# Independent Generation of the Major Adduct of Hydroxyl Radical and Thymidine. Examination of Intramolecular Hydrogen Atom Transfer in Competition With Thiol Trapping.
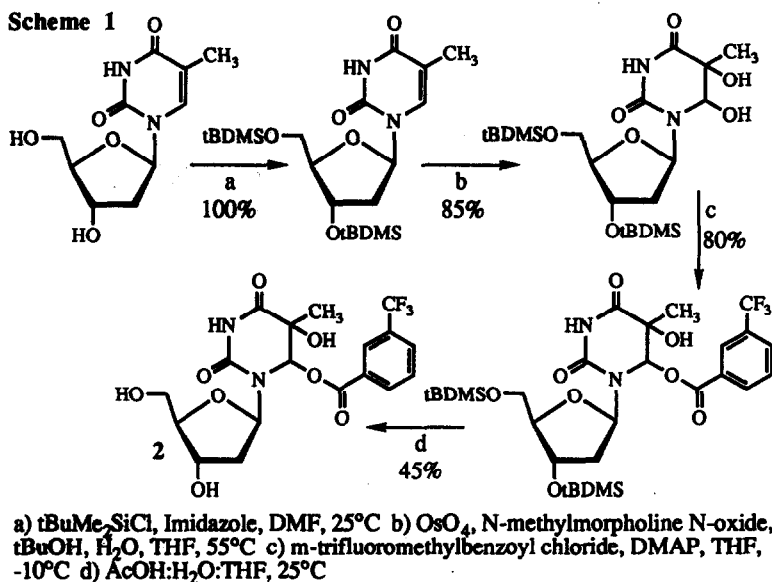
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Abstract: 5,6-Dihydro-5-hydroxythymid-6-yl (**1**) has been generated from **2** via photoinduced electron transfer from N-methylcarbazole. In agreement with prior reports, deuterium incorporation in conjunction with ^{2}H NMR analysis of 5,6-dihydro-5-hydroxythymidine (**3**) formed from **1** provides no evidence for intramolecular hydrogen atom abstraction.

Hydroxyl radical is produced during γ-radiolysis via the ionization of water.[1] It may also be generated by a number of Fenton like processes and during the photorearrangement of phthalimide hydroperoxides.[2,3] Studies involving pyrimidine nucleosides, nucleotides and polynucleotides suggest that their predominant reaction with OH• involves addition to the pyrimidine double bond at C5 of the nucleobase.[1,4] Under anoxic conditions hydrogen atom abstraction from the sugar backbone by the nucleobase radical is necessary in order for these adducts to induce strand scission. However, time resolved EPR experiments and product studies involving γ-irradiation of thymidine under anoxic conditions suggest that intramolecular hydrogen atom abstraction by the nucleobase radicals is inefficient, and that strand scission results from hydrogen atom abstraction from a neighboring deoxyribose moiety.[5] In agreement with these observations, we report indirect evidence which indicates that intramolecular hydrogen atom abstraction by (5R)-5,6-dihydro-5-hydroxythymid-6-yl (**1**) does not compete with intermolecular quenching by t-butylthiol.
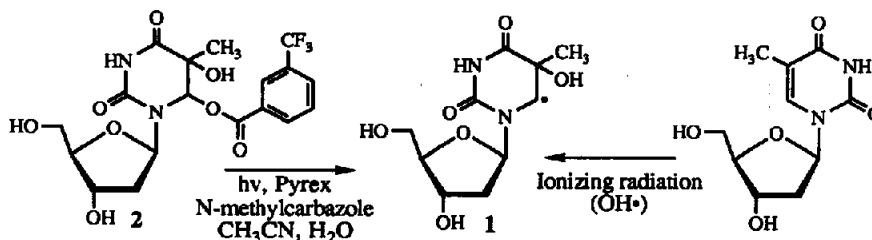
We have approached this problem by independently generating **1** from an appropriate photochemical precursor, thereby assuring that the chemistry of a single reactive intermediate is under investigation. Photosubstrate **2** is prepared in gram quantities from thymidine (Scheme 1), and is designed to utilize established photochemistry for generating alkyl radicals from m-trifluoromethylbenzoates via photoinduced electron transfer.[6,7] To our knowledge, this is the first independent generation of 5,6-dihydro-5-hydroxythymidin-6-yl (**1**).

Scheme 1

a) tBuMe$_2$SiCl, Imidazole, DMF, 25°C b) OsO$_4$, N-methylmorpholine N-oxide, tBuOH, H$_2$O, THF, 55°C c) m-trifluoromethylbenzoyl chloride, DMAP, THF, -10°C d) AcOH:H$_2$O:THF, 25°C

Photolysis of **2** in the presence of equimolar N-methylcarbazole and various types and concentrations of hydrogen atom donors leads to the formation of **3** and **4** as the sole tractable products in high yield. Stereochemical assignments were made based upon comparisons to genuine materials.[8] Mass balances obtained by HPLC using internal standards were in excess of 90%. Negligible consumption of **2** occurs after several hours of photolysis in the absence of N-methylcarbazole. The ratio of **3** to **4** is dependent upon the nature and concentration of hydrogen atom donor.[9] When **2** was photolyzed in H$_2^{18}$O (98% enriched), mass spectral analysis of pertrimethylsilylated glycol (**4**) formed indicated greater than >96.5% ^{18}O incorporation. Control experiments with independently prepared **4** revealed that ^{18}O enrichment did not result from washing in via equilibration with the free aldehyde. These observations support the formation of **4** via oxidation of **1** rather than acyl bond cleavage in the radical anion of **2**.[6b]
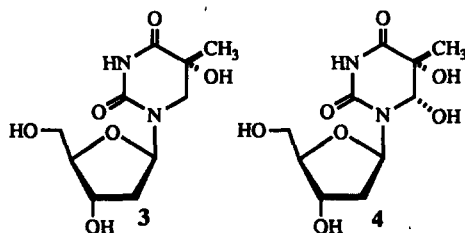
In principle, intramolecular hydrogen atom abstraction within **1** could be detected via analysis of the sugar fragmentation products. However, the variety of hydrogen atoms and subsequent accessible reaction pathways available encouraged us to seek a more expedient, albeit indirect approach to this problem. We rationalized that analysis by ^{2}H NMR of **3** formed during photolysis of **2** in the presence of a deuterium atom donor whose rate

