DNA Damage Induced via 5,6-Dihydrothymid-5-yl in Single-Stranded Oligonucleotides

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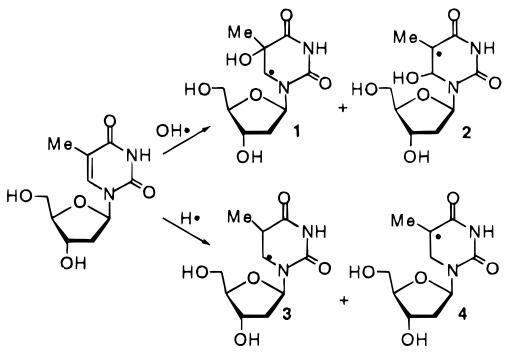
Abstract: 5,6-Dihydrothymid-5-yl (**4**) is generated via Norrish type I cleavage of isopropyl ketone **7**. Ketone **7** was site specifically incorporated into chemically synthesized polythymidylates and an oligonucleotide containing all four native deoxyribonucleotides. No damage is induced in oligonucleotides containing **7** upon photolysis under anaerobic conditions. In the presence of O_2, strand breaks and alkaline labile lesions are formed at the original site of **7**, and at nucleotides adjacent to the 5′-phosphate of **7**. Kinetic isotope effect experiments reveal that direct strand scission at the thymidine adjacent to the 5′-phosphate of **4** arises from C1′ hydrogen atom abstraction. The observed KIE (∼3.9) is attributed to hydrogen atom abstraction from C1′ by the peroxyl radical **35** derived from **4**. Enzymatic end group analysis and measurement of free base release are consistent with a process involving C1′ hydrogen atom abstraction. Cleavage experiments carried out in the presence of *t*-BuOH (1.05 M) and NaN_3 (10 mM) indicate that damage does not result from hydroxyl radical, but that $^1O_2$ is responsible for a significant amount of the observed strand damage.

Ionizing radiation is the agent that has been used the longest to purposefully damage nucleic acids.[1] Unlike many of the synthetic and natural product based nucleic acid damaging agents, which predominantly react with the carbohydrate component of nucleic acids, nucleobase-centered reactive intermediates comprise the majority of reactive intermediates produced by ionizing radiation (Scheme 1).[2−6] In addition to answering fundamental mechanistic questions regarding the effects of ionizing radiation on nucleic acids, the elucidation of the role that individual nucleobase reactive intermediates play in producing nucleic acid strand breaks presents the possibility that new reaction pathways will be uncovered. This, in turn, could provide the impetus for the design of new nucleic acid damaging agents, or improve ones already in use.

The effects of ionizing radiation on pyrimidine substrates (such as thymidine and its respective nucleotide and polymeric congeners) have been the focus of numerous investigations.[1] The principal radical species produced via ionization of water, hydroxyl radical and hydrogen atom, show opposite regioselectivity in their addition to the thymine double bond (Scheme 1). The regioselectivity is maintained for each reactive species, whether thymine is present as a monomer, or as part of a biopolymer. The preferred C6 hydrogen atom addition product, 5,6-dihydrothymid-5-yl (**4**), is also formed via the direct effect of ionizing radiation. The direct effect of ionizing radiation is believed to give rise to **4** via addition of an electron, followed by protonation of the resulting radical anion.[7] In order for nucleobase-centered radicals, such as **4**, to induce a direct strand break, spin must be transferred from the nucleobase to a sugar moiety. Pyrimidine-based reactive intermediates have been proposed to induce strand breaks by abstracting a hydrogen atom from the carbohydrate portions of the nucleotides covalently bound to their 5′- or 3′-phosphates.[1,7a,8] It has also been suggested that the more reactive σ radical, 2′-deoxyurid-5-yl (**5**), translates damage in duplex DNA in the 5′ direction via a mechanism that involves hydrogen atom abstraction from an adjacent carbohydrate substrate.[9,10]

Scheme 1



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(1) von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor and Francis: London, 1987.

(2) (a) Duff, R. J.; de Vroom, E.; Geluk, A.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1993**, *115*, 3350. (b) McGall, G. H.; Rabow, L. E.; Ashley, G. W.; Wu, S. H.; Kozarich, J. W.; Stubbe, J. *J. Am. Chem. Soc.* **1992**, *114*, 4958. (c) Hangeland, J. J.; De Voss, J. J.; Heath, J. A.; Townsend, C. A. *J. Am. Chem. Soc.* **1992**, *114*, 9200. (d) Zein, N.; Poncin, M.; Nilakantan, R.; Ellestead, G. A. *Science* **1989**, *244*, 697. (e) Goldberg, I. H. *Acc. Chem. Res.* **1991**, *24*, 191. (f) Myers, A. G.; Cohen, S. B.; Kwon, B-M. *J. Am. Chem. Soc.* **1994**, *116*, 1670.

(3) (a) Goyne, T. E.; Sigman, D. S. *J. Am. Chem. Soc.* **1987**, *109*, 2846. (b) Sigman, D. S. *Acc. Chem. Res.* **1986**, *19*, 180.

(4) (a) Cheng, C-C.; Goll, J. G.; Neyhart, G. A.; Welch, T. W.; Singh, P.; Thorp, H. H. *J. Am. Chem. Soc.* **1995**, *117*, 2970. (b) Neyhart, G. A.; Cheng, C-C.; Thorp, H. H. *J. Am. Chem. Soc.* **1995**, *117*, 1463.

(5) Sitlani, A.; Long, E. C.; Pyle, A. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 2303.

(6) Pitié, M.; Bernadou, J.; Meunier, B. *J. Am. Chem. Soc.* **1995**, *117*, 2935.

(7) (a) Symons, M. C. R. *J. Chem. Soc., Faraday Trans. 1* **1987**, *83*, 1. (b) Bernhard, W. A. *J. Phys. Chem.* **1989**, *93*, 2187.

(8) (a) Becker, D.; Sevilla, M. D. In *Advances in Radiation Biology, Volume 17, DNA and Chromatin Damage Caused by Radiation*; Lett, J. T., Sinclair, W. K., Eds.; Academic Press: New York, 1987. (b) Cullis, P. M.; Langman, S.; Podmore, I. D.; Symons, M. C. R. *J. Chem. Soc., Faraday Trans.* **1990**, *86*, 3267.

(9) (a) Sugiyama, H.; Tsutsumi, Y.; Saito, I. *J. Am. Chem. Soc.* **1990**, *112*, 6720. (b) Murray, V.; Martin, R. F. *Nucleic Acids Res.* **1989**, *17*, 2675. (c) Hutchinson, F.; Köhnlein, W. *Prog. Subcell. Biol.* **1980**, *7*, 1.

(10) Cook, G. P.; Greenberg, M. M. *J. Am. Chem. Soc.* **1996**, *118*, 10025.

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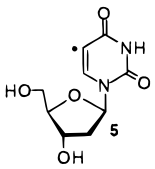
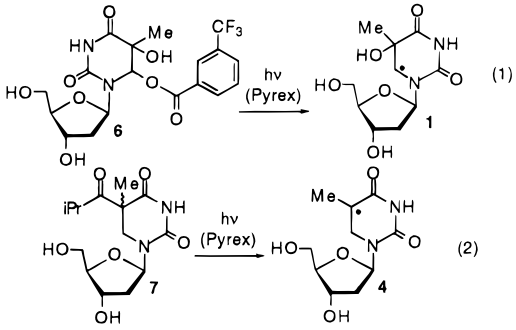


To help elucidate the mechanisms by which ionizing radiation cleaves DNA, we have examined the reactivity of nucleobase-centered radicals under biologically relevant conditions (eqs 1 and 2). Generating **1** and **4** in solution by irradiation of **6** and

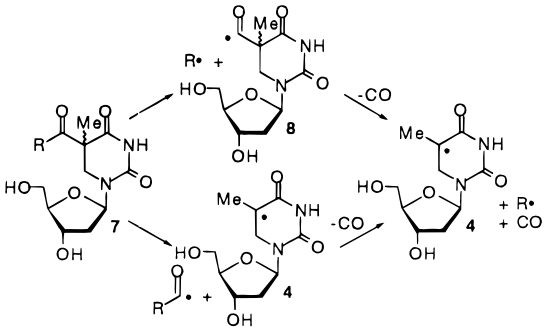


7, respectively, enables us to investigate the chemistry of these radicals under conditions in which other radicals derived from nucleosides are not formed.[11,12] This approach offers a distinct advantage over the use of ionizing radiation, in which multiple reactive intermediates are formed upon irradiation. Contrary to previous proposals regarding DNA damage amplification via hydrogen atom abstraction by **4**, we demonstrated that this species is incapable of inducing strand breaks in single-stranded polythymidylate via hydrogen atom abstraction under anaerobic conditions.[7a,8,12] Direct strand breaks and alkaline labile lesions are produced when **4** is generated in the presence of O_2. Further characterization of the nature of this damage and proposals for its formation are described below.

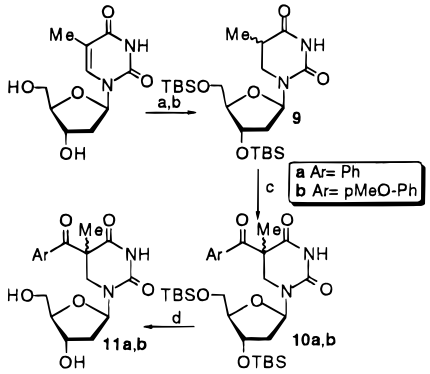
Results and Discussion

Synthesis of Photosubstrates and Reaction Products. The C5-hydroxyl radical adduct of thymidine (**1**) was previously generated from **6** via a photoinduced single electron transfer process.[11,13] This process is less useful for producing radicals in biopolymers, due to the possibility of electron transfer between the photosensitizer (e.g. *N*-methylcarbazole) and random nucleotides. This method is also incompatible with studying radical processes under aerobic conditions, because the benzoate radical anion is readily oxidized by O_2. Ketones contain a weak n,π^* transition, which can be populated via direct irradiation at >300 nm. Consequently, we sought to generate **4** via Norrish type I photocleavage.[14,15] In designing ketone precursors to **4**, we wished to minimize the formation of other reactive intermediates (such as the acyl derivative of **4** (**8**) Scheme 2). On the basis of the comparison between **4** and the *tert*-butyl radical, we expected decarbonylation of the acyl

Scheme 2

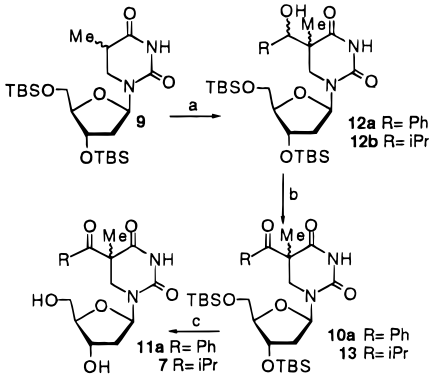


Scheme 3[a]



[a] Key: (a) H_2, Rh/Al_2O_3, $MeOH/H_2O$ (1:1), (b) TBSCl, pyridine, (c) *sec*-BuLi (2.5 equiv), THF and then aroyl chloride, (d) HOAc, H_2O.

Scheme 4[a]



[a] Key: (a) *sec*-BuLi (2.5 equiv), THF and then isobutyraldehyde or benzaldehyde, (b) Dess−Martin periodinane, CH_2Cl_2, (c) NH_4F, MeOH or $HOAc/H_2O$.

radical progenitor of **4** (**8**) to be $\geq 10^5$ s^{-1}.[16] Nonetheless, we chose to synthesize aryl and alkyl ketones for which the type I cleavage could be expected to yield **4** directly, and not via **8**.

Formation of the C5-acylated dihydropyrimidines was accomplished via acylation of the dianion of **9** (Schemes 3 and 4). The protected forms of aromatic ketones **10a**,**b** were obtainable directly via trapping of the dianion by the respective acyl chloride (Scheme 3). However, employing a two-step aldol condensation/oxidation sequence (Scheme 4) gave rise to more reproducible yields, and facilitated product purification. Desilylations of the aromatic ketones **10a**,**b** and the isopropyl ketone **13** were carried out using either aqueous acetic acid or NH_4F in MeOH.[17]

(11) Barvian, M. R.; Greenberg, M. M. *J. Am. Chem. Soc.* **1995**, *117*, 4894.

