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Independent Generation and Reactivity of Uridin-2′-yl Radical

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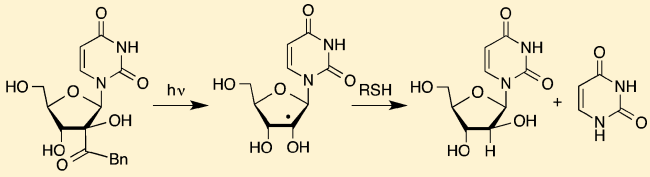
Supporting Information

ABSTRACT: The uridin-2′-yl radical (**1**) has been proposed as an intermediate during RNA oxidation. However, its reactivity has not been thoroughly studied due to the complex conditions under which it is typically generated. The uridin-2′-yl radical was independently generated from a benzyl ketone (**2a**) via Norrish type I photocleavage upon irradiation at λ_{max} = 350 nm. Dioxygen and β-mercaptoethanol are unable to compete with loss of uracil from **1** in phosphate buffer. Thiol trapping competes with uracil fragmentation in less polar solvent conditions. This is ascribed mostly to a reduction in the rate constant for uracil elimination in the less polar solvent. Hydrogen atom transfer to **1** from β-mercaptoethanol occurs exclusively from the α-face to produce arabinouridine. Mass balances range from 72 to 95%. Furthermore, the synthesis of **2a** is amenable to formation of the requisite phosphoramidite for solid-phase oligonucleotide synthesis. This and the fidelity with which the urdin-2′-yl radical is generated from **2a** suggest that this precursor should be useful for studying the radical's reactivity in synthetic oligonucleotides.

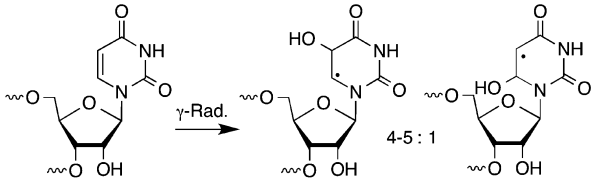


INTRODUCTION

Nucleic acid oxidation is an important analytical tool for describing RNA structure and folding.[1−4] Whereas DNA oxidation has long been associated with disease etiology and treatment, as well as aging, the physiological relevance of RNA oxidation has been recognized more recently.[5−9] Hydroxyl radical (OH•) is the primary reactive oxygen species produced from water by ionizing radiation, which is the most common cytotoxic, therapeutic modality that targets nucleic acids. Hydroxyl radical is also the principal reactive species formed from the reaction of Fe·EDTA with hydrogen peroxide, which is a commonly used tool in RNA structure and folding studies.[10] Although a variety of lesions are formed, direct strand breaks are of particular interest for experiments in which gel electrophoresis is used as an analytical tool. Direct strand scission by OH• is often attributed to hydrogen atom abstraction from the C4′- and C5′-positions. It is notable that the cited source for this statement is a study on OH• cleavage of DNA.[11] In addition, the primary reaction pathway for OH• with nucleic acids is nucleobase addition (Scheme 1). Nucleobase addition accounts for as much as 93% of the reactions of OH• with RNA, yet ∼40% of the oxidative events result in strand scission.[12−14] Although nucleobase addition of OH• is also the dominant pathway in DNA, strand cleavage resulting from the nucleobase radicals that are formed is much less efficient.[15] Strand scission from the originally formed nucleobase radicals and/or their respective peroxyl radicals requires hydrogen atom abstraction from the sugar backbone. RNA cleavage following C2′- and C4′-hydrogen atom abstraction by nucleobase (peroxyl) radicals has been proposed in experiments where damage is initiated by γ-radiolysis.[13,14,16−18] The C2′-carbon−hydrogen bond dissociation energy in a ribonucleotide is at least 4.5 kcal/mol weaker than any other carbon−hydrogen bond in a 2′-deoxy- or ribonucleotide.[19] The much weaker C2′-carbon−hydrogen bond in RNA suggests why OH• strand scission is more efficient in this biopolymer than in DNA. Recently, evidence for strand scission following C2′-hydrogen atom abstraction by independently generated dihydropyrimidine radicals has been reported (Scheme 2).[20−22] However, C2′-radical reactivity is not well understood. Herein, we describe the synthesis of a ketone designed to produce uridin-2′-yl radical (**1**) upon photolysis and product analysis that supports generation of **1**.

