# *Communications*

Independent Generation and Reactivity of 2′-Deoxyurid-1′-yl

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The anomeric hydrogen of nucleotides is abstracted by a variety of nucleic acid damaging agents.[1−5] Upon quenching by thiol, the C1′ radical gives rise to the premutagenic α-anomer of the respective nucleoside *in vitro* (Scheme 1).[5b] However, little is known about the level of α-nucleoside formation in cellular DNA. When trapped by O_2, the C1′ lesion is "fixed" and ultimately results in the formation of either a direct strand break, or an alkaline labile lesion. However, the ultimate fate of the C1′ radical is highly dependent upon the environment in which it is generated. For example, generation of the C1′ radical by the neocarzinostatin chromophore (NCS) leads to the formation of the alkaline labile 2′-deoxyribonolactone lesion.[1] In contrast, oxidation of nucleic acids by the bis-phenanthroline complex of Cu $(Cu\bullet(OP)_2)$ produces direct strand breaks.[2] Although the source of this bifurcation in reactivity is uncertain, 2′-deoxyribonolactone formation is postulated to involve reduction of the intermediate peroxyl radical by the thiol present during activation of NCS.[1a] It is interesting to note that thiol is also present during the reaction between $Cu(OP)_2$ and DNA, in which direct strand breaks are produced.[2] As part of an effort aimed at elucidating the role of individual radicals in nucleic acid damage, we wish to report the first independent generation of 2′-deoxyurid-1′-yl (**1**, eq 1).
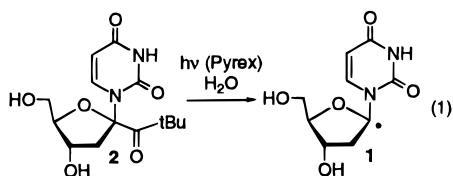
The choice of photosubstrate (**2**) was based upon recent successes in the utilization of the Norrish type I photoprocess for the independent generation of other nucleoside-based reactive intermediates.[6] The requisite ketone (**2**) was synthesized from fructose via the previously reported 1-[3-deoxy-β-D-psicofuranosyl]uracil (**3**, Scheme 2).[7] Formation of the disiloxane proceeded with poor selectivity (26%) due to the similar reactivity of the two

Scheme 1

Scheme 2[a]
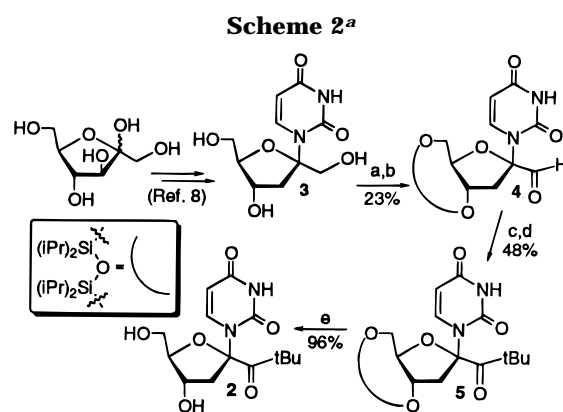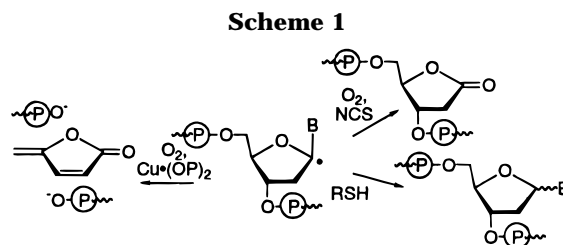
[a] Key: (a) 1,3-dichlorotetraisopropyldisiloxane, pyridine; (b) Sern; (c) t-BuLi, THF; (d) Dess−Martin; (e) NH_4F, MeOH.

primary alcohols in **3**.[8] However, 47% of **3** was recovered via deprotection of the undesired silylated products. Swern oxidation proceeded extremely cleanly, and in practice the crude aldehyde (**4**) was subjected to nucleophilic addition without any purification. The diastereomeric mixture of secondary alcohols obtained from the addition of *t*-BuLi was unstable and was quickly purified and oxidized to the ketone.[9] The respective phenyl and isopropyl ketones were also prepared via this strategy, but **2** showed the best combination of synthetic accessibility, stability, and photochemical properties.[10]

The quantum yield for the disappearance of **2** exhibits Stern−Volmer behavior in the presence of *trans*-piperylene, suggesting that **2** reacts through its triplet excited state (Figure 1). Assuming $k_q \leq 1 \times 10^{10}$ M^{-1} s^{-1}, the n,π* state of **2** has a lifetime of ≥ 2 ns.[11] Anaerobic photolysis of **2** (10 mM) in the presence of β-mercaptoethanol (100 mM) produces α,β-2′-deoxyuridine in a 2:3 ratio as the sole products in 77% yield. The same ratio of epimers is observed (58% yield) when **2** is irradiated in the presence of cyclohexa-1,4-diene.[12] No deuterium is incorporated in either anomer of the 2′-deoxyuridine that is formed in the presence of cyclohexa-1,4-diene when the photolysis is carried out in CH_3CN/D_2O (3:2; v:v). Furthermore, no evidence of photoreduction of **2** is observed. Small amounts of α,β-2′-deoxyuridine (16.2%), and an approximately equal amount of uracil

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(9) The desilylated alcohol releases uracil upon standing in H_2O. This is attributed to a cationic rearrangement.