(12) (a) Barvian, M. R.; Greenberg, M. M. *J. Am. Chem. Soc.* **1995**, *117*, 8291. (b) Barvian, M. R.; Greenberg, M. M. *J. Org. Chem.* **1995**, *60*, 1916.

(13) (a) Saito, I.; Ikehira, H.; Kasatani, R.; Watanabe, M.; Matsuura, T. *J. Am. Chem. Soc.* **1986**, *108*, 3115. (b) Masnovi, J. *J. Am. Chem. Soc.* **1989**, *111*, 9081.

(14) Bohne, C. In *CRC Handbook of Organic Photochemistry and Photobiology*; Horspool, W. M., Song, P.-S., Eds.; CRC Press: Boca Raton, FL, 1995.

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

(16) (a) Fischer, H.; Paul, H. *Acc. Chem. Res.* **1987**, *20*, 200. (b) Lunazzi, L.; Ingold, K. U.; Scaiano, J. C. *J. Phys. Chem.* **1983**, *87*, 529. (c) Hany, R.; Fischer, H. *J. Chem. Phys.* **1993**, *172*, 131.

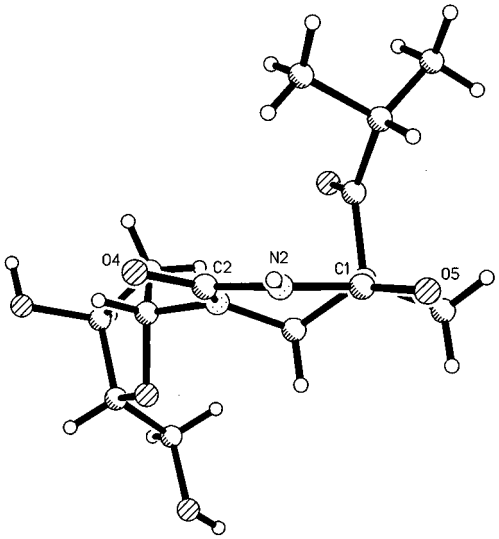
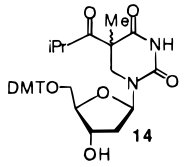


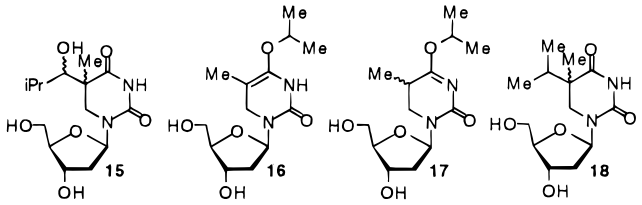
Figure 1. X-ray crystal structure of **7**.

In general, the diastereoselectivity of the condensation reactions was approximately 3:1. On the basis of previous electrophilic reactions with the double bond of protected thymidine derivatives, the 5*S* stereoisomer was expected to be the major product.[18] Structural assignments were complicated by the fact that the four stereoisomers obtained from the condensation reaction, as well as the diastereomers of protected (**10**, **13**) and deprotected (**7**, **11**) ketones, were inseparable by column chromatography. However, separation of the diastereomers of **7** was achieved following formation of the respective 5′-*O*-dimethoxytritylated ketones **14**. Following detritylation

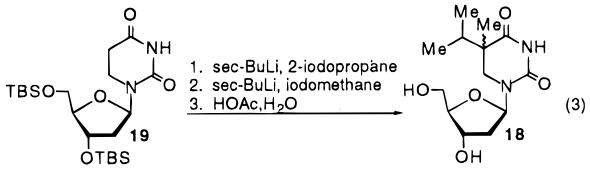


of the major diastereomer using aqueous acetic acid, unambiguous assignment of the major stereoisomer of **7** as 5*S* was achieved via X-ray crystallography (Figure 1). In addition to verifying the anticipated stereochemistry of **7**, and the nonplanar nature of the dihydropyrimidine ring, the crystal structure shows that the nucleoside exists in the C2′-*endo*,*anti* conformation. The *anti* conformation of **7** suggests that it could participate in base pairing in duplex DNA. However, the isopropyl group is projected toward what would be the adjacent 3′-nucleotide, and thus could adversely affect base stacking in the helix. The effect of **7** on duplex stability awaits experimental investigation.

It was necessary to verify the fidelity of the photochemical decomposition of the ketones **7** and **11a** prior to incorporation of these radical precursors into oligonucleotides. This required the independent preparation of some of the potential reaction products. While the hydrogen atom trapping product of **4** (5,6-dihydrothymidine) was readily available, other possible products were unknown. The photoreduction product of **7** (**15**) was obtained as a complex mixture of diastereomers via deprotection of **12b** (Scheme 4). The analogous product **23** from **11a** was isolated from preparative scale photolyses of the ketone.



Additional products can be formed via reaction of the radical pairs formed from **7**. Radical pair chemistry can follow two pathways. Disproportionation is expected to produce dihydrothymidine and/or thymidine, while recombination can occur at two sites in the conjugated radical **4**. The product resulting from recombination on oxygen (**16**, an *N*,*O*-ketene acetal, or tautomeric **17**, an *O*-alkyl imidate) proved to be too unstable to be isolable by column chromatography or HPLC. Compounds similar to **16** and **17** are known to be unstable.[19,20] Similar difficulties were not anticipated for the independent preparation of recombination product **18**. Accordingly, while alkylation of the dianion of **9** with 2-iodopropane was unsuccessful, **18** was obtained via sequential alkylation of **19** (eq 3).

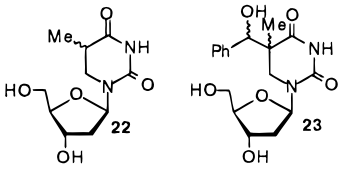


Monomer Photochemistry. As mentioned above, the photolabile radical precursors were designed to take advantage of the stepwise nature of the Norrish type I photocleavage reaction.[14,15,21] Aromatic ketones such as **11** can only cleave to yield **4** and the aroyl species. However, photochemical cleavage of alkyl ketones (e.g., **7**) can yield **4** directly, or via decarbonylation of **8** (Scheme 2). The acyl radical **8** is not expected to be proficient at hydrogen atom abstraction. Furthermore, decarbonylation rate constants of acyl radicals that yield relatively stable alkyl radicals (such as tertiary or benzylic radicals) are sufficiently great that bimolecular reactions involving the acyl radical should not compete with this unimolecular process.[16] Comparison of the expected stability of **4** to that of the *tert*-butyl radical led us to predict that, if formed, **8** would decarbonylate at rates equal to, or greater than that of the pivaloyl system ($\geq 1.3 \times 10^5$ s^{-1}).[16b]

Photolysis (λ_{max}= 350 nm) of **11a** (10 mM) in the presence of pseudo-first-order concentrations of cyclohexa-1,4-diene (0.2 M), in a mixture of CH_3CN/D_2O (1:19), produces 5,6-dihydrothymidine (**22**) in ∼12% yield when the crude photolysate is analyzed directly by reversed phase HPLC. GC/MS analysis reveals that all of the 5,6-dihydrothymidine (**22**) is protonated. For technical reasons, it was desirable to remove nonpolar components (e.g., cyclohexa-1,4-diene and byproducts of the benzoyl radical) prior to HPLC analysis. The aqueous workup employed to remove organics, as well as heating the sample at 90 °C for 10 min prior to HPLC analysis, increases the yield of **22** to ∼65%. The 5,6-dihydrothymidine (**22**) formed following heating contains ∼80% deuterium, indicating that the majority of this product did not arise from trapping of **4** by the hydrogen atom donor. Deuterated **22** can arise via radical pair combina-

(17) Zhang, W.; Robins, M. J. *Tetrahedron Lett.* **1992**, *33*, 1177.
(18) Barvian, M. R.; Greenberg, M. M. *J. Org. Chem.* **1993**, *58*, 6151.
(19) Hegedus, L. S.; Schultze, L. M.; Montgomery, J. *Organometallics* **1989**, *8*, 2189.
(20) Schmir, G. L.; Cunningham, B. A. *J. Am. Chem. Soc.* **1965**, *87*, 5692.
(21) (a) Engel, P. S. *J. Am. Chem. Soc.* **1970**, *92*, 6074. (b) Robbins, W. K.; Eastman, R. H. *J. Am. Chem. Soc.* **1970**, *92*, 6077. (c) Robbins, W. K.; Eastman, R. H. *J. Am. Chem. Soc.* **1970**, *92*, 6079.

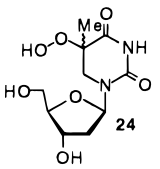
tion on oxygen (to form **16** and/or **17**), followed by hydrolysis, or photoreduction (e.g., formation of **23**) and subsequent



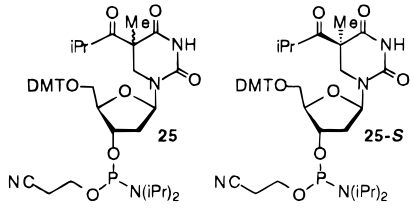
retrocondensation. As mentioned above, we were unable to isolate either tautomer of the oxygen recombination product. Furthermore, neither **16** nor **17** was detectable in crude photolysates by ^{1}H NMR. Similarly, attempts at deprotecting **12a** resulted in formation of **22**, illustrating the lability of **23** as well. Fortunately, **23** was isolable from preparative scale photolyses of **11a** in the presence of cyclohexa-1,4-diene (0.2 M). Consequently, we suggest that the majority of **22** formed via the photolysis of **11a** results from retrocondensation of photoreduction product **23**. It was hoped that the *p*-methoxyphenyl ketone **11b** would undergo less photoreduction in the presence of hydrogen atom donors, but this was not observed when **11b** was irradiated in the presence of cyclohexa-1,4-diene.[22]

An investigation of the photochemistry of **7** was undertaken to determine if the same complications would arise as was observed for the aryl ketones **11a** and **11b**. While the *O*-alkylation (*O*-acylation) products were again undetectable, the photoreduction (**15**) and carbon alkylation (**18**) products were prepared as described above. Upon irradiation of **7** in CH_3CN/D_2O (1:19), less than 5% of **15** was observed by HPLC, even in the presence of 0.2 M cyclohexa-1,4-diene. In addition, the 5,6-dihydrothymidine (**22**) formed in the presence of cyclohexa-1,4-diene was completely protonated. In the absence of exogenous hydrogen atom donor, small amounts (<10%) of 5,6-dihydrothymidine (**22**) containing large percentages (>33%) of deuterium were formed.[12b] Under these conditions, protonated 5,6-dihydrothymidine can arise from disproportionation within a radical pair, or hydrogen atom abstraction from CH_3CN, with the former being more likely. It is possible that deuterated 5,6-dihydrothymidine arises from the analogous *O*-alkylation pathway discussed above. If so, then the fact that none of the radical pair recombination product **18** was observed could be rationalized on the basis of a large steric preference for reaction at O^4 of **4** within the radical pair.

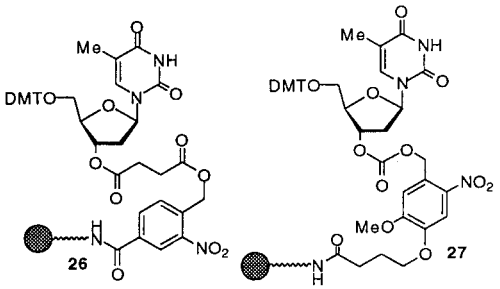
The above trapping results are consistent with the generation and trapping of **4** from **7**. Isolation of the diastereomeric mixture of hydrogen peroxides **24** further supports the generation of **4**, even in the presence of high concentrations of hydrogen atom donor. Isolated yields of **24** were low due to difficult purification via reversed phase HPLC, as well as its instability.[12b]



Oligonucleotide Synthesis. The isopropyl ketone **7** is stable to the reagents (trichloroacetic acid, tetrazole, iodine/pyridine/H_2O, and acetic anhydride/*N*-methylimidazole) used in automated chemical DNA synthesis. However, **7** is extremely unstable in concentrated NH_4OH, which is the method traditionally used for cleaving oligonucleotides from their solid phase supports, while simultaneously removing the phosphate and amino protecting groups. This problem was circumvented by site specifically incorporating **7** as its β-cyanoethyl phosphoramidite **25** into chemically synthesized oligonucleotides, which were prepared in a manner that takes advantage of methodology that obviates the use of NH_4OH to effect deprotection.[23,24]



For most experiments, **7** was introduced into the biopolymer as a mixture of diastereomers. The separate diastereomers of **7** were incorporated individually, following separation of the 5′-*O*-dimethoxytritylated ketones **14**. All oligonucleotides were synthesized on one of two photolabile solid phase synthesis supports (**26**, **27**).[23] Oligonucleotides containing only thymidine, an abasic site model (**28**), and **7** were treated with anhydrous diisopropylamine prior to cleavage from the solid phase supports to remove the β-cyanoethyl protecting groups. Deoxyadenosine, deoxycytidine, and deoxyguanosine were incorporated into **31** as their allyloxy phosphoramidites.[23,24] Thymidine and **7** were incorporated as their β-cyanoethyl phosphoramidites. The β-cyanoethyl groups were removed from thymidine and **7** with anhydrous diisopropylamine prior to removing the allyloxy and allyloxycarbonyl protecting groups with Pd(0).