constants for reaction with alkyl radicals (k_{inter}) are known would enable us to estimate the rate constant for intramolecular hydrogen atom transfer (k_{intra}). Intramolecular hydrogen atom abstraction within **1** would be evident in such an experiment by the appearance of deuterium in the deoxyribose moiety.

Thymidine C5-hydrate (**3**) and glycol (**4**) were isolated in a 4:1 ratio via reverse phase HPLC (80% isolated yield) following anaerobic photolysis of **2** (5mM) in the presence of N-methylcarbazole (5 mM) and t-butylthiol (15 mM) in CH_3CN/D_2O (65:35 v:v). GC/MS analysis of persilylated **3** and **4** indicated at least 86%
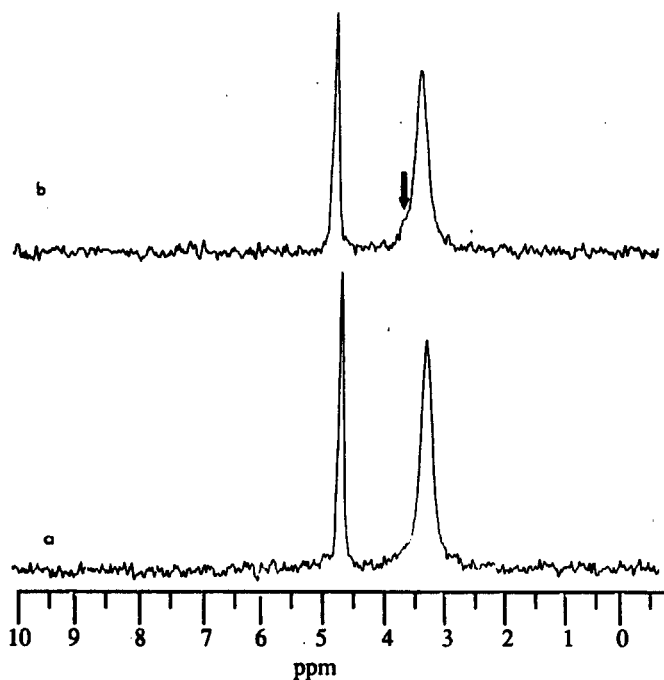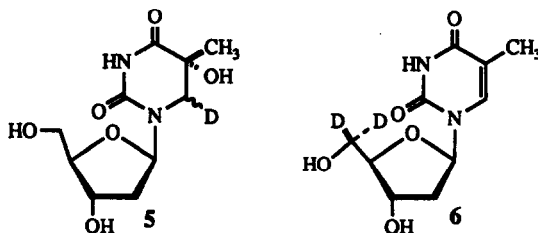

Figure 1. ^2H NMR analysis of **3** isolated from photolysis of **1**. a) **3** in H_2O b) **3** spiked with (2.5% by moles, 5% by atom) of 5',5''-dideuterothymidine.

deuterium incorporation in **3** and none in the latter.[10] ^2H NMR of **3** (38.4 mM) in H_2O (Figure 1) revealed the presence of only the diastereotopic C6 deuterons of (6R,S)-6-^2H-3 (**5**), which were not resolvable at 45 MHz.

Detection limits were established by standard addition of an aqueous solution of 5',5"-dideuterothymidine (6, 95% dideutero), which indicated 5% of any single positional isomer of monodeuterated 3 could have been detected. 5',5"-Dideuterothymidine (6) was purposely chosen, because the chemical shift of the 5' deuterons were expected to overlap with those of C6 in 3, and would therefore represent a worst case scenario for detection of other deuterated isomers of 3. We believe that the 5% detection limit is probably a conservative estimate, and it is likely that we would have been able to observe a lesser amount of 3 deuterated at C3' or C2' since the chemical shifts of these positions would not be partially occluded by those of the C6 deuterons.

$$0.05 = \frac{k_{intra}}{k_{inter}[\text{t-BuSD}]} \qquad (1)$$

An upper limit for the rate constant of intramolecular hydrogen atom transfer within 1 can be assumed if three assumptions are made (equation 1). (1) A lower limit of $k_{inter}= 1 \times 10^6 \, M^{-1}s^{-1}$ is placed on the rate constant for reaction of t-BuSD with 1.[11] This value attempts to take into account any kinetic isotope effect, and the effect of the stability of 1 relative to simple alkyl radicals on the rate of reaction.[12] (2) Quenching of any subsequently formed radical by the trap is fast with respect to further reaction. (3) Five percent of any one intramolecular process can be detected in the NMR experiment. These assumptions place an upper limit for any single intramolecular hydrogen atom abstraction event in 1 to be $k_{intra}= 7.5 \times 10^2 \, s^{-1}$.

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