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(12) Photolyses in the presence of cyclohexa-1,4-diene were carried out in a mixture of acetonitrile and water (3:2 by volume).
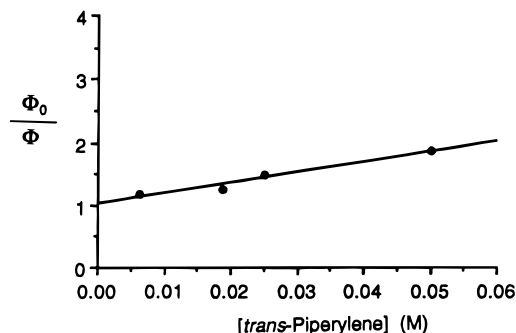
Figure 1. Stern-Volmer plot of the quenching of the disappearance of **2** by *trans*-piperylene.

Figure 2. Plot of the ratio of [dU]/[Uracil] formed versus [β-mercaptoethanol]/[O$_2$].

(14.0%), are also formed under anaerobic conditions in the absence of trap. We attribute the formation of α,β-2′-deoxyuridine and uracil in the absence of trap to disproportionation of the *tert*-butyl and 2′-deoxyurid-1′-yl radicals, as opposed to hydrogen atom abstraction from the CH$_3$CN. Support for this proposal is gleaned from the observation that no deuterium is incorporated in the α,β-2′-deoxyuridine when CD$_3$CN is used as cosolvent. The uracil presumably arises via hydrolysis of the 1′,2′-dehydrodeoxyuridine, which could be formed via disproportionation.[13] An amount of 2′-deoxyribonolactone commensurate with the observed uracil is also observed, consistent with this proposal.[14]

Aerobic photolysis of **2** in the absence of exogenous hydrogen atom donors results in the formation of equal amounts of uracil and 2′-deoxyribonolactone. When O$_2$ is present as a limiting reagent, 2′-deoxyuridine is still observed, presumably through radical pair chemistry. However, under photolysis conditions where O$_2$ is not limiting, uracil and 2′-deoxyribonolactone are the only products observed from **2**.

The results of the photolysis of **2** described above are fully consistent with the formation of **1**. In order to address the feasibility of the formation of α-nucleosides in cellular DNA, the competition between O$_2$ and β-mecrcaptoethanol for **1** was examined under conditions where O$_2$ is not limiting (Figure 2). The ratio of anomers of 2′-deoxyuridine formed was independent of thiol concentration. Large concentrations of thiol were needed in order to compete with O$_2$ for **1**. This competition is important because the formation of α-nucleosides, such as α-2′-deoxyadenosine, has been investigated only *in*
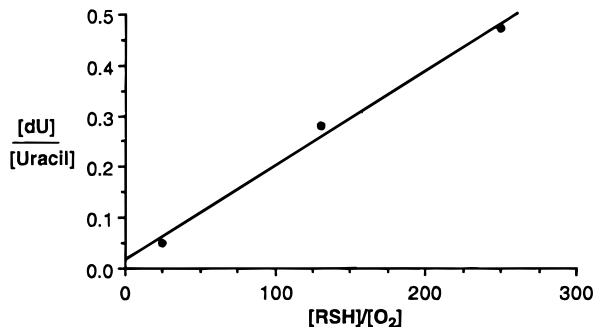
vitro under anaerobic conditions.[15] Hence, determining the relative rate constants for trapping of **1** by a thiol versus O$_2$ would be useful for estimating the likelihood that α-nucleotides would be formed in nucleic acids *in vivo*. Assuming that the rate constant for trapping of **1** by O$_2$ is 2×10^9 M^{-1} s^{-1} and [O$_2$] $= 2 \times 10^{-4}$ M, the results shown in Figure 2 indicate that **1** is trapped by β-mecrcaptoethanol with a bimolecular rate constant (k_{RSH}) equal to 3.7×10^6 M^{-1}s^{-1} (eq 2).[16] The rate constant

$$\frac{[dU]}{[uracil]} = \frac{k_{RSH}[RSH]}{k_{O_2}[O_2]} \qquad (2)$$

determined by us indicates that **1** is trapped by the thiol at rates comparable to other alkyl radicals.[17] The concentration of thiol *in vivo* is less than 10 mM and [O$_2$] $\leq$ 0.2 mM.[5a] Therefore, if the anomeric radicals are trapped in nucleic acids with similar stereoselectivity as observed in solution, then α-nucleotides will account for approximately 4% of the C1′ nucleotide radicals formed in nucleic acids *in vivo*.[5a] These results suggest that the generation of the C1′ radical is of consequence *in vivo*. Further investigations regarding the role that the radicals resulting from abstraction of the C1′ hydrogen of nucleotides (i.e., **1**) in nucleic acids play in strand damage are underway.

Supporting Information Available: Experimental procedure and characterization data (3 pages).

JO951769A

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(14) Uracil and 2′-deoxyuridine were quantitated by reversed phase HPLC. 2′-Deoxyribonolactone was quantitated as its bis(trimethylsilyl) ether via GC/MS using selected ion monitoring. Response factors versus appropriate internal standards were measured for each product.

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