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Generation of Thiyl Radicals by the Photolysis of 5-Iodo-4-thiouridine

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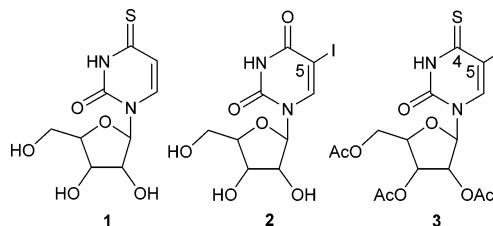
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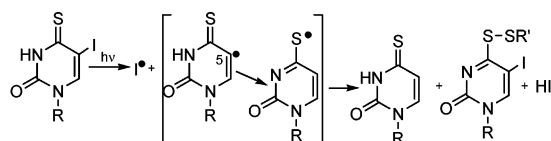
R=tri-O-acetylribosyl; R'=1-(tri-O-acetylribosyl)-pyrimidin-2-one

or 1-(tri-O-acetylribosyl)-5-iodo-pyrimidin-2-one

The photochemistry of 2′,3′,5′-tri-*O*-acetyl-5-iodo-4-thiouridine (**3**) in deoxygenated 1:1 CH₃CN−H₂O pH 5.8 (phosphate buffer) solution has been studied by means of steady-state and nanosecond laser flash photolysis methods. Under steady-state irradiation ($\lambda \geq 334$ nm), the stable photoproducts were iodide ion, 2′,3′,5′-tri-*O*-acetyl-4-thiouridine (**4**), and two disulfides. The disulfides were the symmetrical bis-(2′,3′,5′-tri-*O*-acetyl-5-iodo-4-thiouridine) (**5**) and unsymmetrical **6**, which contains both 4-thiouridine and 5-iodo-4-thiouridine residues. The formation of the dehalogenated photoproduct suggests that C(5)−I bond cleavage is a primary photochemical step. Attempts to scavenge the resulting C(5)-centered radical by suitable addends, bis-(*N*-α-acetyl)cystine-bis-*N*-ethylamide or benzene, were unsuccessful. Analysis of the photoproducts formed under these conditions showed that the S-atom is the reactive center. The photoproduct **4**, obtained by irradiation of **3** in CD₃-CN−H₂O, followed by reversed-phase HPLC isolation using nonlabeled eluents, did not contain deuterium. An analogous experiment performed in CH₃CN−D₂O gave deuterated product **4**-*d* with 88% of the deuterium incorporated at C(5). Transient absorption observed upon laser excitation (λ = 308 nm) of **3** was assigned to the 4-uridinylthiyl radical on the basis of the similarity of this spectrum with that obtained upon laser photolysis of the disulfide: bis-(2′,3′,5′-tri-*O*-acetyl-4-thiouridine) **14**. On the basis of the results of steady-state and laser photolysis studies, a mechanism of the photochemical reaction of **3** is proposed. The key mechanistic step is a transformation of the C(5)-centered radical formed initially by C(5)−I bond cleavage into a long-lived S-centered radical via a 1,3-hydrogen shift. Theoretical calculations confirmed that the long-lived S-centered radical is the most stable radical derived from the 4-thiouracil residue.

Introduction

Photochemical reactions of 4-thiouridine (**1**, Chart 1)[1,2] and 5-iodouridine (**2**)[3,4] have been extensively studied by

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(1) Favre, A. In *Bioorganic Photochemistry*; Morrison, H., Ed.; Wiley: New York, 1990; Vol. 1, pp 379−425.
(2) Favre, A.; Saintome, C.; Fourrey, J.-L.; Clivio, P.; Laugaa, P. *J. Photochem. Photobiol. B: Biol.* **1998**, *42*, 109−124.
(3) Norris, C. L.; Meisenheimer, P. L.; Koch, T. H. *J. Am. Chem. Soc.* **1996**, *118*, 5796−5803.
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CHART 1

steady-state photolysis methods. These compounds are of interest because they have wide application in various studies of biological systems. In particular, both are used

as photo-cross-linking agents in nucleic acids or in nucleic acid−protein complexes.[5,6]

The main photoreactions of **1** are photocycloaddition (forming thiethane) and H-abstraction. The results of photochemical studies of the nucleoside **2**,[3,4] 5-iodouracil,[7−12] and other derivatives of pyrimidine[13,14] indicate that the primary photochemical event, following electronic excitation of the chromophore, is C−I bond homolysis leading to the 5-uracilyl radical.[3,13,14] Subsequent reactions of this radical and eventually the structures of isolated, stable photoproducts depend on the presence of various reactive agents such as H atom donors, radical scavengers, and olefinic or aromatic compounds present in the irradiated systems. In the absence of reactive addends in deoxygenated aqueous or acetonitrile−water solutions, the major product obtained from **2** or 5-iodouracil was uridine or uracil, respectively.[3,4,12] Irradiation of 2′,3′-*O*-isopropylidene-5-iodouridine[15] or 1,3-dimethyl-5-iodouracil[16] in the presence of benzene, pyrene, or phenantrene gave uracil derivatives with an appropriate aromatic substituent at the C-5 position of the uracil ring in addition to the dehalogenated pyrimidine or its nucleoside. The (C-5)-uridinyl radical is trapped by bis(2-amino-1-thioethane) to give 5-(2-aminoethylthio)-uracil[9,11,17] upon irradiation of 5-iodouracil in the presence of the disulfide.