Although the C2′-carbon−hydrogen bond is the weakest such bond in ribonucleotides, hydrogen atom abstraction from this position is presumably not a major pathway for duplex RNA damage by diffusible species such as OH• because of the hydrogen atom's relatively low solvent accessibility.[11,19] However, the C2′-hydrogen atom, which is present in the major groove of a duplex is readily accessible to nucleobase (peroxyl) radicals resulting from addition of OH• to the C5- or C6-position of a pyrimidine. C5- and C6-dihydropyrimidine radicals are well positioned to abstract the C2′-hydrogen atom from 5′-adjacent nucleotides. C6-radicals can also effect intranucleotidyl C2′-hydrogen abstraction (Scheme 2).[20−22]

Scheme 1



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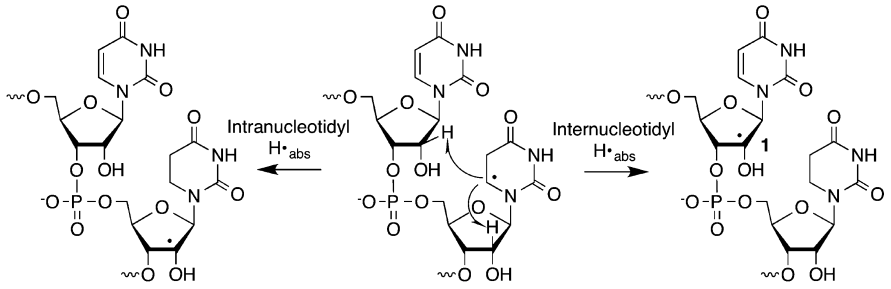
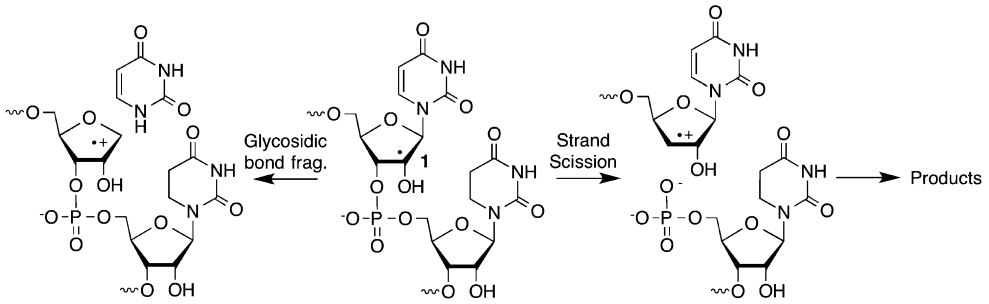
Scheme 2



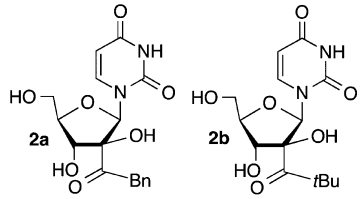
Scheme 3



The C2′-radical is believed to yield the respective nucleobase via glycosidic bond fragmentation (Scheme 3). In polymers, the C2′-radical is believed to yield strand breaks via elimination of the phosphate leaving group (Scheme 3).[14,16−18] Independent generation of radical **1** within RNA oligonucleotides should facilitate characterization of its reactivity. However, prior to carrying out such studies in a biopolymer, it is important to characterize the photochemical generation of the reactive species at the monomeric level in order to validate the integrity of the process.

RESULTS AND DISCUSSION

Independent generation of putative radical intermediates is an effective approach for studying oxidative damage in nucleic acids. It was invaluable for elucidating the rules governing hole transfer in DNA and enabled detecting unknown mechanisms for strand scission.[23−26] Independent generation of reactive intermediates at defined sites in synthetic oligonucleotides has also provided insight into how γ-radiolysis damages nucleic acids by simplifying this unselective, complex chemistry that produces a variety of intermediates throughout heteropolymers.[27−33] As mentioned above, this approach has been applied more recently to studying RNA oxidative damage.[20−22] The majority of the radicals studied have been generated via Norrish Type I photocleavage of appropriate ketones. We reasoned that this strategy would be well suited for generating uridin-2′-yl radical (**1**) as well. In fact, the presence of a α-hydroxyl substituent in **1** necessitates using a radical precursor containing a carbon bonded to the incipient radical center. Methyl, isopropyl, phenyl, benzyl, and *tert*-butyl ketones have been used to generate nucleoside radicals, although the latter are most common and benzyl ketones[34] often exhibit favorable excited state properties for Norrish type I photocleavage.[30,35−44]