5'- d(TTT TTT TTT$_9$ T$_{10}$T$_{11}$**7** T$_{13}$TT TTT TT)

29

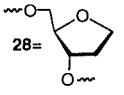
5'- d(TTT TTT TTT$_9$ T$_{10}$(**28**)(**7**) T$_{13}$TT TTT TT)

30

5'- d(TTG TTT CTT TTT TCG **7**AC TTT TTG TTT TTT)

31

5'- d(TTT TTT TTT T**X7** TTT TTT TTT T**7**T TTT TTT TT)



32a X= T
32b X= C1′-^{2}H-T
32c X= C2′-^{2}H-T
32d X= C4′-^{2}H-T

Structural Effects on Strand Damage. We previously demonstrated that 5,6-dihydrothymid-5-yl (**4**) requires O_2 to induce either strand breaks or alkaline labile lesions in **29** and **30**. The observed oxygen dependence of damage is independent of which diastereomer of **7** is present in **29**.[12a] Strand damage in **29** is readily observed in the 5′ direction up to three nucleotides away from the original site at which **4** is generated,

(22) Yang, N. C.; McClure, D. S.; Murov, S. L.; Houser, J. J.; Dusenbery, R. *J. Am. Chem. Soc.* **1967**, *89*, 5466.

(23) (a) Matray, T. J.; Greenberg, M. M. *J. Am. Chem. Soc.* **1994**, *116*, 6931. (b) Greenberg, M. M.; Gilmore, J. L. *J. Org. Chem.* **1994**, *59*, 746. (c) Venkatesan, H.; Greenberg, M. M. *J. Org. Chem.* **1996**, *61*, 525.

(24) Hayakawa, Y.; Wakabayashi, S.; Kato, H.; Noyori, R. *J. Am. Chem. Soc.* **1990**, *112*, 1691.

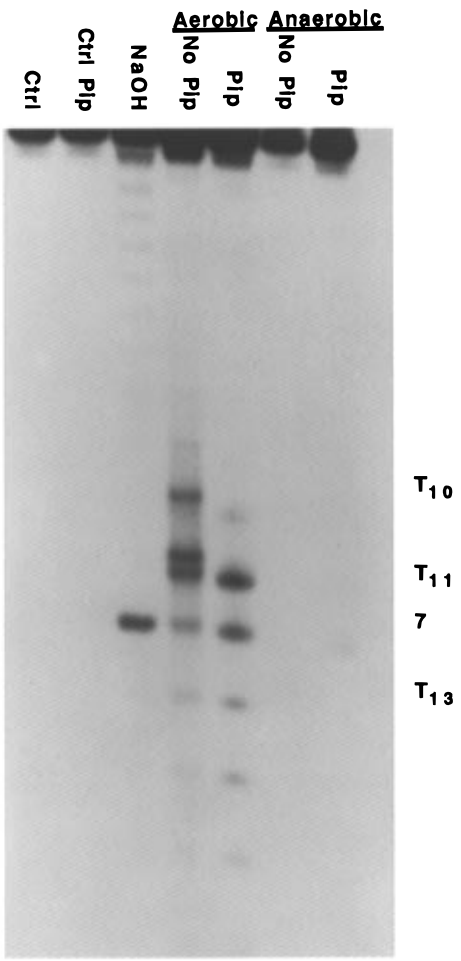


Figure 2. Autoradiogram of a 20% denaturing polyacrylamide gel of photolyzed (6 h) 3′-^{32}P labelled **29**.

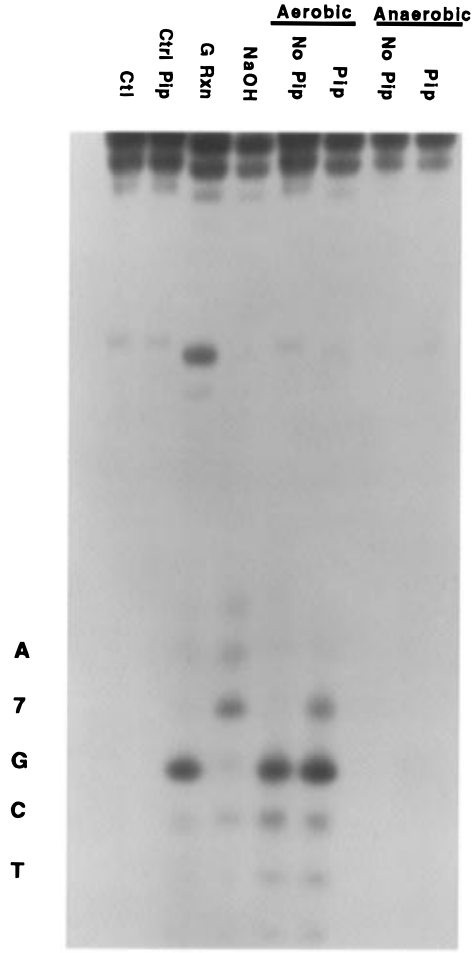


Figure 3. Autoradiogram of a 20% denaturing polyacrylamide gel of photolyzed (6 h) 5′-^{32}P-labeled **31**.

but is significantly diminished past the site of **28** in **30**.[12a] Piperidine treatment of photolyzed, 3′-^{32}P-labeled **29** significantly alters the cleavage pattern (Figure 2). Whereas two cleavage products are observed at T_{11} prior to piperidine treatment, only the faster moving product is present after alkaline treatment. In addition, reduction of cleavage at T_{10} is observed upon piperidine cleavage, while cleavage at **7** is increased (Figure 2). The respective behavior of the two cleavage products at T_{11} upon piperidine treatment was verified following their isolation from the gel. The products were isolated and desalted together as previously described.[10] Piperidine treatment confirmed that the slower moving product is converted into an oligonucleotide which comigrates with the cleavage product produced upon NaOH treatment of intact 3′-^{32}P-labeled **29**.[25] The disparate susceptibilities of the two products produced upon cleavage at T_{11} to piperidine are consistent with the interpretation that the faster moving product contains an intact molecule of **7** (which is stable to piperidine treatment), whereas the slower moving oligonucleotide fragment contains a more labile molecule in place of **7**. Finally, the disappearance of the product resulting from cleavage at T_{10} (Figures 2 and 5) upon piperidine treatment suggests that it too is associated with the formation of a product containing an alkaline labile lesion within the fragmented biopolymer.

Oxygen dependent strand damage is also observed when **4** is produced upon irradiation from **7** in a polymer containing naturally occurring nucleotides other than thymidine (**31**; Figure 3). In contrast to **29**, alkaline labile and direct strand breaks are observed predominantly at the nucleotide (deoxyguanosine; G in Figure 3) bound to the 5′-phosphate of **7**. Direct strand cleavage at deoxyguanosine is at least 5 times greater than at the deoxycytidine, which is two nucleotides removed from the 5′-phosphate of **7**. The reasons for the different cleavage patterns observed upon irradiation of **29** and **31** are addressed below. Regardless of the structure of the biopolymer containing **7** (**29** or **31**), phosphorimaging analysis reveals that cleavage is significantly enhanced upon piperidine treatment of photoirradiated samples (Table 1).[26]

Table 1. Effect of *tert*-Butyl Alcohol on Strand Cleavage in Photolyzed **29** and **31**[a–c]

oligonucleotide	− −	− +	+ −	+ +	piperidine *t*-BuOH
29	2.70 ± 0.6	2.46 ± 0.1	28.1 ± 3.0	28.7 ± 1.7	
31	2.27 ± 0.2	1.94 ± 0.2	19.2 ± 0.8	20.1 ± 1.6	

[a] Cleavage is expressed in terms of percent of total DNA cleaved. [b] [*t*-BuOH] = 1.05 M. [c] $h\nu$ = 8 h.

Enzymatic End Group Analysis of Oligonucleotide Fragmentation Products. Information regarding the nature of the 3′-terminal end groups of products obtained upon irradiation of oligonucleotides containing **7** was obtained via T4 polynucleotide kinase treatment.[27] In the absence of triphosphate, this enzyme dephosphorylates 3′-terminal phosphates, yielding

(25) See the Supporting Information for this paper.

(26) Previous estimates of the enhancement of strand damage upon piperidine treatment were made using densitometric analysis of autoradiograms.[12a] These measurements underestimated the extent of cleavage upon piperidine treatment, due to the limited linear range of the X-ray film. The current results were obtained using phosphorimaging analysis.

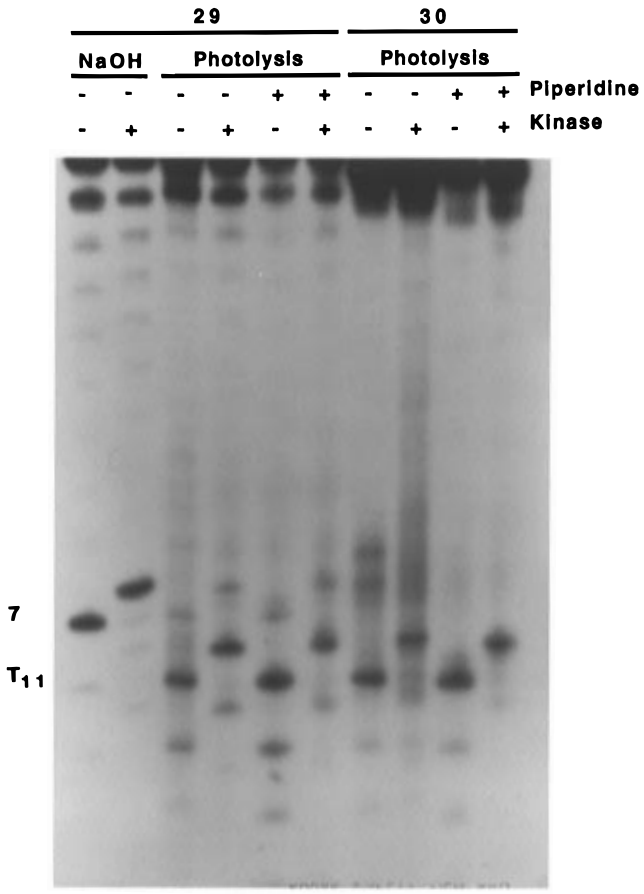


Figure 4. Autoradiogram of a 20% denaturing polyacrylamide gel showing the effects of polynucleotide T_4 kinase on photolyzed (6 h) 5′-^{32}P-labeled **29** and **30**.