Although several nucleic acid−protein photo-cross-linking agents have been developed,[1,5] there is still a need for new, long-wavelength-absorbing, highly reactive, and selective probes. Recently, we have started systematic studies of the photophysical and photochemical properties of uridine derivatives that contain both C=S and C=C−X fragments, where X = halogen. In the series of 4-thiouridine derivatives with 5-fluoro-, 5-chloro-, and 5-bromo-substituents, it was found that the presence of the C=S function in the molecules dominated their photochemistry in aqueous solution. These compounds give photoproducts resulting from cycloaddition of the C=S group of the excited molecule to an ethylenic fragment of the substrate in its ground state.[18] The results of preliminary photochemical studies of the remaining member of the family, i.e., 5-iodo-substituted derivative, in CCl$_4$ solution indicated that the nucleoside behaves differently and undergoes dehalogenation.[19,20] This finding prompted us to
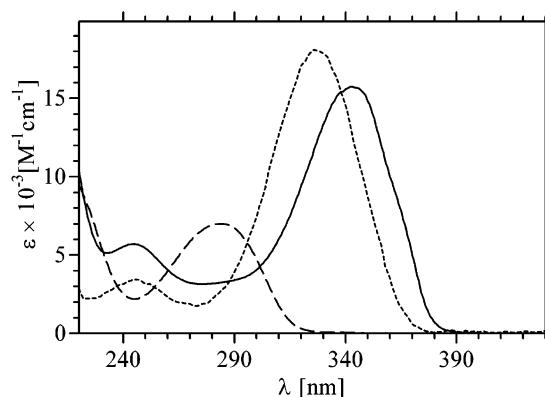

FIGURE 1. UV absorption spectra of **1** (dotted line), **2** (broken line), and **3** (solid line) in a 1:1 mixture of CH$_3$CN−H$_2$O pH 5.8 (phosphate buffer).

study the photochemistry of this latter compound in more detail. In this paper, we report the results of the steady state and laser flash photolysis of 2′,3′,5′-tri-*O*-acetyl-5-iodo-4-thiouridine (**3**) in deoxygenated 1:1 CH$_3$CN−H$_2$O pH 5.8 (phosphate buffer) solution. In the nucleoside **3**, the sugar hydroxyl groups are converted into *O*-acetyl groups to improve the solubility of the compound.

Results and Discussion

Steady-State Photochemistry. The electronic absorption spectra of nucleosides **1**−**3** in a 1:1 mixture of CH$_3$CN−H$_2$O pH 5.8 (phosphate buffer) are presented in Figure 1. Among these uridine derivatives, the nucleoside **3** absorbs at the longest wavelengths. The shape of the absorption bands of **3** resembles that of **1**. The similarity of the spectra suggests that **3**, like **1**, exists in solution in the thione tautomeric form. The absorption band with its maximum at $\lambda_{max} = 345$ nm is due to the strong $\pi \rightarrow \pi^*$ (S$_0 \rightarrow$ S$_2$) and weak $n \rightarrow \pi^*$ (S$_0 \rightarrow$ S$_1$) electronic transitions in molecule **3**.[19]

In aqueous media at pH ≈ 6, nucleoside **3** exists as a neutral molecule. The pK_a value for the neutral molecule−anion equilibrium was determined to be p$K_a = 7.7 \pm 0.1$ (see Supporting Information) by spectrophotometric titration. The nucleoside **3** is a stronger acid than **1** (pK_a = 8.2),[1] which does not contain an iodo substituent, and **2** (deoxynucleoside, p$K_a = 8.34$),[21] an oxygen analogue of the thione **3**.

Irradiation ($\lambda > 334$ nm) of a deoxygenated solution of **3** in the concentration range [**3**] = 0.1−1 mM in a 1:1 mixture of CH$_3$CN−H$_2$O pH 5.8 (phosphate buffer) leads to three organic photoproducts, **4**−**6**, and iodide ions; see Scheme 1. The structures of the photoproducts were established by the spectral data and confirmed by independent synthesis. Disulfides **5** and **6** are new compounds. They are quite stable (several days) as solids at 5 °C. However, the unsymmetrical disulfide **6**, in contrast to symmetrical compound **5**, is unstable in