Attempted Synthesis of a Ketone Photochemical Precursor to Urdin-2′-yl Radical (1). Initial attempts to prepare **2a**,**b** were the most direct. These involved nucleophilic addition to the 2′-keto nucleoside in which the 3′- and 5′-hydroxyl groups were protected as either the dibenzyl ether (**3**) or the di-*tert*-butylsiloxane (**4**).[45,46] Attempts to directly form the *tert*-butyl ketone by reacting either **3** or **4** with pivaloyl chloride in the presence of SmI_2 were unsuccessful. The ketones were recovered unchanged from these reactions. The Bom-protected siloxane ketone (**5**) also did not react with SmI_2. Suspecting that the lack of addition was due to sterics, we tried a less commonly used *tert*-butyl acyl anion equivalent.[47] The *tert*-butyl acyloin (**6**) has been successfully used to produce α-hydroxy *tert*-butyl ketones from hindered ketones.[48] However, we could not obtain any addition product from **4** or **5** under these conditions. We were able to add cyanide to **3** and **4** and trap the oxy anions as the respective silyl ethers (**7**, **8**). However, subsequent *tert*-butyllithium addition in the presence of CuI was unsuccessful. Given the difficulty of adding the *tert*-butyl group to the C2′-ketone or cyano ether, we pursued an approach in which the alkyl group was introduced first. We chose to incorporate the benzyl group via a Wittig reaction.[49] However, the desired product (**9**, Scheme 4) was obtained in low yield (<15%) from reaction of siloxane **4**, and the Wittig reagent and the dibenzyl ketone (**3**) yielded none of the respective alkene. Alkene **9** did yield a mixture of diols (**10**) upon treatment with OsO_4, but despite being two steps from our target ketone (**2a**), this route was abandoned because we could not obtain sufficient quantities of **9**.

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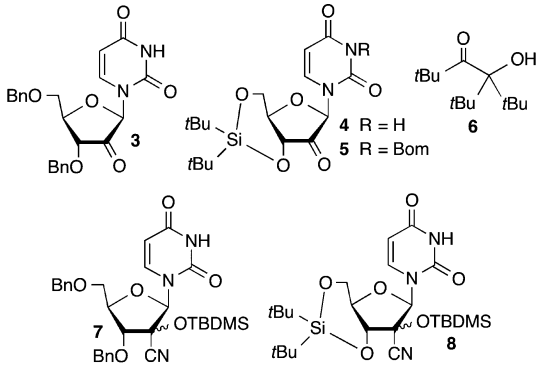
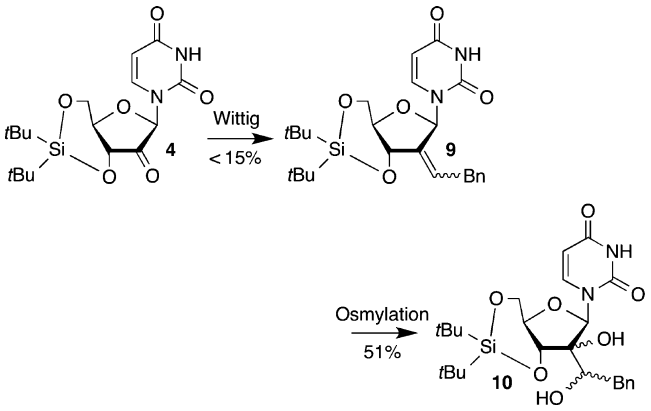
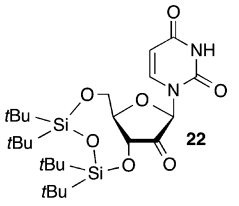


