Independent Generation of 5,6-Dihydrothymid-5-yl and Investigation of Its Ability To Effect Nucleic Acid Strand Scission via Hydrogen Atom Abstraction

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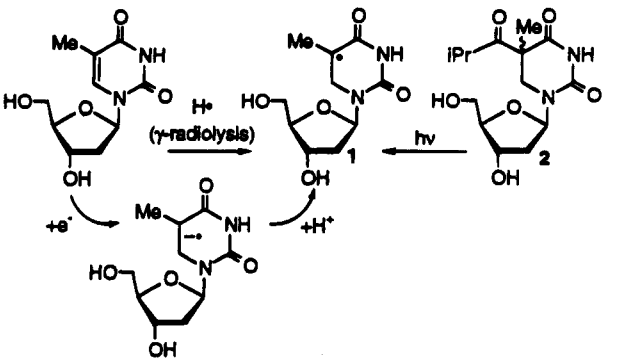
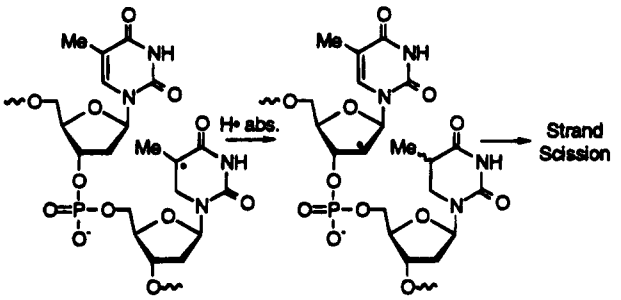
Nucleobase radicals account for the majority of reactive intermediates produced via the interaction of nucleic acids with ionizing radiation.[1] Oxidation of nucleic acids by the radiomimetic systems Fe·EDTA and copper phenanthroline also results in the formation of mutated nucleosides that are attributable to nucleobase radical intermediates.[2] 5,6-Dihydrothymid-5-yl (**1**) is the major reactive intermediate produced upon reaction of thymidine and hydrogen atoms (H•).[1] Reactive intermediate **1** is also postulated to be formed within the chromatin via the direct effect of ionizing radiation (Scheme 1).[3] While the relative importance of the indirect and direct effects of ionizing radiation on nucleic acid strand scission within cells is a point of contention, the formation of **1** via both pathways is well accepted.[3,4] The role of **1** in nucleic acid strand scission is also an active subject of debate. We report the first unambiguous generation of **1** from a designed precursor and the results of experiments involving the reactivity of **1** in solution which shed light on its participation in nucleic acid cleavage processes.

The formation of 5,6-dihydrothymid-5-yl (**1**) via ionizing radiation has been directly established via EPR spectroscopy.[5] When generated within a biopolymer (Scheme 2), this tertiary, resonance-stabilized radical (**1**) is believed to induce strand scission via hydrogen atom abstraction from C2′ and/or C1′ and C4′ of the adjacent (deoxy)ribonucleotide.[4] Consideration of thermodynamics leads one to predict that transfer of a hydrogen atom from an adjacent nucleotide (particularly from C2′) to sterically hindered and resonance-stabilized **1** will be an unfavorable process. However, one could argue that the effective concentration of the reactive centers within a biopolymer enables **1** to overcome the anticipated unfavorable thermodynamics. This proposal has been questioned by others, who suggest that **1** is incompetent at inducing nucleic acid strand scission via direct hydrogen atom abstraction, despite the juxtaposition of **1** and the sugar moiety.[4a] Independent generation of reactive intermediates is a common approach to probing the plausibility of reaction mechanisms involving such species. Recently, we and others have investigated mechanistic issues involving nucleic acid oxidation by independently generating putative reactive intermediates involved in these processes.[6]