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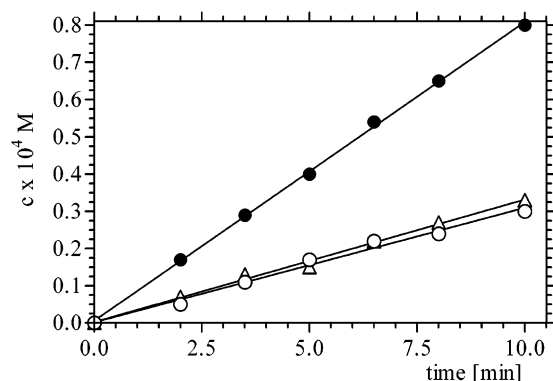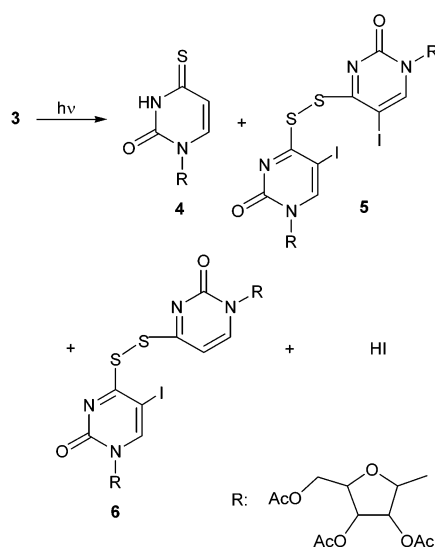
FIGURE 2. Production of **4** (full circles), disulfides **5** (open circles), and **6** (triangles) during the irradiation $\lambda \geq 334$ nm of **3** (0.5 mM) in a 1:1 mixture CH$_3$CN–H$_2$O pH 5.8 (phosphate buffer) in the absence of oxygen.

SCHEME 1

solution even at room temperature as indicated by NMR spectroscopy. Therefore, its complete spectral characterization (^{13}C NMR spectrum) could not be performed. Iodide anions, in the irradiated solutions, were detected by HPLC. In addition, they were identified on the basis of their absorption spectrum measured with an HPLC-PDD detector (I$^-$: $\lambda_{max} = 226$ nm, $\epsilon_{max} = 13\,500$ M^{-1} cm^{-1})[14] and by their characteristic reaction with AgNO$_3$.

All of the organic photoproducts absorb the incident light and are photolabile. However, as indicated by the linear plots presented in Figure 2, they are all primary photoproducts and did not undergo secondary reactions when the irradiation of **3** was performed at low (<40%) conversion of the substrate. Under these conditions, they account for 80–95% of the substrate that reacted.

The quantum yield of disappearance of **3** was essentially independent of the excitation wavelength ($\lambda = 313$ or 366 nm) and concentration in the 0.1–1 mM range.

In an attempt to rationalize the formation of the photoproducts presented in Scheme 1, it appeared reasonable to assume that the primary reaction of the excited molecule **3** is photodissociation of its C–I bond. Photoinduced homolysis of carbon–iodide bonds is a

CHART 2
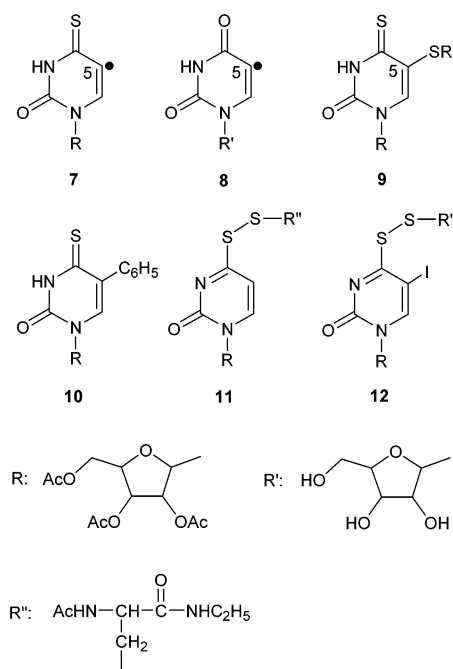
common reaction of organic halides, e.g., aromatic and heteroaromatic compounds, containing a variety of functional groups in addition to iodine substituents.[3,22,23] However, to the best of our knowledge, there are no reports in the literature on the photochemistry of iodine-substituted aromatic thiones. Photoinduced homolysis of C(5)–I in **3**, in analogy to the photochemistry of its oxygen congener **2**,[3,4,7–9,11–16] should lead to the formation the C(5)-centered radical 4-thiouridine-5-yl radical **7** (Chart 2) and an iodine atom. However, attempts to trap radical **7** by suitable reagents were unsuccessful. The trapping reagents were chosen as suitable on the basis that the chemistry of radical **7** should be similar to the uridine-5-yl radical **8** (Chart 2), an analogue of **7** that has a C(4)=O group instead of a C(4)=S group.