Scheme 4



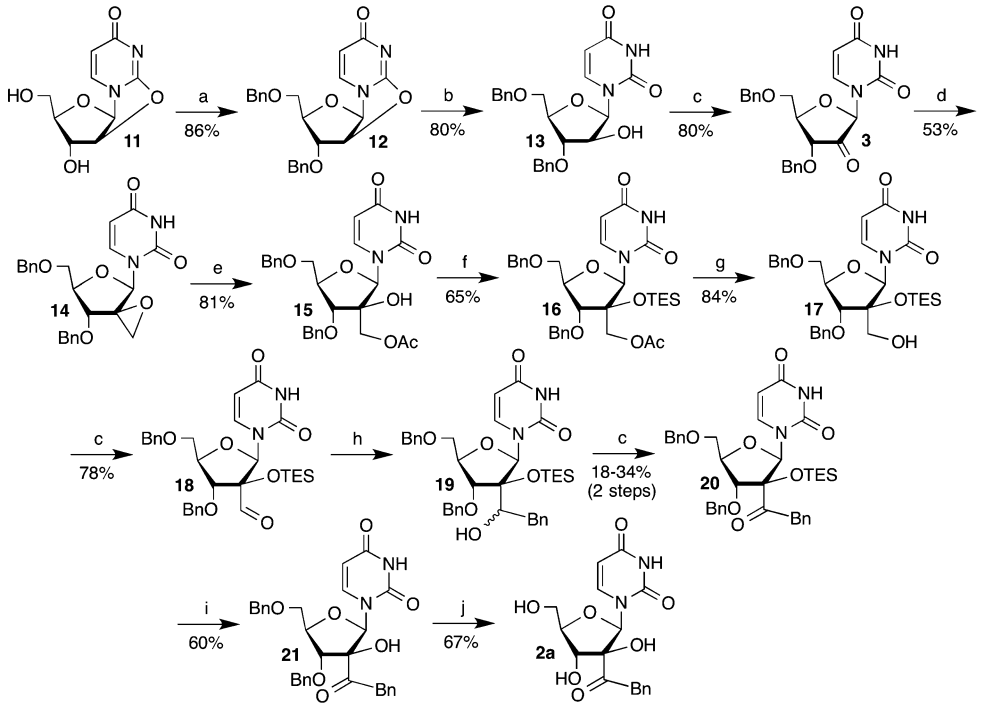
Successful Synthesis of a Benzyl Ketone Precursor to 1 (2a). The aborted approaches outlined above suggested that we needed a more electrophilic C2′-appendage to generate the ultimate ketone from. Furthermore, thinking that **1** would ultimately be generated in chemically synthesized RNA oligonucleotides, the 2′-hydroxyl was protected as a silyl ether. The 3′- and 5′-hydroxyl groups were protected as their benzyl ethers because this protecting group would withstand strongly basic conditions and could be removed without cleaving the 2′-silyl ether.

Dibenyl ketone was prepared from the known cyclonucleoside (**11**),[50,51] which provided **3** upon dibenzylation (**12**), hydrolysis of the cyclonucleoside (**13**), and oxidation (Scheme 5). Epoxidation of dibenzyl ketone **3** by reaction with the trimethylsulfoxonium ylide yielded a single diastereomer (**14**).[52] We anticipated that addition would occur to the pro-*R* face of the carbonyl based upon literature precedent in which **22** was subjected to the same conditions and the stereochemistry of the epoxide was established via subsequent chemical transformations.[53] The stereochemistry in **14** was independently verified by NMR characterization of a downstream intermediate, aldehyde **18**.[54] The identities of the sugar protons in **18** were determined via COSY NMR. Subsequent NOESY experiments showed interactions between the C3′-proton and the methyl groups of the triethylsilyl protecting group, as well as between the aldehyde and C1′-protons.



Following nucleophilic ring opening of the epoxide by acetate, the 2′-hydroxyl group in **15** was silylated (**16**). The

Scheme 5[a]



[a]Key: (a) NaH, BnBr, DMF; (b) KOH, EtOH,reflux; (c) Dess−Martin periodinane, CH_2Cl_2; (d) Me_3SOI, NaH, DMSO/THF; (e) NaOAc, AcOH, reflux; (f) TESOTf, 2,6-lutidine, CH_2Cl_2; (g) EtMgBr, Et_2O; (h) BnMgCl, THF; (i) TBAF, THF; (j) H_2, Pd/C, THF.