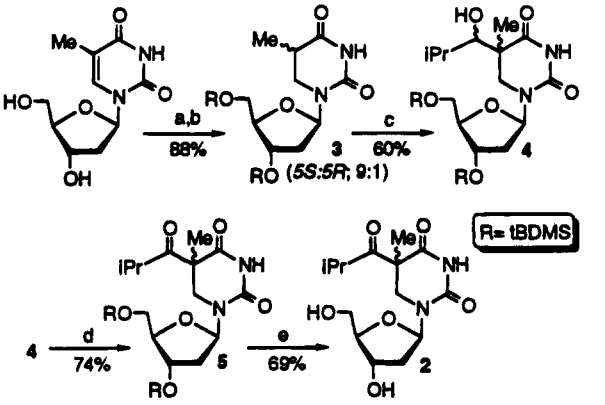
Scheme 1



Scheme 2



Scheme 3



[a] Key: (a) H_2, Rh/Al_2O_3, $MeOH/H_2O$ (1:1); (b) tBDMSiCl, pyridine; (c) s-BuLi (2.5 equiv), THF then isobutyraldehyde (1.5 equiv); (d) Dess–Martin periodinane (1.5 equiv), CH_2Cl_2, 0–25 °C; (e) NH_4F, MeOH.

In designing a stable precursor to 5,6-dihydrothymid-5-yl (**1**), we sought a molecule that enables us to generate it in H_2O via direct photolysis, under aerobic conditions. Consequently, we designed dihydrothymidine derivatives such as **2** to generate **1** via Norrish Type I photocleavage. A variety of alkyl- and aryl-substituted ketones (as a mixture of epimers at C5) were readily obtained starting from the bis-tBDMS ether of 5,6-dihydrothymidine (**3**). The isopropyl analogue was found to display the best combination of thermal stability and photoreactivity.

Irradiation of **2** (10 mM) under anaerobic conditions in a mixture of CH_3CN and D_2O (3:2 by volume) produced

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(5) Cullis, P. M.; McClymont, J. D.; Malone, M. E.; Mather, A. N.; Podmore, I. D.; Sweeney, M. C.; Symons, M. C. R. *J. Chem. Soc., Perkin Trans. 2* **1992**, 1695.

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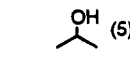
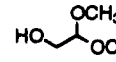
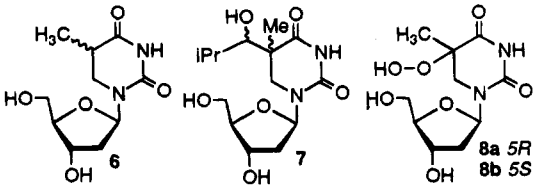
Table 1. Yield of 5,6-Dihydrothymidine (6) Obtained via Photolysis of 2 under Anaerobic Conditions

[Trap] (M)	[2] (mM)	% Yield 6	^{1}H-6:^{2}H-6
None	10[c]	9.8 ± 2.6[a]	1.4
	10[d]	5.3 ± 0.6[b]	1.9
	0.1[c]	5.6 ± 2.8[a]	1.6
 (5)[c]	10	15.5 ± 5.8[a]	1.0
	0.1	8.0 ± 4.2[a]	1.1
 (5)[c]	0.1	8.6[a]	0.5
 (0.2)[d]	10	85.0 ± 2.8[b]	>25
	0.1	82.6 ± 3.2[b]	>25

[a] Measured by GC/MS. [b] Measured by HPLC. [c] Solvent: CH_3CN:D_2O 5:95. [d] Solvent: CH_3CN:D_2O 3:2.