In particular, irradiation of **3** in the presence of bis-(N-α-acetyl)cystine-bis-N-ethylamide in aqueous media and irradiation of **3** in benzene solution did not lead to C(5)-substituted derivatives of 4-thiouridine, i.e., photoproducts **9** or **10** (Chart 2), both of which are expected on the basis of the above analogy. In the presence of an equimolar amount of bis-(N-α-acetyl)cystine-bis-N-ethylamide, the disulfides **11** and **12** were formed (Chart 2) in addition to the photoproducts identified when the nucleoside **3** was irradiated without addends (Scheme 1). The resulting disulfides **11** and **12** were unstable during the preparative-scale isolation procedure. However, the UV and MS spectral data (Experimental Section) provided sufficient information about their structure. The structural assignment was confirmed by the quantitative splitting of disulfides **11** and **12** to N-α-acetyl-cysteine N-ethylamide and equimolar amounts of the respective nucleosides **4** and **3** by treatment with dithiotreitol, Cleland's disulfide reducing agent.
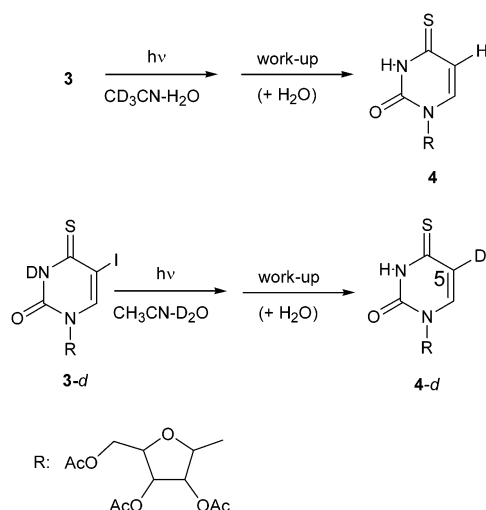
When **3** in benzene solution was irradiated to 33% conversion, the major organic photoproducts were the

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TABLE 1. Quantum Yield[a] of Substrate Decomposition, $\phi(3)$, and Formation of Photoproducts, $\phi(4)$, $\phi(5)$, and $\phi(6)$, upon Irradiation of 3 at $\lambda = 366$ nm in a Deoxygenated 1:1 Mixture of CH_3CN-H_2O pH 5.8 (Phosphate Buffer)

solvent	[3] mM	$\phi(3)$	$\phi(4)$	$\phi(5)$	$\phi(6)$
CH_3CN-H_2O pH 5.8	0.12	0.0039	0.0017	0.0006	0.0007
CH_3CN-H_2O pH 5.8	1.20	0.0037	0.0018	0.0006	0.0005
C_6H_6	0.12	0.0028	0.0012	0.0003	0.0002

[a] mean of three independent measurements (error $\pm$ 20%).

SCHEME 2

FIGURE 3. (a) Transient absorption spectra following laser flash photolysis ($\lambda_{exc} = 308$ nm) of **3** ([**3**] = 0.043 mM) in a deoxygenated 1:1 mixture of CH_3CN-H_2O pH 5.8 (0.01 M phosphate buffer); triangles 5–20 μs, circles 20–40 μs after the laser pulse. (b) Time profile of absorption at 450 nm.

same as in the 1:1 mixture of CH_3CN-H_2O pH 5.8 (phosphate buffer), although they were formed with smaller quantum yields (Table 1). None of the several minor photoproducts were identified as 2′,3′,5′-tri-*O*-acetyl-5-phenyl-4-thiouridine (**10**, Chart 2), as would normally be expected when the solvent is benzene. A sample of the latter compound was synthesized independently (Experimental Section). Upon irradiation of **3** in benzene, the inorganic photoproducts were I_2 and I^-. The presence of the former was suggested by the pink color of the irradiated solution. Iodide anions were detected by a reversed-phase HPLC analysis of the irradiated sample after the volatile I_2 was removed in the process of evaporating the sample to dryness.

Finally, to identify the source of hydrogen at the C(5) position of the photoproduct **4**, irradiations of **3** were performed in deuterium-labeled solvents. The analysis was limited to the photoproduct **4** (Scheme 2). The procedure involved irradiation in CD_3CN-H_2O pH 5.8 (phosphate buffer) and isolation of the photoproduct **4** by HPLC, which was performed in nonlabeled aqueous media. The result was 2′,3′,5′-tri-*O*-acetyl-4-thiouridine (**4**), which did not contain any deuterium as determined by mass spectrometric analysis. In contrast, an analogous experiment performed in CH_3CN-D_2O pH 5.8 (phosphate buffer), with the identical isolation procedure as the other deuterated solvent, gave 2′,3′,5′-tri-*O*-acetyl-4-thiouridine with 88% incorporation of deuterium (**4**-*d*, Scheme 2). The deuterium was introduced into the C(5) position of the uracil ring of **4**-*d* as shown by ¹H NMR spectroscopy. In a test experiment, it was confirmed that, in this solvent system, no dark (C-5)-H/D exchange in **4** molecules takes place in the absence of light. The results of the labeling experiments point to the involvement of an exchangeable
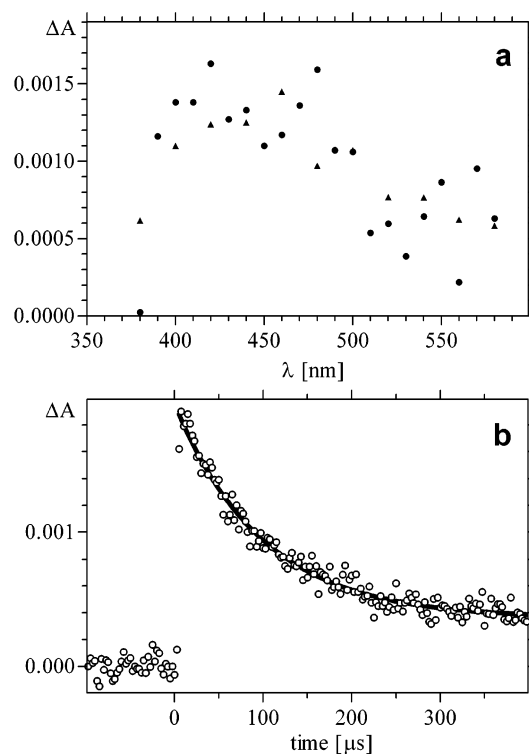
hydrogen (N–H) from the molecule **3** rather than a hydrogen from the solvent. This leads to the formation of the C(5)–H bond in the photoproduct **4**.