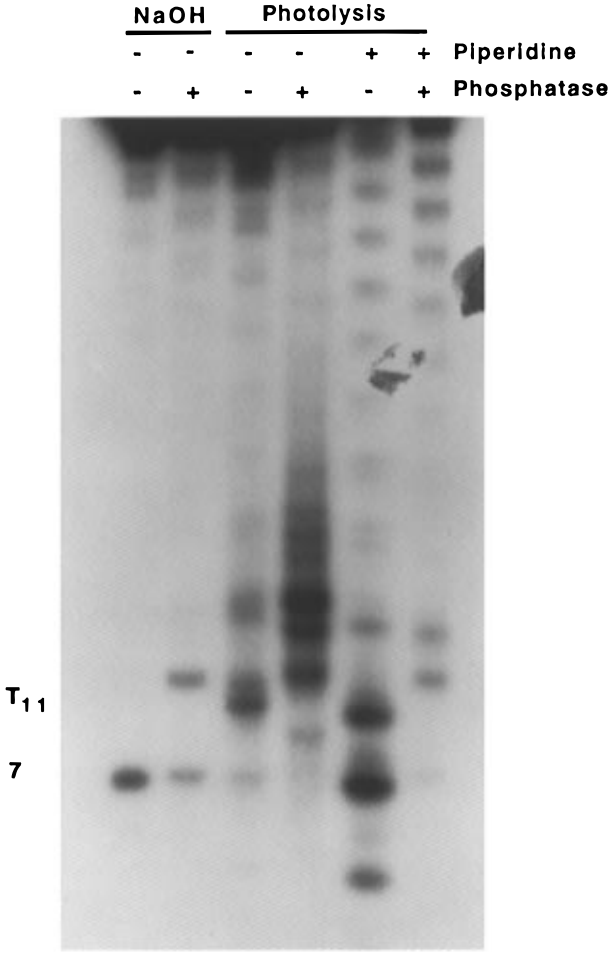


Figure 5. Autoradiogram of a 20% denaturing polyacrylamide gel showing the effects of calf intestine alkaline phosphatase on photolyzed (6 h) 3′-^{32}P-labeled **29**.

oligonucleotides containing 3′-terminal hydroxyl groups. Oligonucleotides containing the respective end groups are separable by gel electrophoresis. Although the cleavage patterns observed in **29**−**31** are different, enzymatic end group analyses show that the strand breaks and alkaline labile lesions formed in these biopolymers consist solely of 3′-phosphate termini (Figure 4).[25] The absence of phosphoglycolate termini indicates that hydrogen atom abstraction from the C4′ position does not occur upon irradiation of oligonucleotides containing **7**.[28]

Similarly, treatment of 3′-^{32}P-labeled **29** (Figure 5) and **31**[25] with calf intestine alkaline phosphatase, which dephosphorylates the 5′-terminus of oligonucleotides, reveals that the 5′-termini produced via photolysis in the presence of O_2 also consist exclusively of phosphate groups. The observation that the mobility of both cleavage bands at T_{11} in **29** is affected upon treatment with calf intestine alkaline phosphatase is significant. It suggests that the structures of these cleavage products differ at a position other than their 5′-termini. We attribute this surprising behavior to the presence of two oligonucleotide fragmentation products, each containing a 5′-phosphate, but a different nucleotide at the position where **7** is incorporated (see above). As evidenced by the effect of **28** on the migration of oligonucleotides via gel electrophoresis, structural alterations of nucleosides and even sequence alterations can exert significant effects on the migratory aptitudes of short oligonucleotides.[12a,29] Also consistent with this proposal was the observation of a substantial change in migration for two oligonucleotide fragments having the same sequence and charge, but differing solely by the presence of a 3′-phosphoglycoaldehyde in one case, and a 3′-phosphoglycol in another.[5]

The structures of the oligonucleotide fragmentation products at T_{11} are uncertain and warrant further characterization. However, the above data (piperidine lability, enzymatic end group analysis) and other information concerning the results obtained upon photolysis of **7** lead us to infer that the faster and slower moving bands arising from cleavage at T_{11} (Figures 2 and 5) are **33** and **34**, respectively (Scheme 5). Aerobic photolysis of **7** produces **24**, which undergoes decomposition upon storage at −20 °C.[12b] We expect that an oligonucleotide containing **24** (**34**) would migrate more slowly than **33** on account of its additional hydrogen bonding group, and that this biopolymer would undergo cleavage upon piperidine treatment.

Hydrogen Atom Abstraction and Free Base Release. Gel electrophoresis has previously been used to measure kinetic isotope effects, in order to glean mechanistic information on nucleic acid damage processes.[10,30] The experiment utilized here enables one to determine an observed KIE by comparing the

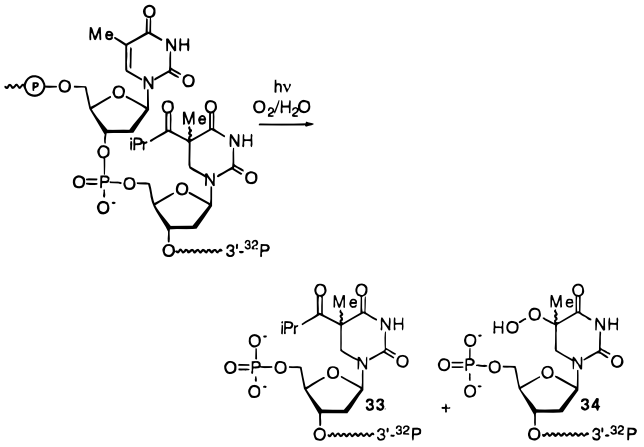
(27) Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning: A Laboratory Manual*, 2nd ed.; Cold Spring Harbor Laboratory Press: New York, 1989.

(28) (a) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107. (b) Giese, B.; Beyrich-Graf, X.; Erdmann, P.; Giraud, L.; Imwinkelried, P.; Müller, S. N.; Schwitter, U. *J. Am. Chem. Soc.* **1995**, *117*, 6146.

(29) Shibutani, S.; Takeshita, M.; Grollman, A. P. *Nature* **1991**, *349*, 431.

(30) (a) Greenberg, M. M.; Dervan, P. B. (California Institute of Technology). Unpublished results. (b) Kozarich, J. W.; Worth, L., Jr.; Frank, B. L.; Christner, D. F.; Vanderwall, D. E.; Stubbe, J. *Science* **1989**, *245*, 1396. (c) Absalon, M. J.; Wu, W.; Kozarich, J. W.; Stubbe, J. *Biochemistry* **1995**, *34*, 2076. (d) Kappen, L. S.; Goldberg, I. H.; Wu, S. H.; Stubbe, J.; Worth, L., Jr.; Kozarich, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 2797. (e) Kappen, L. S.; Goldberg, I. H.; Frank, B. L.; Worth, L., Jr.; Christner, D. F.; Kozarich, J. W.; Stubbe, J. *Biochemistry* **1991**, *30*, 2034.

Scheme 5



Scheme 6

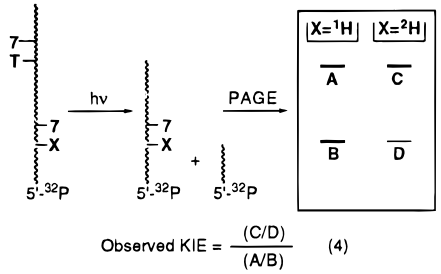


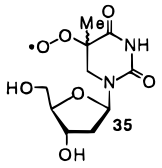
Table 2. Observed Kinetic Isotope Effects in **32**

position deuterated	non-piperidine	piperidine
C1′	3.9 ± 0.5	1.3 ± 0.2
C2′	1.0 ± 0.1	1.0 ± 0.2
C4′	1.0 ± 0.1	1.0 ± 0.1

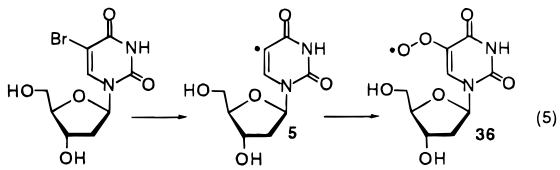
measured ratio of strand breaks at two cleavage sites in two different oligonucleotides (Scheme 6, eq 4).[10,30a] One oligonucleotide (control) contains two molecules of **7** where neither carbohydrate of the adjacent 5′-nucleotides is isotopically enriched. The ratio of cleavage at the two nucleotides adjacent to **7** in the control is compared to the relative cleavage of an oligonucleotide of identical sequence, where the nucleotide adjacent to the 5′-phosphate of one of the molecules of **7** is enriched in deuterium at a single position in the carbohydrate moiety. By carrying out the experiment in this manner (preferably to a small extent of cleavage), one does not have to measure the extent of conversion, because the protio site serves as an internal standard.

The kinetic isotope effects were measured using the above method with deuterium enrichment at C1′, C2′, and C4′ using **32a**−**d**. Measurements were made for direct strand breaks, as well as alkaline labile lesions. The deuterated thymidines were prepared via known procedures, and incorporated into **32b**−**d** as their phosphoramidite analogues.[30a,31,32] In a typical experiment, 4−5 samples of each oligonucleotide were irradiated. The KIEs reported are derived from the averages of the cleavage ratios measured in these independent experiments (Table 2).[32] Essentially no KIE is observed upon deuteration of C2′ or C4′ of the thymidine at position **X** in **32**, while a significant effect is observed upon deuteration of C1′. Also of interest is the fact that the diminution of strand damage at this nucleotide does not affect strand damage at nucleotides displaced further in the 5′ direction from **7**. This observation eliminates a possible free radical chain propagation mechanism, analogous to that observed in lipid peroxidation, as the reason for the cleavage pattern observed for **29**.[12a] As explained below, we believe that a portion of the strand damage at sites distal to **7** in polythymidylates is due to the participation of an additional pathway.

The significant KIE observed upon deuteration of C1′ strongly suggests that hydrogen atom abstraction from this position of the deoxyribonucleotide covalently bound to the 5′-phosphate of **7** is involved in the direct strand scission produced during aerobic photolysis of **32**. The absence of a KIE upon dideuteration of the C2′ and deuteration of the C4′ positions suggests that the reactive species responsible for hydrogen atom abstraction is a very selective one. We expect that the peroxyl radical derived from **4** (**35**) would react selectively with an adjacent



deoxyribonucleotide. On the basis of recent calculations of enthalpies for hydrogen atom abstraction from deoxyribose, and the approximate O−H bond strength in a hydroperoxide (∼90 kcal/mol), the C1′ position may be the only thermodynamically favorable position within a deoxyribonucleotide for hydrogen atom abstraction by a peroxyl radical.[33] Further justification for proposing that strand breaks are produced via C1′ hydrogen atom abstraction by **35** is provided by the observation that the analogous peroxyl radical derived from aerobic photolysis of 5-bromodeoxyuridine (**36**; eq 5) also selectively abstracts a hydrogen atom from the C1′ position of the 5′ adjacent nucleotide in single-stranded DNA.[34]



Despite the arguments presented above in favor of participation of **35** in the observed strand scission, the magnitude of the KIE (∼4.0) observed upon deuteration of C1′ is lower than one might expect for the involvement of a peroxyl radical.[35] The observed KIE is also smaller than one might expect if an alkoxyl radical was the species responsible for hydrogen atom abstraction.[35] The selectivity of the process implied by the absence of a KIE upon deuteration at C4′ and C2′ is more consistent with the involvement of a peroxyl radical than a more reactive alkoxyl radical.[36,37] Finally, while the magnitude of the observed KIE could be considered low for hydrogen atom abstraction by a peroxyl radical, one must realize that the

(31) (a) Cook, G. P.; Greenberg, M. M. *J. Org. Chem.* **1994**, *59*, 4704. (b) Kohn, P.; Samaritano, R. H.; Lerner, L. M. *J. Am. Chem. Soc.* **1965**, *87*, 5475. (c) Vorbrüggen, H.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1279.

(32) A sample autoradiogram demonstraing the KIE experiment is included in the Supporting Information.

(33) (a) Benson, S. W. *Thermochemical Kinetics*, 2nd ed.; John Wiley: New York, 1976. (b) Miaskiewicz, K.; Osman, R. *J. Am. Chem. Soc.* **1994**, *116*, 232.

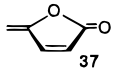
(34) Cook, G. P.; Koppisch, A. T.; Greenberg, M. M. (Colorado State University) Unpublished results.

(35) (a) Lewis, E. S.; Ogino, K. *J. Am. Chem. Soc.* **1976**, *98*, 2264. (b) Howard, J. A. In *Free Radicals, Volume II*; Kochi, J. K., Ed.; John Wiley and Sons, New York, 1973. (c) Green, M. K.; Boyle, B. A.; Vairamani, M.; Mukhopadhyay, T.; Saunders, W. H., Jr.; Bowen, P.; Allinger, N. L. *J. Am. Chem. Soc.* **1986**, *108*, 2381. (d) For an example where KIEs for peroxyl radicals are relatively low see: Evans, C.; Scaiano, J. C.; Ingold, K. U. *J. Am. Chem. Soc.* **1992**, *114*, 4589.

observed KIE in this experiment is a weighted average of the true KIE for the process, multiplied by the fraction of total product (strand break) measured that is attributable to this pathway.[30e] Alternate reactive species and/or pathways would result in a reduction of the observed KIE for a process involving **35**.