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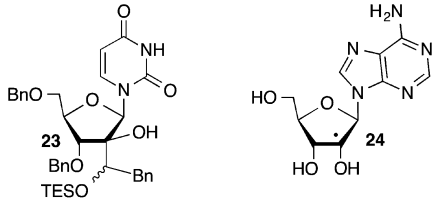
triethylsilyl group was chosen because we hoped that it would be more robust than a trimethylsilyl group but smaller than a *tert*-butyldimethylsilyl group. We were concerned that the latter group would adversely affect nucleophilic addition to the aldehyde that would ultimately be introduced. The aldehyde (**18**) was prepared by deprotecting the primary alcohol (**17**) by treating the acetate with a Grignard reagent, followed by Dess-Martin periodinane oxidation of **17**. Cleavage of the acetate under these conditions provided a good yield of the alcohol (**17**) without loss or migration of the silyl group.

We were unable to prepare the corresponding *tert*-butyl ketone by reacting **18** with *t*-BuLi in the presence of $CeCl_3$. Despite several attempts under a variety of conditions, no addition product was obtained. Alcohol **17**, resulting from net hydride addition, was the sole product observed. Addition of the benzyl group to the aldehyde in **18** was achieved using the respective Grignard reagent. Although ^{1}H NMR of the crude reaction mixture indicated that two diastereomers of **19** were formed, only a single isomer could be isolated and characterized in pure form. The product mixture and yields obtained from the Grignard reaction were very sensitive to the reaction conditions. Purification of **19** was complicated by side reactions, such as triethylsilyl group migration and addition of 2 equiv of the nucleophile. The position of the triethylsilyl group was determined on the basis of the coupling between the CH and OH protons in the COSY spectrum of the desired product (**19**),[54] whereas such coupling was absent in the spectrum of the migrated product (**23**). Better results were obtained when the crude mixture containing **19** was oxidized and ketone **20** purified by flash chromatography. However, even then the yields were variable and never very high. Nonetheless, serviceable quantities of photolabile precursor **2a** were obtained from **20** via straightforward desilylation, followed by hydrogenolysis of **21**. THF was used as the solvent in the hydrogenolysis reaction to prevent reduction of the benzyl ketone in other solvents such as methanol.



Photochemical Generation of Uridin-2′-yl Radical (1) from 2a. Uracil is the only product observed upon photolysis of **2a** in phosphate-buffered (10 mM, pH 7.2) saline (100 mM NaCl). Independent experiments confirmed that uracil, uridine, and arabinouridine (**25**) are stable to the photolysis conditions. Uracil is observed under aerobic or anaerobic conditions, with or without thiol (glutathione, β-mercaptoethanol (BME)). In addition, uracil grows continuously with respect to photolysis time, and its absolute yield correlates with the disappearance of **2a** (Figure 1). The yield of uracil based upon converted ketone (**2a**) is unchanged within experimental error over the course of 2−7 h of photolysis, as expected for a product that is stable under the reaction conditions. (The yield of uracil based upon converted **2a** ranged from 56.8 ± 4.0% to 58.8 ± 9.3%.) Uridine and arabinouridine are not detected, even when **2a** is irradiated under degassed conditions in the presence of 250 mM BME. Previous reports suggest that uracil results from heterolytic cleavage from **1** (Scheme 3).[16,17,55] Furthermore, the inability to trap **1** with hydrogen atom donors is also