Laser Flash Photolysis. Solutions of **3** ([**3**] = 0.043 mM) in 1:1 mixtures of CH_3CN-H_2O pH 5.8 (phosphate buffer) were irradiated at $\lambda = 308$ nm using an excimer laser. Transient absorptions were observed in the region 350–600 nm. The resulting spectrum is presented in Figure 3a. It exhibits a broad band centered at $\lambda = 430$ nm. The absorption decays monoexponentially, and the lifetime $\tau = 95.4$ μs was determined from the time profile of the 450 nm absorbance (Figure 3b). The long lifetime of the species responsible for the observed absorption precludes it from being the lowest excited triplet state of **3**. This conclusion follows because the room-temperature phosphorescence lifetime of compound **3** in CCl_4 was determined to be 0.024 μs, and its triplet lifetime is expected to be even shorter in aqueous solution.[19,24]

Identification of the species responsible for the observed transient absorption was hampered by the lack of any reported spectrum of C-centered radicals derived from heterocyclic thione, which could serve as a model for C(5)-centered radical **7** (Chart 2). On the other hand, the spectrum of the thiyl radical **13** (Scheme 3) was easy to obtain. Laser flash photolysis of the readily available disulfide **14** (Experimental Section) is expected to give thiyl radicals **13**. Disulfides are known to undergo

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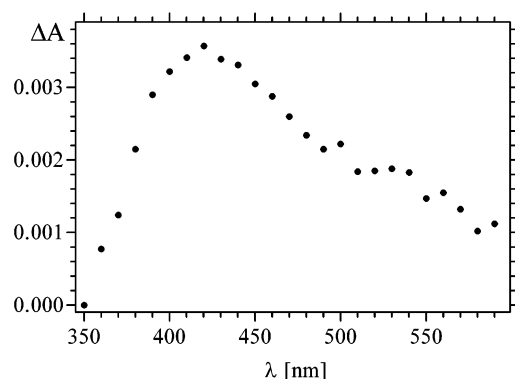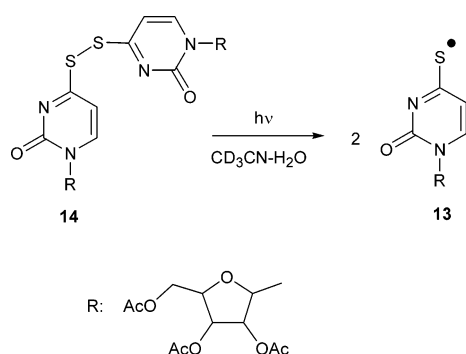
FIGURE 4. Transient absorption spectrum following laser flash photolysis (λ_{exc} = 308 nm) of **14** ([**14**] = 0.05 mM) in a deoxygenated 1:1 mixture CH_3CN-H_2O pH 5.8 (0.01 M phosphate buffer); 5−10 μs after the laser pulse.
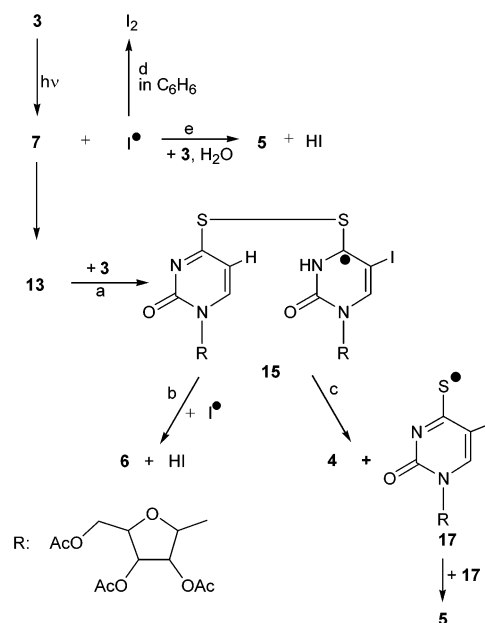
SCHEME 3

homolytic scission of their S−S bond upon laser excitation as well as in steady-state photolysis.[25−27]

The transient absorption spectrum observed after laser photolysis of the disulfide **14** is presented in Figure 4. The shape of the spectrum and the location of the maximum closely resemble that observed after laser flash photolysis of **3**. Thus, we conclude that the only observable species in the transient spectrum of **3** is the thiyl radical **13**. The failure to observe C(5)-centered radical **7** could be due to it being short-lived and/or to its spectrum being located in a shorter wavelength region. By analogy to the properties of the radicals formed from *N*-hydroxypyridine 2(1*H*)-thione, the former possibility (radical **7** being short-lived) appears likely. In particular, even though the O-centered *N*-oxypyridine-2(1*H*)-thione and S-centered *N*-oxy-2-pyridylthiyl radicals absorb in similar spectral regions, the lifetimes of the latter (25 μs) are 2 orders of magnitude longer than the former.[28]