Since strand scission observed upon irradiation of oligonucleotides containing **7** is dependent upon oxygen, alternative pathways might also be expected to involve oxygen. Furthermore, any reactive species in addition to **35** is (are) not required to induce strand damage via hydrogen atom abstraction from the carbohydrate moiety. In fact, the significant diminution of the observed KIE (Table 2), as well as the large overall increase in strand scission (Table 1) observed upon piperidine treatment, is consistent with the production of a second reactive species that does not react via a pathway similar to that followed by **35**.

In order to corroborate the KIE experiments, we investigated the degradation products formed upon irradiation of **29**. Abstraction of the C1′ hydrogen atom of a nucleotide in DNA typically results in concomitant release of the respective nucleobase.[2e,3b,4] The yield of thymine was determined by reversed phase HPLC as a function of direct strand break formation in **29**. Irradiation of unlabeled **29** resulted in the release of ∼40% thymine compared to direct strand breaks, which were measured by anion exchange HPLC. We were unable to determine whether thymine degradation products were formed, due to limitations of HPLC detection. Methylenefuranone (**37**) has also been associated with DNA oxidation at C1′



37

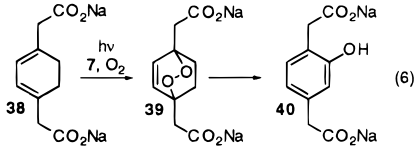
in some, but not all, instances.[3a,4,6] We did not detect significant photodegradation of independently synthesized **37** under our irradiation conditions.[4,38] However, our detection limits (∼25 nmol via GC/MS) prohibited direct observation of the methylenefuranone (**37**) when **29** was irradiated.

Photochemical Generation of $^1O_2$ and Its Role in Strand Damage. The observations regarding the cleavage pattern in **32** that were made while KIE experiments were carried out, as well as the very different cleavage patterns produced for irradiation of polythymidylates **29**, **30**, and **32** and **31**, led us to investigate the possibility that a reactive species other than **35** is responsible for a portion of the O_2 dependent strand damage of irradiated oligonucleotides containing **7**.[12a] The different cleavage patterns observed in **29**−**32**, as well as the dependence on O_2, suggest that a highly reactive, nonselective diffusible species, such as hydroxyl radical, was not responsible for the oligonucleotide damage. This proposal was supported by photolyses carried out in the presence of *t*-BuOH (1.05 M), a hydroxyl radical trap, which had no effect upon the extent of damage in either **29** or **31** (Table 1).[1] Other pathways considered by us included (1) energy transfer to the neighboring nucleotides from the excited state of **7**, (2) involvement of the isopropyl and/or respective acyl radical produced via the Norrish type I cleavage of **7**, and (3) production of $^1O_2$ via quenching of the excited state of **7** by O_2.

We previously discounted the involvement of the excited state of **7** in strand damage, on the basis of the independence of strand scission on the stereochemistry of the ketone.[12a] The observed dependence of the cleavage pattern on the oligonucleotide sequence is also inconsistent with this explanation, as it has recently been shown that the excited states of all four nucleotides have very similar energies, and are all sensitized by ketones.[39]

Isopropyl and/or the respective acyl radical is produced in solution via the Norrish type I photocleavage. These radicals are capable of inducing nucleic acid damage, and might well contribute in some manner to the observed cleavage. However, carbon-centered radicals do not require O_2 in order to damage nucleic acids.[40] Hence, we do not believe that such species, or the peroxyl radicals derived from them (which one would expect would be even less reactive), are major contributors to the strand damage derived from photolysis of **7**.

The above arguments against hydroxyl, alkyl, and peroxyl radicals do not rule out all diffusible species as being participants in strand damage. Various excited states, including those of ketones, can be quenched by O_2 to produce singlet oxygen ($^1O_2$). Indeed, photolysis of **7** in the presence of $^1O_2$ trap **38** indicated that this reactive species is produced under conditions in which DNA strand damage is observed.[41] The product observed was **40** and not the initially formed endoperoxide **39**, which was shown to decompose to the phenol **40** under the conditions in which **7** is photolyzed (eq 6). Singlet oxygen is most often



(6)

associated with damage of guanine-containing nucleosides.[42] Absolute rate measurements show that $^1O_2$ reacts at least an order of magnitude faster with deoxyguanosine than with other nucleosides.[43] However, it has also been shown that thymidine monophosphate deactivates $^1O_2$ with a bimolecular rate constant greater than 10^5.[43b] Furthermore, early studies showed that thymidine was degraded by $^1O_2$.[44] Both direct and indirect measurements indicate that thymidine reacts more rapidly than deoxycytidine and deoxyadenosine with $^1O_2$.[43,44] Hence, while $^1O_2$ does react preferentially with deoxyguanosine, it is erroneous to assume that this oxidant does not react with other nucleotides.

The selectivity observed for cleavage in **31**, relative to that observed in polythymidylates (e.g., **29**), is consistent with the involvement of $^1O_2$ in the observed strand damage. Consequently, we probed for the possible involvement of $^1O_2$ in strand damage, by examining the effect of azide on the direct and alkaline labile strand breaks in **29** (Table 3).[42,43] Addition of

(36) On the basis of the calculated enthalpies for hydrogen atom abstraction from deoxyribose, and the approximate bond dissociation energy for an O−H bond in an alcohol (∼104 kcal/mol), we expect that an alkoxyl radical will exhibit much less selectivity in its reactions with deoxyribonucleotides than a peroxyl radical.[33] In a related study, less selective reactivity has been observed for **5** in single-stranded DNA.[34]

(37) (a) Foti, M.; Ingold, K. U.; Lusztyk, J. *J. Am. Chem. Soc.* **1994**, *116*, 9440. (b) Ingold, K. U. In *Free Radicals, Volume I*; Kochi, J., Ed.; John Wiley: New York, 1973. (c) Bérces, T.; Dombi, J. *Int. J. Chem. Kinet.* **1980**, *12*, 183.

(38) Grundmann, C.; Kober, E. *J. Am. Chem. Soc.* **1955**, *77*, 2332.

(39) (a) Wood, P. D.; Redmond, R. W. *J. Am. Chem. Soc.* **1996**, *118*, 4256. (b) Gut, I. G.; Wood, P. D.; Redmond, R. W. *J. Am. Chem. Soc.* **1996**, *118*, 2366.

(40) (a) Milligan, J. R.; Ward, J. F. *Radiat. Res.* **1994**, *137*, 295. (b) Hiramato, Z.; Johkoh, H.; Sako, K-I.; Kikugawa, K. *Free Radical Res. Commun.* **1993**, *19*, 323.

(41) Nardello, V.; Azaroual, N.; Cervoise, I.; Vermeersch, G.; Aubry, J-M. *Tetrahedron* **1996**, *52*, 2031.

(42) (a) Sies, H.; Menck, C. F. M. *Mutat. Res.* **1992**, *275*, 367. (b) Sheu, C.; Foote, C. S. *J. Am. Chem. Soc.* **1995**, *117*, 474. (c) Devasagayam, T. P. A.; Steenken, S.; Obendorf, M. S. W.; Schulz, W. A.; Sies, H. *Biochemistry* **1991**, *30*, 6283.

(43) (a) Lee, P. C. C.; Rodgers, M. A. J. *Photochem. Photobiol.* **1987**, *45*, 79. (b) Rougeé, M.; Benasson, R. V. *C. R. Acad. Sci., Ser. II* **1986**, *302*, 1223.

(44) Simon, M. I.; Van Vunakis, H. *J. Mol. Biol.* **1962**, *4*, 488.

Table 3. Effect of Azide on Strand Scission in **29**[a,b]

[azide] (mm)	non-piperidine	piperidine
0	0.66 ± 0.22	15.0 ± 1.1
10	0.25 ± 0.04	8.1 ± 1.1

[a] Cleavage is expressed in terms of percent of total DNA cleaved. [b] $h\nu = 6$ h.

Table 4. Effect of D_2O on Strand Scission in **29**[a,b]

solvent	non-piperidine	piperidine
H_2O	2.4 ± 0.6	27.4 ± 3.1
D_2O	1.7 ± 0.1	22.7 ± 2.4

[a] Cleavage is expressed in terms of percent of total DNA cleaved. [b] $h\nu = 8$ h.

NaN_3 (10 mM) to aerobic photolyses of **29** results in approximately a 2-fold diminution in overall strand damage. Independent experiments showed that the azide does not affect the quantum yield for disappearance of **7**. These observations suggest that $^1O_2$ is partially responsible for the observed strand damage. In contrast to these experiments, photolytic cleavage in D_2O (which typically enhances strand damage by increasing the lifetime of $^1O_2$) appears to slightly reduce the extent of cleavage at **7** and the nucleotides adjacent to its 5′-phosphate (Table 4). Statistically, the extents of cleavage in H_2O and D_2O are within experimental error of each other. A possible reason for this lack of an effect by D_2O can be explained by considering the fact that $^1O_2$ is generated in the vicinity of the oligonucleotides that are cleaved. This is far different from the situation when $^1O_2$ is produced in the bulk of a dilute solution of DNA. The lifetime of $^1O_2$ in H_2O is >40 μs, and it is estimated to have a diffusion radius of ~100 Å.[42a] Consequently, $^1O_2$ diffuses many angstroms greater than that required to span the distance (<20 Å) of the nucleotides (**7** to T_9) over which damage is measured in **29**. Hence, increasing the $^1O_2$ lifetime by adding D_2O does not necessarily have to increase the amount of strand damage at nucleotides **7** through T_9 in **29**.

Finally, the involvement of $^1O_2$ in strand damage is also consistent with the cleavage pattern observed for 3′-^{32}P-labeled oligonucleotides, as well as the enzymatic end group analysis of oligonucleotides labeled at this terminus. Production of $^1O_2$ has no effect on the structure of **7**. Hence, the formation of two cleavage (**33**, **34**) products containing 5′-phosphate termini upon cleavage at the nucleotide adjacent to the 5′-phosphate of **7** (Scheme 5) is consistent with cleavage induced via **35** (yielding a cleavage product, **34**, that is itself alkaline labile) and $^1O_2$ (yielding a product, **33**, containing an intact molecule of **7**, which is not susceptible to piperidine treatment).

Summary and Conclusions. Isopropyl ketone **7** was chosen as a precursor to **4** in order to influence the initial bond cleavage during the Norrish type I photocleavage. However, this choice of ketone inadvertently complicated the photochemistry. Previously observed strand damage, and that characterized above, are consistent with the involvement of **35** (from **4**) and $^1O_2$ (due to quenching of the excited state of **7** by O_2).[12]

Kinetic isotope effect experiments and enzymatic end group analysis are consistent with a reaction mechanism involving hydrogen atom abstraction by **35** from the C1′ position at the 5′ adjacent nucleotide (Scheme 7).[3,6] Estimates of the thermodynamics of this process are consistent with the observed selectivity of this process.[33] The details for the formation of a strand break following hydrogen atom abstraction from the C1′ position, and the nature of the deoxyribose degradation product(s), are currently under investigation. Utilizing photochemical precursors to **4** that are less prone to $^1O_2$ generation (e.g., *tert*-butyl and/or benzyl ketones) will simplify these studies, as will independent generation of the radical produced upon C1′ hydrogen atom abstraction.[45]

Despite being partially obscured by the generation of $^1O_2$, the experiments described here support the proposal that two covalently linked nucleotides in a single strand of DNA can be damaged via the transposition of spin from an initially damaged nucleobase to an adjacent carbohydrate moiety.[46] This nucleic acid damage pathway is distinct from those examined involving other agents, and could provide the impetus for the design of a new family of nucleic acid damaging agents.[2−4]

Experimental Section

General Methods. ^{1}H NMR spectra were recorded at 300, 270, or 200 MHz. IR spectra were obtained using a Perkin-Elmer 1600 Series FT-IR. All reactions were carried out in oven-dried glassware, under a nitrogen atmosphere, unless specified otherwise. *N*,*O*-Bis(trimethylsilyl)(trifluoromethyl)acetamide containing 1% TMSCl (BSTFA/TMS) was obtained from Sigma. T4 polynucleotide kinase and calf intestine alkaline phosphatase were from New England Biolabs. Terminal deoxynucleotidyl transferase was from US Biochemical. [α-^{32}P]ddATP and [γ-^{32}P]ATP were from Amersham. Pyridine, *N*,*N*-diisopropylethylamine (Hünig's base), and CH_2Cl_2 were distilled from CaH_2. THF was distilled from Na^0/benzophenone ketyl. Acetonitrile was purified and dried by passing over anhydrous $CuSO_4$, followed by distillation from CaH_2.