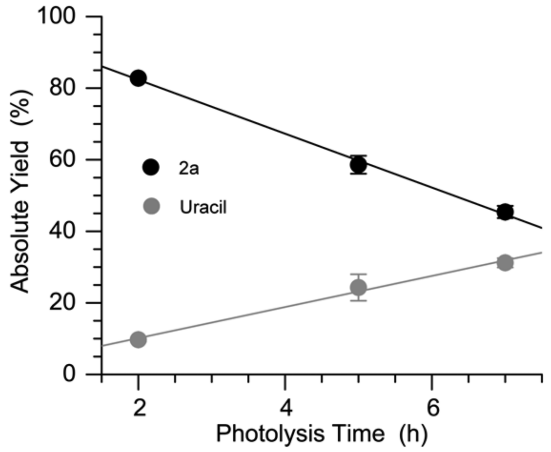
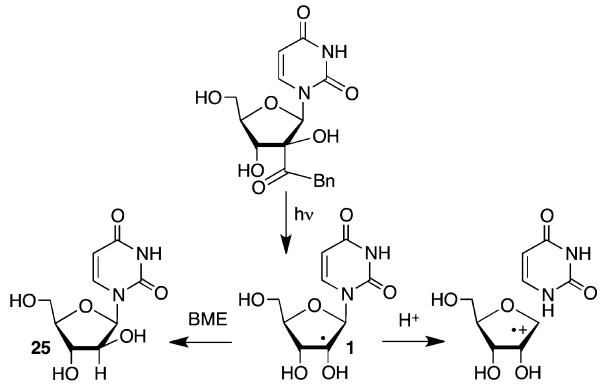


Figure 1. Absolute yield % of uracil and percent of **2a** remaining as a function of photolysis time. Photolysis conditions: 10 mM phosphate (pH 7.2), 100 mM NaCl, O_2. Values presented are the average ± std dev of three experiments.

consistent with previous experiments that suggested that nucleobase fragmentation from nucleoside 2′-radicals is rapid. However, the adenosin-2′-yl radical (**24**), independently generated from the respective methyl ketone, was trapped by glutathione in aqueous buffer (pH 7.0).[43] The rate constant for adenine elimination from **24** was estimated to be 1.1×10^5 s^{-1}. More facile thiol trapping of **24** than the uridin-2′-yl radical (**1**) could be reflective of more rapid cleavage of uracil than adenine. The respective pK_a's of the free bases (uracil, 9.4; adenine, 9.8) are at least consistent with this possibility.

Nonetheless, the absence of hydrogen atom trapping products prohibited us from eliminating the possibility that free base release upon irradiation of **2a** results from one or more mechanisms that do not involve **1**. Observation of hydrogen atom trapping product(s) in competition with uracil formation would provide stronger evidence for nucleobase release from **1**. If uracil fragmentation from **1** proceeds heterolytically, as proposed (Schemes 3 and 6), we reasoned

Scheme 6



that reducing the polarity of the solvent would decrease the rate constant for this process.[16,17,55] Newcomb and Crich rigorously demonstrated this phenomenon in a number of structurally related radicals.[56−62] The rate constant for trapping of **1** by thiol should also decrease as the solvent polarity is reduced.[63] However, we expected the solvent effect on hydrogen atom transfer to be smaller than that on formation of the charged

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cation radical. Consequently, we examined the photochemistry of **2a** in solvents containing between 0 and 99% acetonitrile in water (not buffer) and BME (0.1 M) (Scheme 6, Figure 2).

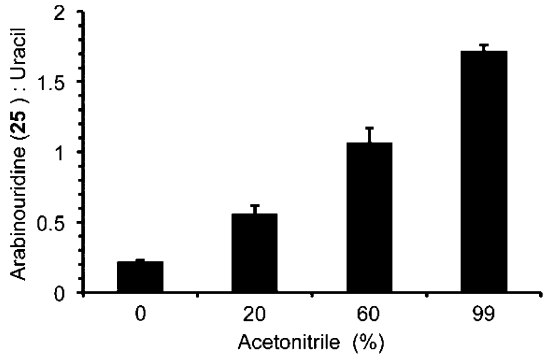

Figure 2. Solvent effect on the ratio of arabinouridine (**25**): uracil from uridin-2′-yl radical (**1**) in the presence of BME (0.1 M). Values presented are the average ± std dev of three experiments.