Mechanism of Photoreaction. The suggested mechanism of the photoreaction of **3**, which takes into account the results of steady-state and laser flash photolysis studies of **3**, is presented in Scheme 4. The initially formed radical **7** is short-lived (estimated lifetime τ < 0.1 μs). It subsequently transforms into the more stable, long-

SCHEME 4

lived thiyl radical **13** by the 1,3-hydrogen shift: N(3)−H → C(5)−H. The deuterium-labeling evidence that the exchangeable hydrogen (N−H) of **3** appears as nonexchangeable C−H in photoproduct **4** supports the proposed 1,3-hydrogen shift in Scheme 4.

Presumably this shift is not a one-step process because of the unfavorable spatial arrangement of the orbitals. An alternative explanation would be the tautomerization of the initially formed C(5)-centered radical **7** to the thiol form, followed by a 1,2-H shift from the C−SH to the neighboring C(5) radical center. This would lead to the more stable thiyl radical **13**. Furthermore, the DFT calculations support this mechanistic step. The DFT calculation was performed on simplified models of radicals **7** and **13**. The simplified models replaced the ribosyl substituent with a methyl group. The results show that the S-centered (thiyl) radical is more stable than the thione C(5)-centered radical or its tautomeric thiol form by 80 and 135 kJ mol^{-1}, respectively, in vacuo. In acetonitrile, the thiyl radical is about 70 kJ mol^{-1} more stable than the thione C(5)-centered radical and about 135 kJ mol^{-1} more stable than the tautomeric thiol form of the C(5)-centered radical.

Although thiyl radicals are known to undergo inefficient, reversible addition to olefins,[26] they add readily to the carbon−sulfur double bonds of thiones, with large rate constants approaching the diffusion limit.[28−31] The rapid reaction between thiyl radical **13** and ground-state molecule **3** (route a, Scheme 4), combined with the long lifetime of **13**, is consistent with the proposed mechanism. These two kinetic features account for the observation that the photoreaction quantum yields (Table 1) remain unchanged as the substrate concentration was varied between 0.1 and 1 mM. Another way of interpreting this is that almost all of the radicals that escape geminate

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recombination react with the substrate. This is why the quantum yield of degradation of the substrate matches the yield of photoproducts. The radical adduct **15**, the product of this bimolecular reaction, could be a precursor of the stable photoproducts identified in steady-state photochemistry. Oxidation of radical **15** by iodine gives iodide and disulfide **6** (route b, Scheme 4). Decomposition of the radical **15** gives **4** and radical **17** (route c, Scheme 4). The latter species finally gives symmetrical sulfide **5**. Iodine formed in the primary photochemical step in benzene solution recombines to give I₂ (route d, Scheme 4). In an aqueous solution, the iodine atoms may undergo a reaction with the solvent to give iodide and other species.[13,32] Moreover, in aqueous solutions of **3**, dark iodine chemistry may also be responsible for the oxidation of the thione **7** to the disulfide **5** (route e, Scheme 4). It has been established that 4-thiouracil and its nucleosides are instantaneously and quantitatively converted into disulfides upon treatment with iodine/iodide in aqueous solution.[33] In a control experiment, we demonstrated that addition of an equimolar amount of I₂ to CH₃CN−H₂O solution of **3** ([**3**] = 0.5 mM) leads instantaneously to the disulfide **5** as the sole product. No dark oxidation of **3** to disulfide was observed in benzene. The dark oxidation may be responsible for the ca. 2 times greater quantum yields for the production of disulfides upon irradiation of **3** in aqueous solution as compared to that in benzene solution (Table 1).

Experimental Section

Preparation of Disulfides 5, 6, and 14. The disulfides were prepared by iodine oxidation of the corresponding thiones. A standard published procedure[33] was accordingly modified. See Supporting Information.

5-Iodo-4-{[1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-5-iodo-2-oxo-1,2-dihydropyrimidin-4-yl]dithio}-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrimidin-2(1*H*)-one (5). From **3** (51.2 mg, 0.1 mmol) was obtained disulfide **5** as a colorless oil (36.8 mg, 72% yield): ¹H NMR δ 8.08 (s, 2H), 6.03 (d, *J* = 3.6 Hz, 2H), 5.38 (m, 2H), 5.31 (m, 2H), 4.43−4.33 (m, 6H), 2.23 (s, 6H), 2.11 (s, 6H), 2.08 (s, 6H); ¹³C NMR δ 175.0, 170.1, 169.3, 169.1, 151.7, 146.3, 121.3, 89.2, 80.1, 73.9, 69.3, 63.5, 62.4, 21.1, 20.4; UV λ_max nm (ε_max M⁻¹ cm⁻¹) 229 (39 000), 265 (14 700), 338 (16 800); HR-LSIMS calcd for C₃₀H₃₃I₂N₄O₁₆S₂ (M + H⁺) 1022.94198, obsd 1022.94087.