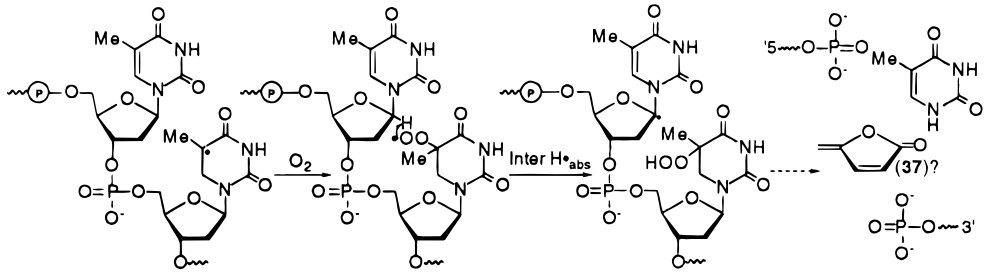
Oligonucleotides were synthesized on an Applied Biosystems Inc. 380B DNA synthesizer using previously reported cycles.[23a,b] Allyloxy-protected phosphoramidites and photolabile solid phase synthesis supports **26** and **27** were prepared as described.[23,24] DNA manipulations were carried out using standard procedures.[27] Chemical sequencing reactions were performed as described.[23a,47] Photolyzed oligonucleotides were electrophoresed on 20% polyacrylamide denaturing gels (5% cross-link, 45% (urea by weight)). The gels were quantitated either using a Molecular Dynamics phosphorimager equipped with Imagequant Version 3.3 software or by densitometry.

All photolyses of nucleosides and oligonucleotides were carried out in Pyrex tubes (0.25 in. i.d.) using a Rayonet photoreactor equipped with lamps having a maximum output at 350 nm. Oligonucleotide photolyses were carried out in 10 mM phosphate buffer (pH 7.0) and 100 mM NaCl. In experiments involving photolysis of monomeric materials, yields of **7**, **11a**, and **22** were determined by reversed phase HPLC analysis (Rainin Microsorb-MV C_{18}, 0.4 × 25 cm column; A, H_2O; B, CH_3CN; 0−35% B linearly over 35 min; flow rate, 1.0 mL/min) using 5-bromo-2′-deoxyuridine as an internal standard (added after irradiation). The response factors versus 5-bromo-2′-deoxyuridine were determined using independently prepared **7** (0.9), **11a** (0.5), and **22** (1.4) at 205 nm. The yield of free thymine released during the photolysis of **29** was determined by reversed phase HPLC using the above C_{18} column (A, 10 mM KH_2PO_4 (pH 6.8), 2.5% MeOH; B, 10 mM KH_2PO_4 (pH 6.8), 20% MeOH; 0−100% B linearly over 15 min; flow rate, 1.0 mL/min). Uracil was added as an internal standard after photolysis (response factor, 1.2 at 254 nm). The loss of **29** was determined using anion exchange HPLC (Vydac weak anion-exchange oligonucleotide column; 0.4 × 25 cm; A, 0.1 M $(NH_4)_2HPO_4$ (pH 6.7), 20% CH_3CN; B, 0.3 M $(NH_4)_2HPO_4$ (pH 6.7), 20% CH_3CN; 0−67% B linearly over 20 min; flow rate, 1.5 mL/min). Singlet oxygen trapping experiments were analyzed by reversed phase HPLC using the above C_{18} column (A, H_2O; B, CH_3CN; 5% B from $t = 0$ to $t = 5$ min; 5−50% B linearly over 25 min; 50−5% B linearly over 10 min; flow rate, 1 mL/min).

Quantitation of Thymine Release as a Function of Strand Break Formation in 29. Oligonucleotide **29** (56 μM, total volume 100 μL) was photolyzed for 28 h as described above. Prior to, and after photolysis, 5 μL of the photolysis sample was removed, spiked with a 24 nucleotide long oligonucleotide (11.8 μM, 5 μL), and analyzed by

(45) (a) Goodman, B. K.; Greenberg, M. M. *J. Org. Chem.* **1996**, *61*, 2. (b) Gimisis, T.; Chatgilialoglu, C. *J. Org. Chem.* **1996**, *61*, 1908.
(46) Box, H. C.; Budzinski, E. E.; Freund, H. G.; Evans, M. S.; Patrzyc, H. B.; Wallace, J. C.; Maccubbin, A. E. *Int. J. Radiat. Biol.* **1993**, *64*, 261.
(47) Maxam, A. M.; Gilbert, W. S. *Methods Enzymol.* **1980**, *65*, 499.

Scheme 7



anion exchange HPLC. After photolysis, the sample was filtered through an Amicon membrane filter. The membrane was washed with H_2O (100 μL), prior to speed-vacing the filtrate to dryness. The filtrate was resuspended in an aqueous solution of uracil (9.8 μM, 100 μL), and analyzed by reversed phase HPLC.

Detection of Singlet Oxygen upon Photolysis of 7. A methanol solution (0.2 mL) of **7** (3 mM) was photolyzed in a RPR-100 photoreactor for 6 h at 25 °C in the presence of **38** (20 mM).[41] The crude photolysate was analyzed by reversed phase HPLC and ^{1}H NMR. In a control experiment, **38** (10 mM) was photolyzed in the presence of Rose Bengal (1 mg), as described in methanol (5 mL).[41] An aliquot of the crude photolysate was analyzed as described above by reversed phase HPLC and ^{1}H NMR. Following HPLC analysis, the photolysis mixture was subjected to the identical photolysis conditions used for **7**, and the crude photolysate was again analyzed by reversed phase HPLC and ^{1}H NMR.

Preparation of 9. Rhodium on alumina (10%, 100 mg) was added to a solution of thymidine (10 g, 41.28 mmol) in a mixture of methanol and water (1:1, 75 mL) in a high-pressure bottle. The mixture was stirred under 50 psi of hydrogen gas for 48 h. The catalyst was removed by filtration through a pad of Celite and the filtrate lyophilized to give 9.6 g (96%) of dihydrothymidine. TBSCl (5.65 g, 37.50 mmol) was dissolved in pyridine (25 mL) and added to dihydrothymidine (3.0 g, 12.38 mmol) that had been previously dried from pyridine (3.0 mL) under vacuum. The reaction was allowed to stir at 40 °C for 24 h, quenched with MeOH (0.5 mL), poured into H_2O (250 mL), and extracted with diethyl ether (3 × 150 mL). The combined organic layers were washed with brine (50 mL) and dried over $MgSO_4$. Flash chromatography (CH_2Cl_2/EtOAc; 9:1) yielded **9** (5.1 g, 88%): mp 103−104 °C; ^{1}H NMR ($CDCl_3$) δ 7.31 (br d s, 1H), 6.26 (dd, 1 H, J = 6.6, 6.6 Hz), 4.34 (m, 1H) 3.77 (m, 1H), 3.69 (m, 2H), 3.37 (dd, 1H, J = 5.4, 12.9 Hz), 3.25 (dd, 1H, J = 8.9, 13.2 Hz), 2.60 (m, 1H), 1.97 (m, 2H), 1.24 (d, 3H, J = 7 Hz), 0.89 (s, 9H), 0.87 (s, 9H), 0.06 (s, 6H), 0.05 (s, 6H); IR (film) 3211 (br d), 3077, 2955, 2922, 2888, 2844, 1705, 1472, 1383, 1250, 1111, 1022 cm^{-1}. Anal. Calcd for $C_{22}H_{44}N_2O_5Si_2$: C, 55.88; H, 9.40; N, 5.92. Found: C, 55.67; H, 9.15; N, 5.94.

Preparation of 12b. A cyclohexane solution of *sec*-BuLi (1.98 mL, 1.2 M) was added via syringe to **9** (450 mg, 0.96 mmol) in THF (4.5 mL) at −78 °C. After 1 h, isobutyraldehyde (104 mg, 1.44 mmol) was added dropwise via syringe. After stirring for an additional 2 h at −78 °C, the reaction was quenched with saturated NH_4Cl (0.5 mL), warmed to room temperature, and poured into H_2O (50 mL). The aqueous layer was extracted with ether (2 × 125 mL), and the combined organic layers were washed with brine (50 mL) and dried over $MgSO_4$. Flash chromatography (CH_2Cl_2/EtOAc; 9:1) yielded **12b** (314 mg, 60%) as a mixture of diastereomers: mp 58−62 °C; ^{1}H NMR ($CDCl_3$) δ 7.62 (br d s,1H), 6.34 (dd, 1H, J = 6.2, 7.0 Hz, minor), 6.24 (dd, 1H, J = 7,7 Hz, major), 4.31 (m, 1H), 3.77 (m, 1H), 3.64−3.22 (m, 5H), 1.91−1.73 (m, 3H), 1.29 (s, 2H), 1.19 (s, 1H), 0.98 (m, 6H), 0.88 (s, 9H), 0.86 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H), 0.04 (s, 3H), 0.03 (s, 3H); IR (film) 3214 (br d), 3084 (br d), 2955, 2927, 2853, 1699, 1469, 1384, 1356, 1215, 1120, 1092 cm^{-1}. Anal. Calcd for $C_{26}H_{52}N_2O_6Si_2$: C, 57.30; H, 9.64; N, 5.14. Found: C, 57.17; H, 9.48; N, 5.04.

Preparation of 13. Dess−Martin periodinane reagent (650 mg, 0.87 mmol) was added to a solution of **12b** (314 mg, 0.58 mmol) in CH_2Cl_2 (6.5 mL) at 0 °C. The reaction was stirred and allowed to warm to room temperature over 24 h, at which time the reaction was quenched with saturated $NaHCO_3$ (20 mL) containing $Na_2S_2O_3$ (2.5 g). The aqueous layer was extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were washed with brine (25 mL) and dried over $MgSO_4$. Flash chromatography (CH_2Cl_2/EtOAc; 12:1) afforded **13** (229 mg, 74%) as a hygroscopic foam: ^{1}H NMR ($CDCl_3$) δ 7.68 (br d s, 1H, minor), 7.63 (br d s, 1H, major), 6.25 (dd, 1H, J = 6.2, 6.3 Hz, minor), 6.12 (dd, 1H, J = 6.0, 6.0 Hz, major), 4.39 (m, 1H, minor), 4.29 (m, 1H, major), 4.08 (d, 0.55H, J = 13 Hz, major), 3.84−3.79 (m, 1H), 3.75−3.65 (m, 2.45H, major + minor), 3.15−2.98 (m, 2H), 2.30−2.21 (m, 0.45H, minor), 2.01−1.81 (m, 1.55H, major + minor), 1.37 (s, 3H), 1.07−0.95 (m, 6H), 0.92−0.84 (m, 18H), 0.08−0.02 (m, 12H); IR (film) 3083 (br d), 2947, 2920, 2857, 1691, 1473, 1437, 1383, 1360, 1257, 1224, 1089, 1021 cm^{-1}.