5,6-dihydrothymidine (**6**) in less than 10% yield (Table 1).[7] GC/MS analysis of the persilylated crude photolysate revealed the 5,6-dihydrothymidine (**6**) formed in this reaction consisted of an approximately 1.9:1 mixture of protio and deuterio material. Under these conditions, we suggest that [^{2}H]-**6** is derived from recombination of the photolytically produced radical pair on oxygen, followed by hydrolysis of the *O*-alkyl imidate, rather than photoreduction and subsequent retrocondensation.[10] [^{1}H]-**6** is believed to result from disproportionation of the radical pair, as opposed to trapping by CH_3CN. Photolysis in the presence of 1,4-cyclohexadiene (0.2 M) produced 5,6-dihydrothymidine (as a 1:1 mixture of *5R* and *5S* diastereomers) in >82% yield. Mass spectral analysis indicated a >25:1 ratio of [^{1}H]-**6**:[^{2}H]-**6**. These observations indicate that >95% of the 5,6-dihydrothymidine produced in the photoreaction is attributable to the trapping of **1** by the exogenous hydrogen atom donor.

The ratio of [^{1}H]-**6**:[^{2}H]-**6** formed is unchanged when **2** is irradiated under aerobic conditions in the presence of 1,4-cyclohexadiene (200 mM). However, under these conditions the yield of **6** decreases to 58%. Examination of the HPLC chromatogram revealed the presence of two new peaks which elute earlier than **6**. Isolation and characterization by ^{1}H NMR, MS, and hydroperoxide reactivity tests revealed that these materials were the respective hydroperoxides (**8a,b**).[11,12] The hydroperoxides



are assumed to arise via trapping of **1** with O_2, followed by hydrogen atom transfer. The stereochemistry of **8a** and **8b** is based upon their relative HPLC retention times, compared to those of (5*S*)- and (5*R*)-thymidine C5-hydrate.[9,10] The ratios of **6**:**8** obtained from these photoreactions are not indicative of the respective rate constants that describe their formation, because [O_2] is limiting. When **2** is irradiated under conditions in which O_2 is present in excess (but not pseudo first order), the yield of **6** drops to the background value observed in the absence of any trap (<10%), and that of **8** is 35%. This latter observation is not surprising, based upon the known rate constants for the reaction of **1** with O_2 and alkyl radicals with 1,4-cyclohexadiene.[11]

In order to more accurately gauge the ability of **1** to effect nucleic acid strand scission via direct hydrogen atom abstraction, trapping of **1** by 2-propanol and glycoaldehyde dimethyl acetal was examined. These substrates were chosen instead of D-ribose as models for the C1′ and C2′ centers of ribose in RNA because of the latter's limited solubility. Anaerobic irradiation of **2** (0.1 mM) in the presence of 2-propanol (5 M) produces **6** in approximately the same yield and proportion of deuterium, as in experiments carried out in the absence of any potential trap (Table 1). A slightly higher yield of **6** is detected when **2** is irradiated under higher concentration conditions (10 mM), but its isotopic content is not indicative of a radical mechanism being responsible for the increased amount of **6** produced. Similar observations (Table 1) regarding **6** are obtained from the photolysis of **2** in the presence of glycoaldehyde dimethyl acetal (5 M), which was employed as a mimic of the C1′ hydrogen of a (deoxy)ribonucleoside. These results suggest that neither model of the carbohydrate moiety in nucleic acids donates hydrogen atoms to **1**.

One could argue that neither 2-propanol nor glycoaldehyde dimethyl acetal is a suitable model for hydrogen atom abstraction from the D-ribose moiety because of the absence of intramolecular hydrogen bonding in these substrates. However, the hydrogen atom donors employed here should certainly be more reactive than the C2′ hydrogens of a deoxyribose ring. Assuming that the effective molarity of an (deoxy)ribose adjacent to **1** in a nucleic acid is ≤5 M, the above results suggest that the formation of nucleic acid (DNA) strand breaks resulting from hydrogen atom abstraction by **1** will not compete with other processes, such as peroxyl radical formation. This issue could be unambiguously resolved by independently generating **1** site specifically in an oligonucleotide, and examining the formation of strand breaks as a function of O_2 concentration. Efforts directed toward providing this important information are in progress.

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Supplementary Material Available: Experimental procedures for the synthesis of **2**, **7**, and **8** as well as the photolysis of **2** (4 pages).

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(7) See supplementary material.
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