Under these conditions, thiol trapping competed with loss of uracil. Hydrogen atom donation was very stereoselective. Only arabinouridine (**25**) was detected. In 99% acetonitrile, where the yield of **25** was the highest, based on its limit of detection, uridine was formed in <4% yield. Stereoselective attack of 2′-deoxynucleoside-2′-yl radicals from the α-face is generally preferred and in some cases is >99%.[64−66] The 2′-adenosine radical (**24**), which yielded a mixture of the ribose and arabinose trapping products reacts with lower stereoselectivity than most other related systems.[43]

As expected, the yield of uracil decreased with increasing acetonitrile in the solvent (Table 1). In addition to changing

Table 1. Effect of Acetonitrile on Photochemistry of 2a[a]

CH_3CN (%)	yield[b] (%)		% conv of **2a**	mass balance (%)
	25	uracil		
0	9.2 ± 0.5	40.8 ± 0.4	55.2 ± 3.7	72.4 ± 1.8
20	19.0 ± 3.4	33.9 ± 2.5	52.9 ± 4.2	74.9 ± 4.9
60	27.3 ± 2.0	25.4 ± 0.5	39.9 ± 3.3	81.1 ± 2.1
99	49.4 ± 2.3	28.7 ± 1.3	24.4 ± 1.1	94.7 ± 0.9

[a]Values presented are the average ± std dev of three experiments. [b]Yields are based upon unrecovered starting material.

the ratio of **25** to uracil, increasing the percent acetonitrile in the solvent decreased the extent of ketone conversion. We do not know the cause of this effect, but possibilities include solvent effect on the rate constant for bond scission in the ketone excited state and/or the excited-state lifetime. Importantly, the mass balance was never lower than 72%, suggesting that **2a** cleanly generates **1**.

SUMMARY

The uridin-2′-yl radical (**1**) is generated with good fidelity from a benzyl ketone (**2a**) via Norrish type I photocleavage. Radical generation can be carried out under aerobic or anaerobic conditions using a light source (λ_{max} = 350 nm) that causes minimal damage to nucleic acids. Product studies on monomeric **1** indicate that uracil loss is very rapid. Thiol and O_2 do not compete with this reaction in aqueous buffer. Decreasing the solvent polarity enables thiol trapping under anerobic conditions to compete with uracil cleavage. These observations suggest that 3′-phosphate cleavage from **1** generated in RNA will also be very rapid.

EXPERIMENTAL METHODS

General Methods. Solvents used in reactions were purified and dried (using CaH_2 or Na/benzophenone) by distillation before use. Reagents were purchased from commercial sources and were used without further purification. Reactions were carried out under a positive pressure of argon atmosphere and monitored by TLC on silica gel G-25 UV254 (0.25 mm). Spots were detected using UV light and/or by charring with a solution of either ammonium molybdate, ceric ammonium sulfate in water and H_2SO_4, or *p*-anisaldehyde in ethanol and H_2SO_4. Flash chromatography was performed on silica gel 60 (40−60 μm). The ratio between silica gel and crude product ranged from 100:1 to 20:1 (w/w).

Preparation of 3′,5′-*O*-Dibenzylcyclouridine (12). Cyclouridine (**11**) (3.1 g, 13.7 mmol) was azeotropically dried with pyridine (3 × 3 mL) and suspended in 40 mL of DMF. Benzyl bromide (3.58 mL, 5.15 g, 30.1 mmol) was added, and the mixture was cooled to 0 °C. NaH (1.42 g, 35.6 mmol) was added in three portions over 10 min with vigorous stirring, and the mixture was stirred overnight with warming to 25 °C. The reaction mixture was concentrated under diminished pressure, and the residue was purified by flash chromatography on a silica column (20 × 4 cm). Elution with 4 → 8% MeOH in CH_2Cl_2 gave **12** as a colorless solid: yield 4.8 g (86%); silica gel TLC R_f 0.05 (1:1 ethyl acetate−hexanes); ^{1}H NMR ($CDCl_3$) δ 7.42−7.23 (m, 9H), 7.23−7.09 (m, 2H), 6.23 (d, J = 6.0 Hz, 1H), 6.07 (d, J = 7.5 Hz, 1H), 5.38−5.23 (m, 1H), 4.58 (q, J = 11.7 Hz, 2H), 4.40 (d, J = 12.3 Hz, 2H), 4.33−4.17 (m, 2H), 3.28 (qd, J = 10.5, 3.9 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 172.2, 160.2, 136.8, 136.2, 134.9, 128.8, 128.6, 128.6, 128.4, 128.2, 128.2, 110.2, 90.8, 86.7, 86.3, 83.9, 73.8, 72.6, 68.7; IR (KBr plate) 1650, 1539, 1473, 1240, 1091, 825 cm^{-1}; HRMS (ESI-TOF) m/z calcd for $C_{23}H_{23}N_2O_5^+$ (M + H)$^+$, 407.1607, obsd m/z = 407.1620.