Preparation of 7. Anhydrous NH_4F (128 mg, 3.45 mmol) and **13** (229 mg, 0.42 mmol) were stirred in anhydrous MeOH (10 mL) at 50 °C. After 24 h, the mixture was poured into saturated $NaHCO_3$ (100 mL) and extracted with a mixture of isopropyl alcohol and $CHCl_3$ (3:7 (v/v), 5 × 50 mL). The organic layers were pooled, washed with brine (25 mL), and dried over $MgSO_4$. Flash chromatography (CH_2Cl_2/MeOH; 9:1) yielded 92 mg (69%) of **7** as a hygroscopic solid: ^{1}H NMR (MeOH-d_4) δ 6.21 (dd, 1H, J = 7.4, 6.8 Hz, major), 6.10 (dd, 1H, 6.3, 8.0 Hz, minor), 4.29 (m, 1H), 4.08 (d, 1H, J = 12.8 Hz, minor), 3.86 (d, 1H, J = 13.3 Hz, major), 3.78−3.70 (m, 1H), 3.67−3.60 (m, 2H), 3.26 (d, 1H, J = 13.2 Hz, minor), 3.20 (d, 1H, J = 12.8 Hz, major), 3.18−3.12 (m, 1H), 2.41−2.35 (m, 1H, major), 2.2−2.15 (m, 1H, minor), 1.98−1.88 (m, 1H), 1.39 (s, 3H, minor), 1.35 (s, 3H, major), 1.09 (d, 3H, J = 6.9 Hz, minor), 1.07 (d, 3H, J = 6.9 Hz, major), 1.04 (d, 3H, J = 6.9 Hz, minor), 0.96 (d, 3H, J = 6.9 Hz, major); ^{13}C NMR (MeOH-d_4) δ 213.1 (minor), 211.6 (major), 173.0 (minor), 172.7 (major), 154.7 (major), 154.1 (minor), 87.7 (minor), 87.5 (major), 85.7 (minor), 84.9 (major), 72.5 (minor), 72.3 (major), 63.3, 57.1 (minor), 56.9 (major), 45.6 (minor), 44.9 (major), 37.4, 37.2 (major), 37.0 (minor), 20.6 (minor), 20.0 (major), 18.7 (major), 17.9 (minor); IR (KBr) 3493 (br d), 3014, 2979, 2961, 1704, 1702, 1485, 1437, 1384, 1291, 1222, 1091, 1053, 1020 cm^{-1}; HRMS (FAB) calcd 315.1537 (M + H), found 315.1556.

Preparation of 15. Alcohol **12b** (210 mg, 0.39 mmol) was stirred in a mixture of HOAc/H_2O (4:1 (v/v), 3 mL) for 24 h. The solvents were removed *in vacuo*. Flash chromatography (CH_2Cl_2/MeOH, 9:1) yielded **15** (67 mg, 54%) as a mixture of four diastereomers: ^{1}H NMR (MeOH-d_4) δ 6.10−6.35 (m, 1H), 4.25−4.35 (m, 1H), 3.50−4.00 (m, 4H), 3.15−3.40 (m, 2H), 1.75−2.55 (m, 3H), 1.15−1.25 (m, 3H), 0.85−0.95 (m, 6H); HRMS (FAB) calcd 317.1712 (M + H), found 317.1710.

Preparation of Hydroperoxides 24. A mixture of **7** (50 mg, 0.159 mmol) and cyclohexa-1,4-diene (276 mg, 3.0 mmol) in a mixture of water and acetonitrile (3:2 (v/v), 15 mL) was loaded into a Pyrex tube fitted with a Teflon stopcock. The mixture was photolyzed in a Rayonet reaction chamber equipped with λ_{max} = 350 nm bulbs. After 16 h, the solvent was removed using a Savant Speed-Vac concentrator. The gummy residue was dissolved in water (500 μL) and filtered through a 0.45 μm nylon filter. Hydroperoxides **24a** and **24b** were isolated using reversed phase HPLC (Rainin C_{18}, 0.4 × 25 cm column; A, H_2O; B, CH_3CN; 3−35% B linearly over 35 min), with retention times of 5.6 and 6.8 min, respectively. **24a**: ^{1}H NMR (D_2O) δ 6.15 (dd, 1H, J = 6.8, 6.8 Hz), 4.26 (m, 1H), 3.78 (m, 2H), 3.59 (m, 2H), 3.40 (d, 1H, J = 13.2 Hz), 2.24−2.00 (m, 2H), 1.34 (s, 3H); HRMS (FAB) calcd 277.1035 (M + H), found 277.1050. **24b**: ^{1}H NMR (D_2O) δ 6.16 (dd, 1H, J = 6.8, 6.8 Hz), 4.26 (m, 1H), 3.81 (m, 2H), 3.75 (d, 1H, J = 13.5 Hz), 3.61 (m, 2H), 3.39 (d, 1H, J = 13.5 Hz), 2.17−2.02 (m, 2H), 1.35 (s, 3H); HRMS (FAB) calcd 277.1035 (M + H), found 277.1046.

Preparation of 14. Ketone **7** (60 mg, 0.18 mmol) and 4,4′-dimethoxytrityl chloride (77 mg, 0.22 mmol) were stirred in pyridine (3 mL) at 0 °C. After 16 h, the reaction was quenched with MeOH (0.1 mL), poured into saturated $NaHCO_3$ (20 mL), and extracted with EtOAc (3 × 20 mL). The organic layers were combined, washed with brine (25 mL), dried over Na_2SO_4, and concentrated. Flash chromatography (hexanes/EtOAc/MeOH, 4.5:5.0:0.5) yielded **14** (67 mg, 54%): mp 90−92 °C; ^{1}H NMR ($CDCl_3$) δ 7.41−7.17 (m, 10H), 6.82 (d, 3H, J = 8.2 Hz), 6.80 (d, 1H, J = 6.7 Hz), 6.27 (dd, 1H, J = 6.2 Hz, minor), 6.10 (dd, 1H, J = 6.6 Hz, major), 4.50−4.38 (m, 1H), 4.05 (d, 1H, J = 12.7 Hz, major), 3.85 (m,2H, minor), 3.76 (s, 6H), 3.36−3.29 (m, 2H), 3.00 (m, 2H), 2.20−2.05 (m, 2H), 1.54 (s, 3H), 1.25−0.92 (m, 6H); IR (KBr) 3199 (br d), 3077, 2973, 2926, 2832, 1701, 1602, 1504, 1475, 1442, 1377, 1297, 1245, 1174, 1029 cm^{-1}. Anal. Calcd for $C_{35}H_{40}N_2O_8$: C, 68.17; H, 6.54; N, 4.54. Found: C, 67.97; H, 6.44; N, 4.39.

Separation of (5*R*)- and (5*S*)-14. The diastereomers of **14** were prepared as described above and subsequently separated by flash chromatography (hexanes/EtOAc/MeOH, 4.8:5.0:0.2). **(5*S*)-14**: R_f = 0.35; ^{1}H NMR ($CDCl_3$) δ 7.76 (s, 1H), 7.42−7.14 (m, 10H), 6.83−6.76 (m, 4H), 6.27 (dd, 1H, J = 7, 7 Hz), 4.48 (m, 1H), 3.87−3.83 (m, 2H), 3.76 (s, 6H), 3.30 (m, 1H), 3.09 (m, 1H), 3.02 (d, 1H, J = 12.3 Hz), 2.56 (m, 1H), 2.05−1.97 (m, 1H), 1.92 (s, 1H), 1.15 (s, 3H), 1.09 (d, 3H, J = 6.6 Hz), 0.92 (d, 3H, J = 6.7 Hz). **(5*R*)-14**: R_f = 0.28; ^{1}H NMR ($CDCl_3$) δ 7.40−7.19 (m, 11H), 6.83−6.80 (m, 4H), 6.10 (dd, 1H, J = 6.8, 6.8 Hz), 4.41 (m, 1H), 4.05 (d, 1H, J = 12.7 Hz), 3.87 (m, 1H), 3.77 (s, 6H), 3.36 (m, 1H), 3.03−2.94 (m, 2H), 2.56 (m, 1H), 2.17−2.08 (m, 1H), 1.85 (s, 1H), 1.25 (s, 3H), 1.02 (d, 3H, J = 6.6 Hz), 0.91 (d, 3H, J = 6.7 Hz).

Preparation of Phosphoramidite 25. 2-Cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (35 mg, 0.15 mmol) was added to a stirred solution of **14** (74 mg, 0.12 mmol) and Hünig's base (23 mg, 0.18 mmol) in CH_2Cl_2 (3 mL) at 0 °C. After 30 min, the reaction was poured into saturated $NaHCO_3$ (20 mL) and extracted with EtOAc (2 × 15 mL). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, and concentrated. Flash chromatography (CH_2Cl_2EtOAc, 3:1) yielded **5** (50 mg, 51%) as a foam: ^{1}H NMR ($CDCl_3$) δ 7.42−7.14 (m, 10H), 6.83−6.76 (m, 4H), 6.27 (m, 1H, major), 6.12 (m, 1H, minor), 4.60 (m, 1H), 3.75 (s, 6H), 3.64−3.48 (m, 4H), 3.36−3.02 (m, 4H), 2.58 (m, 2H), 2.39 (m, 2H), 2.25−2.02 (m, 2H), 1.30−0.91 (m, 21H); ^{31}P NMR ($CDCl_3$) δ 149.17 (s), 149.08 (s), 149.02 (s).

Phosphoramidites consisting of a single stereoisomer at C5 were prepared as described above using single diastereomers of the dimethoxytritylated ketones **14**.

Preparation of 12a. The dianion of **9** (100 mg, 0.211 mmol) was prepared and acylated with benzaldehyde (28 mg, 0.26 mmol) using the procedure described for the preparation of **12b**. Flash chromatography (CH_2Cl_2/EtOAc, 9:1) afforded 85 mg (70%) of **12a** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.36 (m, 5H), 6.29 (dd, 1H, J = 6, 8 Hz, minor), 6.16 (dd, 1H, J = 5.5, 8.5 Hz, major), 5.00 (s, 1H, major), 4.94 (s, 1H, minor), 4.33 (m, 1H, minor), 4.13 (m, 1H, major), 3.64 (m, 1H), 3.25 (dd, 1H, J = 4.5, 10.5 Hz), 3.15 (d, 1H, J = 12 Hz), 3.05 (dd, 1H, J = 7, 10. 5 Hz), 2.72 (d, 1H, J = 12 Hz, minor), 2.64 (d, 1H, J = 12 Hz), 1.84 (m, 2H), 1.30 (s, 3H, major), 1.18 (s, 3H, minor), 0.89−0.86 (m, 18H), 0.08−0.02 (m, 12H); IR (thin film) 3216 (br d), 3064 (br d), 2953, 2929, 2885, 2857, 1704, 1472, 1387, 1361, 1252, 1222, 1091, 1057, 1029 cm^{-1}; HRMS (FAB) calcd 579.3286 (M + H), found 579.3264.

Preparation of 10a. Alcohol **12a** (85 mg, 0.14 mmol) was oxidized with Dess−Martin periodinane reagent (140 mg, 0.29 mmol) as previously described for the preparation of **13**. Flash chromatography (CH_2Cl_2/EtOAc; 9:1) afforded 79 mg (94%) of **10a** as an oil: ^{1}H NMR ($CDCl_3$) δ 8.88−7.79 (m, 2H), 7.60−7.58 (m, 1H), 7.55−7.37 (m, 2H), 6.25 (dd, 1 H, J = 8, 8 Hz), 4.37−4.34 (m, 1H, major), 4.31−4.27 (m, 1H, minor), 4.15 (d, 1H, J = 14.2 Hz, minor), 3.97 (d, 1H, J = 13 Hz, major), 3.76−3.60 (m, 3H), 3.28 (d, 1H, J = 13 Hz, major), 3.17 (d, 1H, J = 14.2 Hz, minor), 2.19−1.86 (m, 2H), 1.62 (s, 3H, major), 1.58 (s, 3H, minor), 0.87 (m, 18H), 0.07 (s, 3H), 0.06 (s, 3H), 0.05 (s, 3H), 0.04 (s, 3H); IR (thin film) 3200 (br d), 3140 (br d), 2954, 2929, 2857, 1709, 1687, 1472, 1383, 1360, 1252, 1095 cm^{-1}. Anal. Calcd for $C_{29}H_{48}N_2O_6Si_2$: C, 60.37; H, 8.40; N, 4.85. Found: C, 60.15; H, 8.18; N, 4.70.