1-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)-5-iodo-4-{[1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-2-oxo-1,2-dihydropyrimidin-4-yl]dithio}pyrimidin-2(1*H*)-one (6). From a mixture of **3** (25.6 mg, 0.05 mmol) and **4** (19.3 mg, 0.05 mmol) was obtained a colorless solid of disulfide **6** (10.8 mg): ¹H NMR δ 8.07 (s, 1H), 7.79 (d, *J* = 7.14 Hz, 1H), 6.51 (d, *J* = 7.14 Hz, 1H), 6.08 (d, *J* = 4.40 Hz, 1H), 6.04 (d, *J* = 3.84 Hz, 1H), 5.39−5.35 (m, 2H), 5.32−5.27 (m, 2H), 4.46−4.34 (m, 6H), 2.24 (s, 3H), 2.15 (s, 3H), 2.12 (s, 6H), 2.09 (s, 6H); UV λ_max nm (ε_max M⁻¹ cm⁻¹) 220 (26 800), 268 (15 400), 315 (14 400); HR-LSIMS calcd for C₃₀H₃₄IN₄O₁₆S₂ (M + H⁺) 897.04533, obsd 897.04389.

4-{[1-(2′,3′,5′-Tri-*O*-acetyl-β-D-ribofuranosyl)-2-oxo-1,2-dihydropyrimidin-4-yl]dithio}-1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-pyrimidin-2(1*H*)-one (14). From **4** (38.4 mg, 0.1 mmol) was obtained disulfide **14** (22.7 mg, 59% yield) as a colorless oil. ¹H NMR δ 7.88 (d, *J* = 7.2 Hz, 2H), 6.70 (d, *J* = 7.2 Hz, 2H), 6.06 (d, *J* = 3.9 Hz, 2H), 5.40 (dd, *J* = 5.6 Hz, *J* = 3.9 Hz, 2H), 5.29 (t, *J* = 5.6 Hz, 2H), 4.43−4.37 (m, 6H), 2.14 (s, 6H), 2.12 (s, 6H), 2.09 (s, 6H); ¹³C NMR δ 177.0, 170.1,

169.5, 169.4, 152.5, 142.2, 100.5, 89.5, 80.1, 73.8, 69.6, 62.6, 20.8, 20.4; UV λ_max nm (ε_max M⁻¹ cm⁻¹) 309 (18 200), 264 (13 400); HR-LSIMS calcd for C₃₀H₃₅N₄O₁₆S₂ (M + H⁺) 771.14868, obsd 771.14879.

2′,3′,5′-Tri-*O*-acetyl-5-phenyl-4-thiouridine (10). Compound **10** was obtained as a yellow oil (65.6 mg, 33% yield) by treatment of 2′,3′,5′-tri-*O*-acetyl-5-phenyluridine (192 mg, 0.43 mmol) with P₂S₅ according to the literature procedure:[18,34] ¹H NMR δ 9.81 (s, 1H), 7.48 (s, 1H), 7.44−7.43 (m, 5H), 6.15 (d, *J* = 5.4 Hz, 1H), 5.45 (t, *J* = 5.4 Hz, 1H), 5.36 (t, *J* = 5.4 Hz, 1H), 4.44−4.42 (m, 1H), 4.36−4.34 (m, 2H), 2.17 (s, 3H), 2.16 (s, 3H), 1.75 (s, 3H); ¹³C NMR δ 188.7, 170.0, 169.6, 147.1, 134.6, 132.5, 129.8, 128.6, 128.3, 125.4, 87.4, 80.2, 72.8, 70.1, 62.9, 20.5, 20.4, 20.1; UV λ_max nm (ε_max M⁻¹ cm⁻¹) 341 nm, 260 nm; HR-LSIMS calcd for C₂₁H₂₃N₂O₈S (M + H⁺) 463.11751, obsd 463.11936.

Analytical Scale Irradiation and Quantum Yield Determination. Solutions (2.4 mL) of **3** (0.1−1 × 10⁻³ M) in 1:1 (v/v) CH₃CN−H₂O pH 5.8 (0.05 M phosphate buffer) or benzene were placed in a 1 × 1 cm UV cell, deoxygenated by bubbling argon, and irradiated on an optical bench using a 200 W high-pressure mercury lamp equipped with a glass (λ ≥ 334 nm) or an interference filter to isolate the λ = 366 or 313 nm lines. The progress of the reaction was monitored by UV spectroscopy and HPLC. For the quantum-yield determination, the samples were irradiated to <15% conversion of substrate, and the concentrations of substrate **3** and photoproducts **4**−**6** were determined by HPLC analysis. Benzophenone-benzhydrol actinometry was used.[36]

Preparative Irradiation for Product Analysis. A solution of **3** (0.2 mmol) in 400 mL of the above solvents was irradiated, in portions, in an 80 mL photoreactor with a 150 W high-pressure mercury lamp through a Pyrex filter under an argon atmosphere. Irradiation was continued to ~40% conversion of the substrate as checked by HPLC. Irradiated solutions were collected and concentrated under reduced pressure. The photoproducts were isolated from the residue by preparative HPLC and identified by a comparison of their HPLC retention times (coinjections) and spectral data (UV, HR-LSIMS) with those of synthesized samples.

