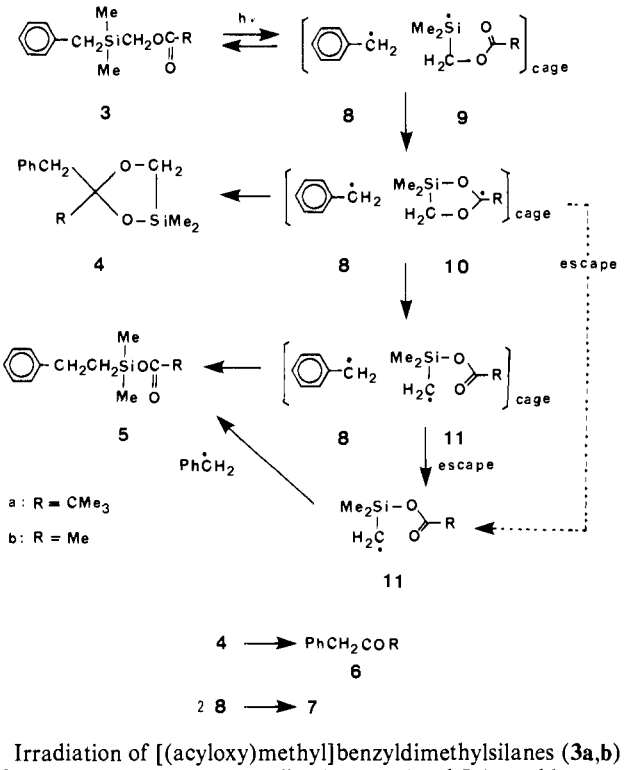
We have designed (acyloxy)methyl-substituted benzylsilanes, which bear an effective intramolecular silyl radical trap, in order to obtain evidence for the formation of the radical pair intermediate in the photoreaction. Intramolecular trapping of a silyl radical by an acyloxy carbonyl group, i.e., free radical 1,2 (C → Si) acyloxy migration,[7] may intercept the radical pair formed initially.

Table I. Product Distributions of the Photolysis of [(Acyloxy)methyl]benzyldimethylsilanes (**3**)[a]

3	conversion, %	products and yields, %[b,c] 4	5	6	7
a, R = *t*-Bu[d]	67	21 (0)	24 (0)	0 (21)	16 (16)
b, R = CH_3	88	11 (0)	13 (0)	4 (14)	13 (14)

[a] Irradiation was performed on a 0.1 M solution of **3** in benzene in a quartz tube with a 10-W low-pressure mercury arc lamp at ambient temperature for 4 h. [b] Yields were determined by GLC. [c] Yields after hydrolysis of the reaction mixtures were shown in parentheses. [d] *t*-$BuCO_2SiMe_3$ was also obtained in ca. 5% yield.

Scheme I



Irradiation of [(acyloxy)methyl]benzyldimethylsilanes (**3a,b**) afforded mainly the corresponding isomers **4** and **5**, benzyl ketones **6**, and bibenzyl (**7**). The reaction conditions, products,[9] and the yields are shown in Table I. The formation of these products may well be explained as shown in Scheme I involving the initial homolysis of the benzyl carbon–silicon bond followed by the 1,2 (C → Si) acyloxy migration of the [(acyloxy)methyl]dimethylsilyl radical **9**.

Detection of a dioxasilolane (**4a,b**) is particularly interesting since the results indicate involvment of the dioxasilolanyl radical **10** as an important intermediate of free radical 1,2 (C → Si) acyloxy migration. In contrast, the corresponding (C → C) acyloxy migration reportedly does not involve such a cyclic intermediate.[10]

In order to determine whether **4** and **5** were cage or escape products, a 51:49 mixture of **3a** and **3b**, where the former was labeled with deuterium at the para position (89% deuterium

(1) Chemistry of Organosilicon Compounds. 204.
(2) Whereas a number of photoreactions including cleavage of benzylic carbon–silicon bonds have been reported,[3-6] these cannot necessarily be regarded as intrinsic photochemistry of benzylsilane chromophore.
(3) Valkovich, P. B.; Ito, T. J.; Weber, W. P. *J. Org. Chem.* **1974**, *39*, 3543.
(4) (a) Nakadaira, Y.; Otsuka, T.; Sakurai, H. *Tetrahedron Lett.* **1981**, *22*, 2417, 2421. (b) Rich, J. D.; Dranhnak, T. J.; West, R. *J. Organomet. Chem.* **1981**, *212*, C1.
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(7) Wilt, J. W.; Keller, S. M. *J. Am. Chem. Soc.* **1983**, *105*, 1395.
(8) In a typical experiment, irradiation of **1** in dry benzene (ca. 0.1 M) with a 10-W low-pressure mercury arc lamp for 9 h afforded **2** in 2% yield with recovered **1** (91%).

(9) All the products except for **4b** were isolated by preparative GLC and identified with mass and ^1H NMR spectroscopy. **4a**: ^1H NMR (200 MHz, $CDCl_3$) δ −0.63 (s, 3), 0.17 (s, 3), 0.97 (s, 9), 2.83 (d, 1, *J* = 13.2 Hz), 3.00 (d, 1, *J* = 13.2 Hz), 3.08 (d, 1, *J* = 12.8 Hz), 3.25 (d, 1, *J* = 12.8 Hz), 7.0–7.4 (m, 5); mass spectrum (70 eV), *m/e* (relative intensity) 264 (0.5, M^+), 249 (3.2, $M^+ - 15$), 173 (90), 91 (34), 57 (100). Whereas **4b** could not be isolated by routine methods, the proposed structure was supported by the analogous mass spectral pattern with **4a** as well as the facile hydrolysis to **6b**.
(10) (a) Beckwith, A. L.; Thomas, C. B. *J. Chem. Soc., Perkin Trans 2* **1973**, 861. (b) Perkins, M. J.; Roberts, B. P. *Ibid.* **1975**, *77*. (c) Barclay, L. R. C.; Griller, D.; Ingold, K. U. *J. Am. Chem. Soc.* **1982**, *104*, 4399. (d) Barclay, L. R. C.; Lusztyk, J.; Ingold, K. U. *Ibid.* **1984**, *106*, 1793. (e) Beckwith, A. L.; Ingold, K. U. In "Rearrangements in Ground and Excited States"; Mayo, P. de, Ed.; Academic Press: New York, 1980; Vol. 1, p 161, and references cited in.

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