Preparation of 11a. Ketone **10a** (91 mg, 0.16 mmol) was stirred in HOAc/H_2O (4:1 (v/v), 3 mL). After 24 h, the solvents were removed *in vacuo*. Flash chromatography (CH_2Cl_2/MeOH, 9:1) yielded **11a** (38 mg, 70%) as a hygroscopic solid: ^{1}H NMR (MeOH-d_4) δ 7.87 (m, 2H), 7.53 (m, 1H), 7.43 (m, 2H), 6.17 (m, 1H), 4.24 (m, 1H), 4.06 (d, 1H, J = 12 Hz), 3.73 (m, 1H), 3.66 (m, 1H), 3.56−3.38 (m, 2H), 2.17−1.81 (m, 2H), 1.62 (s, 3H, major), 1.56 (s, 3H, minor); IR (film) 3188, 3056, 2924, 1682, 1475, 1447, 1432, 1385, 1357, 1235, 1089, 1042 cm^{-1}; ^{13}C NMR (MeOH-d_4) δ 202.1 (minor), 201.6 (major), 176.3 (minor), 175.8 (major), 157.1 (major), 156.7 (minor), 139.6 (minor), 139.5 (major), 136.8 (major), 136.5 (minor), 132.4, 132.2 (major), 132.1 (minor), 90.0 (minor), 89.9 (major), 88.1 (minor), 87.5 (major), 75.2 (minor), 74.9 (major), 65.9, 58.5 (major), 58.4 (minor), 49.5 (minor), 49.2 (major), 40.1 (minor), 40.0 (major), 22.0 (major), 21.9 (minor); HRMS (FAB) calcd 349.1399 (M + H), found 349.1416.

Preparation of 23. A solution of ketone **11a** (20 mg, 57 μmol) in cyclohexa-1,4-diene (200 mM) in CH_3CN/H_2O (3:2; 4 mL) was freeze−pump−thaw−degassed three times and irradiated for 20 h in the Rayonet photoreactor. The solvents were removed *in vacuo*. Flash chromatography (MeOH/CH_2Cl_2; 3:97) yielded a mixture (11 mg, 65%) of four diastereomers of **23** as an oil: ^{1}H NMR (MeOH-d_4) δ 7.4−7.3 (m, 5H), 6.35−6.05 (m, 1H), 5.03 (m, 1H), 4.24 (m, 1H), 3.9−3.32 (m, 4H), 3.10−3.00 (m, 1H), 2.35−1.95 (m, 2H), 1.28 (s, 3H, major), 1.15 (s, 3H, minor), 1.03 (s, 3H); HRMS (FAB) calcd 351.1556 (M + H), found 351.1571.

Preparation of 10b. The dianion of **9** (500 mg, 1.05 mmol) was prepared as described above for the preparation of **12b**, and acylated with *p*-anisoyl chloride (223 mg, 1.31 mmol). After the reaction was worked up, as described for **12b**, flash chromatography (CH_2Cl_2/EtOAc, 9:1) afforded 421 mg (70%) of **10b**, as a 3:1 mixture of diastereomers: ^{1}H NMR ($CDCl_3$) δ 7.93 (d, 2H, J = 8.7 Hz, major), 7.86 (d, 2H, J = 8.7 Hz, minor), 7.48 (br d s, 1H, minor), 7.39 (br d s, 1H, major), 6.88 (d, 2H, J = 8.7 Hz, minor), 6.87 (d, 2H, J = 8.7 Hz, major), 6.25 (t, 1H, J = 6.2 Hz), 4.42−4.25 (m, 1H), 4.12 (d, 1H, J = 13.3 Hz, minor), 3.97 (d, 1H, J = 12.9 Hz, major), 3.84 (s, 3H), 3.80−3.55 (m, 3 H), 3.25 (d, 1H, J = 12.9 Hz, major), 3.16 (d, 1H, J = 13.3 Hz, minor), 2.35−1.85 (m, 2H), 1.60 (s, 3H, major), 1.57 (s, 3H, minor), 0.88 (s, 9H), 0.87 (s, 9H), 0.10−0.08 (m, 12H); IR (thin film) 3202, 3074, 2954, 2929, 2898, 2857, 1711, 1601, 1575, 1511, 1472, 1384, 1360, 1253, 1174, 1096, 1029, 971 cm^{-1}.

Prepartion of 11b. Ketone **10b** (100 mg, 0.165 mmol) was stirred in HOAc/H_2O (4:1 (v/v), 3 mL) for 24 h. The solvents were removed *in vacuo*. Flash chromatography (CH_2Cl_2/MeOH, 9:1) yielded **11b** (41 mg, 66%) as a hygroscopic solid: ^{1}H NMR ($CDCl_3$) δ 8.01−7.90 (m, 3H), 6.86 (d, 2H, J = 8 Hz), 6.18 (t, 1H, J = 7 Hz, major), 6.04 (t, 1H, J = 7 Hz, minor), 4.52 (m, 1H, minor), 4.42 (m, 1H, major), 4.18 (d, 1H, J = 14 Hz, minor), 4.03 (d, 1H, J = 14 Hz, major), 3.81 (s, 3H), 3.80−3.65 (m, 4H), 3.28 (d, 1H, J = 14 Hz, minor), 3.24 (d, 1H, J = 14 Hz, major), 2.54−2.12 (m, 3H), 1.58 (s, 3H, major), 1.56 (s, 3H, minor); ^{13}C NMR (MeOH-d_4) δ 197.0 (minor), 196.5 (major), 173.8 (minor), 173.7 (major), 165.2 (major), 165.1 (minor), 154.6 (major), 154.2 (minor), 132.8, 129.0, 114.9 (major), 114.8 (minor), 88.6 (minor), 88.3 (major), 87.5 (minor), 87.3 (major), 86.2 (minor), 85.8 (major), 85.6 (minor), 85.0 (major), 72.6 (minor), 72.3 (major), 63.3, 56.1 (major), 55.6 (minor), 48.4 (minor), 48.1 (major), 37.5, 19.7 (minor), 19.5 (major); IR (film) 3336, 2932, 2829, 1696, 1596, 1568, 1474, 1455, 1441, 1385, 1347, 1309, 1248, 1173, 1088, 1032 cm^{-1}.

Preparation of 19. 2′-Deoxyuridine was hydrogenated as described above for the preparation of **9**. The dihydronucleoside (500 mg, 2.14 mmol) was dried by repeated azeotroping from anhydrous pyridine (2 mL). TBSCl (825 mg, 5.47 mmol) was dissolved in pyridine (5 mL) and added to the nucleoside. The reaction was stirred for 48 h at 40 °C, quenched with MeOH (0.1 mL), and poured into H_2O (50 mL). The aqueous layers were extracted with ether (3 × 50 mL). The organic layers were combined, washed with brine (25 mL), and dried over $MgSO_4$. Flash chromatography (CH_2Cl_2/EtOAc, 12:1) yielded 875 mg (88%) of **19**: mp 81−85 °C; ^{1}H NMR ($CDCl_3$) 7.33 (br d, 1H), 6.27 (dd, 1H, J = 7, 7 Hz), 4.34 (m, 1H), 3.77 (m, 1H), 3.71−3.60 (m, 3H), 3.29 (m, 1H), 2.58 (m, 2H), 1.96 (dd, 2H, J = 4.6, 7 Hz), 0.88 (s, 9H), 0.87 (s, 9H), 0.06 (s, 6H), 0.05 (s, 6H); IR (film) 3215 (br d), 3086 (br d), 2954, 2928, 2888, 2857, 1713, 1472, 1462, 1337, 1283, 1254, 1218, 1192, 1103, 1032, 1005 cm^{-1}.

Preparation of 20. A cyclohexane solution of *sec*-BuLi (3.0 mL, 1.2 M) was added to **19** (654 mg, 1.43 mmol) and DMPU (549 mg, 4.29 mmol) in THF (5 mL) at −78 °C. After stirring for 1 h, the reaction was warmed to −20 °C and 2-iodopropane (364 mg, 1.56 mmol) was added. After 2 h at −20 °C, the reaction was quenched with saturated NH_4Cl (0.5 mL) and poured into H_2O (30 mL). The aqueous layers were extracted with ether (3 × 50 mL). The combined organic layers were washed with brine (25 mL) and dried over $MgSO_4$. Flash chromatography (CH_2Cl_2/EtOAc, 12:1) yielded 380 mg (55%) of **20**: mp 124−126 °C; ^{1}H NMR ($CDCl_3$) δ 7.29 (br d, 1H), 6.26 (dd, 1H, J = 6, 6 Hz), 4.32 (m, 1H) 3.80 (m, 1H), 3.77−3.54 (m, 3H), 3.17 (m, 1H), 2.40−2.11 (m, 2H), 1.93 (m, 2H), 1.02 (m, 6H), 0.87 (s, 9H), 0.85 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H), 0.04 (s, 3H), 0.03 (s, 3H); IR (film) 3120 (br d), 2970, 2923, 2891, 2852, 1713, 1694, 1682, 1473, 1454, 1371, 1253, 1096 cm^{-1}. Anal. Calcd for $C_{25}H_{50}N_2O_5Si_2$: C, 57.55; H, 9.68; N, 5.59. Found: C, 57.71; H, 9.74; N, 5.54.

Preparation of 21. A cyclohexane solution of *sec*-BuLi (1.9 mL, 1.2 M) was added to **20** (380 mg, 0.76 mmol) and DMPU (293 mg, 2.28 mmol) in THF (3 mL) at −78 °C. After stirring for 1 h, iodomethane (215 mg, 0.915 mmol) was added and the reaction was warmed to 25 °C. After 2 h at 25 °C, the reaction was quenched with saturated NH_4Cl (0.5 mL) and poured into H_2O (30 mL). The aqueous layers were extracted with ether (3 × 50 mL). The combined organic layers were washed with brine (25 mL) and dried over $MgSO_4$. Flash chromatography (CH_2Cl_2/EtOAc, 11:1) yielded 277 mg (71%) of **21** as an oil: ^{1}H NMR ($CDCl_3$) δ 7.31 (br d, 1H), 6.29 (m, 1H), 4.32 (m, 1H), 3.85−3.54 (m, 4H), 3.28−2.85 (m, 2H), 2.20−1.63 (m, 3H), 1.25−0.70 (m, 27H), 0.30−0.06 (m, 12H); IR (film) 3189 (br d), 2957, 2923, 2889, 2845, 1705, 1468, 1438, 1387, 1249, 1219, 1090, 1026 cm^{-1}. Anal. Calcd for $C_{26}H_{52}N_2O_5Si_2$: C, 58.31; H, 9.80; N, 5.44. Found: C, 58.24; H, 9.62; N, 5.29.

Preparation of 18. Protected dihydro derivative **21** (220 mg, 0.43 mmol) was stirred in HOAc/H_2O (4:1 (v/v), 3 mL) for 24 h, after which the solvents were removed *in vacuo*. Flash chromatography (CH_2Cl_2/MeOH, 9:1) yielded **18** (67 mg, 55%) as a hygroscopic solid: ^{1}H NMR (MeOH-d_4) δ 6.26 (dt, 1H, J = 9, 3 Hz, major), 6.21 (dt, 1H, J = 8, 3 Hz, minor), 4.29 (m, 1H, major), 4.01 (m, 1H, minor), 3.78 (m, 1H), 3.66-3.52 (m, 3H), 3.41 (d, 1H, J = 13 Hz, minor), 3.26 (d, 1H, J = 13 Hz, minor), 3.08 (d, 1H, J = 13 Hz, major), 2.20−1.88 (m, 3H), 1.10 (s, 3H, minor), 1.05 (s, 3H, major), 0.99−0.90 (m, 6H); ^{13}C NMR (MeOH-d_4) δ 177.5, 154.5, 87.5 (major), 87.4 (minor), 85.4 (minor), 85.2 (major), 72.9 (major), 72.6 (minor), 63.5 (major), 63.4 (minor), 49.9, 45.3, 45.0 (major), 44.6 (minor), 37.8 (minor), 37.4 (major), 31.5 (minor), 31.0 (major), 17.8 (minor), 17.6 (major), 17.4 (minor), 17.2 (major); IR (KBr) 3385 (br d), 2968, 2879, 1698, 1487, 1439, 1395, 1374, 1226, 1092, 1050 cm^{-1}; HRMS (FAB) calcd 287.1606 (M + H), found 287.1606.

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Supporting Information Available: Autoradiograms of enzymatic end group analysis of **31**, a sample KIE experiment, the results of piperidine treatment of cleavage products from [3′-^{32}P]**29** at T_{11}, the X-ray crystal structure data of **7**, and data analysis of phosphorimager results (17 pages). See any current masthead page for ordering and Internet access instructions.

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