Isolation and Identifications of Photoproducts 11 and 12. A solution of **3** (0.08 mmol) and bis-(*N*-α-acetyl)cystine-bis-*N*-ethylamide (0.08 mmol) in 160 mL of 1:1 (v/v) CH₃CN−H₂O pH 5.8 was irradiated as described above. The photoproducts **11** and **12** were isolated from the concentrated irradiated solution by preparative HPLC and identified on the basis of UV and HR-LSIMS spectral data. The samples of disulfides were treated with 1,4-dimercapto-2,3-butanediol (DTT). Thus, to **11** or **12** (1 mL, *c* ≈ 0.2 mM) in 50% aq CH₃-CN was added a solution of DTT (50 μL, *c* = 11 mM) in H₂O. The reaction mixture was allowed to stand for 15 min at room temperature and then analyzed by HPLC using a gradient of 27% aq CH₃CN (initial) → 80% aq CH₃CN (30 min) as an eluting phase. The products were identified by a comparison of UV spectra and retention times (coinjection) with that of authentic samples of *N*-α-acetyl-L-cysteine *N*-ethylamide and **4** or **3** in the case of **11** and **12**, respectively.

2-(Acetylamino)-*N*-ethyl-3{[1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-2-oxo-1,2-dihydropyrimidin-4-yl]dithio}-propanamide (11): UV λ_max (ε_max M⁻¹ cm⁻¹) 270 nm (8600), 306 nm (12 000); HR-LSIMS *m/z* calcd for C₂₂H₃₁N₄O₁₀S₂ (M + H⁺) 575.14835, obsd 575.14827.

2-(Acetylamino)-*N*-ethyl-3{[1-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-5-iodo-2-oxo-1,2-dihydropyrimidin-4-yl]dithio}-propanamide (12): UV λ_max (ε_max M⁻¹ cm⁻¹) 270 nm

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(12 000), 332 nm (14 000); HR-LSIMS calcd for $C_{22}H_{30}IN_4O_{10}S_2$ (M + H$^+$) 701.04500, obsd 701.04432.

Irradiation in Deuterated Solvents. Solution of **3** (36 mg, 0.07 mmol) in $CD_3CN–H_2O$ pH 5.8 (70 mL) was deoxygenated and irradiated in a photoreactor as described above. Irradiation was followed by HPLC and continued to 50% conversion of the substrate. After irradiation, the solution was concentrated to a small volume under reduced pressure. Photoproduct **4** was isolated by preparative HPLC. The same procedure was used for irradiation of **3** in $CH_3CN–D_2O$ pH 5.8. Samples of photoproduct **4** obtained in the two experiments were subjected to ESI-MS (negative ions) analysis. The percent of deuterium incorporation was calculated[37] from the measured intensities of peaks at *m/z* 385, 386, 387, and 388 in the spectra of the two samples of photoproduct and authentic, unlabeled sample of **4**. Photoproduct isolated from irradiated solution of **3** in $CH_3CN–D_2O$ was also analyzed by ^1H NMR. In the low-field region (6–10 ppm), the ^1H NMR spectrum in $CDCl_3$ shows two intense singlets at δ 7.2 ppm due to C(6)–H and 7.3 due to solvent resonances. Only the trace signal at 6.4 ppm attributable to C(5)-H was observed.

Theoretical Calculation. Density functional theory (DFT) calculations were performed with the Gaussian98 electronic structure software package[38] employing the popular B3LYP functional.[39] This hybrid functional comprises both local[40] and nonlocal[41] (gradient-corrected) exchange and correlation[42,43] contributions mixed with a piece of exact (Hartree–Fock) exchange. The mixing parameters were derived from fits to known thermochemistry of a well-characterized set of small molecules. For open-shell systems (the transient radicals and radical cations), spin-unrestricted DFT (UB3LYP) was employed. For structural studies, the (heavy-atom) polarized split-valence 6-31G* basis set[44] was used. DFT geometries are often converged with modest basis sets. Solvent effects were modeled by self-consistent reaction field theory using a polarized cavity model[45] with COSMO boundary conditions.[46] Thermochemical quantities were derived using standard conversion formulas.[47]

Nanosecond Laser Flash Photolysis. The nanosecond laser flash photolysis setup and its data acquisition system have been described in detail.[48] The excimer laser (Lambda Physik, Compex) provided 20 ns, 10 mJ pulses at 308 nm. The transients were monitored with a pulsed 1 kW xenon lamp, having the monitoring beam perpendicular to the laser beam. All experiments were carried out within a rectangular quartz optical cell (0.5 × 1 cm). The monitoring light path length, *l*, was 0.5 cm.

Supporting Information Available: General experimental methods, experimental procedure for synthesis of disulfides, pH-dependent spectra of **3** and spectrophotometric titration curve, ^1H NMR spectra of **5**, **6**, **10**, and **14**, ^{13}C NMR spectra of **5**, **10**, and **14**, and total energy calculations for 1-methyl-4-thiouracil-5-yl radical, its thiol tautomer, and 1-methyluracil-4-thiyl radical. This material is available free of charge via the Internet at http://pubs.acs.org.

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