Preparation of 3′,5′-*O*-Dibenzylarabinouridine (13). A mixture of **12** (3.9 g, 9.6 mmol) and 0.1 M KOH in 95% EtOH (25 mL) was heated at reflux for 4 h and concentrated under diminished pressure. The residue was diluted with EtOAc (150 mL), washed with brine (50 mL), dried (Na_2SO_4), filtered, and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica column (20 × 2 cm). Elution with 4 → 8% MeOH in CH_2Cl_2 gave **13** as a colorless solid: yield 3.2 g (78%); silica gel TLC R_f 0.10 (1:1 ethyl acetate−hexanes); ^{1}H NMR ($CDCl_3$) δ 10.42 (s, 1H), 7.65 (d, J = 8.1 Hz, 1H), 7.41−7.26 (m, 10H), 6.16 (d, J = 3.8 Hz, 1H), 5.40 (dd, J = 8.1, 1.8 Hz, 1H), 4.76 (d, J = 11.8 Hz, 2H), 4.67−4.49 (m, 4H), 4.20 (d, J = 4.4 Hz, 1H), 4.02 (dd, J = 3.5, 2.3 Hz, 1H), 3.73 (ddd, J = 15.0, 10.4, 4.1 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 165.1, 150.6, 142.7, 137.5, 137.3, 128.7, 128.5, 128.2, 128.1, 128.4, 128.0, 100.6, 87.0, 83.6, 81.8, 73.6, 73.6, 71.9, 69.5; IR (KBr plate) 3033, 1682, 1455, 1279, 1098 cm^{-1}; HRMS (ESI-TOF) m/z calcd for $C_{23}H_{25}N_2O_6^+$ (M + H)$^+$ 425.1713, obsd m/z = 425.1712.

Preparation of 3. Dess−Martin reagent (2.2 g, 5.28 mmol) was added to a solution of alcohol **13** (0.71 g, 1.67 mmol) in anhydrous CH_2Cl_2 (12 mL) at 0 °C, and the mixture was stirred at 25 °C overnight. The reaction mixture was diluted with satd aq $NaHCO_3$ (20 mL) and satd aq $Na_2S_2O_3$ (20 mL) and stirred vigorously at 0 °C for 30 min. The mixture was then extracted with EtOAc (3 × 40 mL). The combined organic layers were washed with brine (30 mL), dried (Na_2SO_4), filtered, and concentrated under diminished pressure. The residue was resuspended in ether (3 × 50 mL) and filtered through a pad (3 × 3 cm) of 1:1 silica gel−anhydrous $MgSO_4$. The filtrate was concentrated under diminished pressure to obtain **3** as a colorless solid which was pure enough for subsequent reactions: crude yield 0.65 g (92%); silica gel TLC R_f 0.18 (1:1 ethyl acetate−hexanes); ^{1}H NMR ($CDCl_3$) δ 10.17 (d, J = 1.2 Hz, 1H), 7.42−7.24 (m, 11H), 7.18−7.07 (m, 1H), 5.67 (dd, J = 8.0, 1.9 Hz, 1H), 5.17 (s, 1H), 5.02 (d, J = 11.5 Hz, 1H), 4.66 (d, J = 11.5 Hz, 1H), 4.53 (s, 2H), 4.42 (d, J = 7.7 Hz,

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1H), 4.30−4.20 (m, 1H), 3.77−3.63 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 206.1, 163.8, 150.1, 143.8, 137.5, 136.8, 128.6, 128.5, 128.4, 128.3, 128.0, 103.2, 85.2, 79.2, 75.3, 73.6, 73.3, 70.0; IR (KBr plate) 3064, 1780, 1693, 1454, 1071 cm^{-1}; HRMS (ESI-TOF) m/z calcd for $C_{23}H_{23}N_2O_6^+$ (M + H)$^+$, 423.1556, obsd m/z = 423.1554.

Preparation of 14. Anhydrous hexanes (25 mL) was added to 200 mg (60% in oil, 5 mmol) of NaH, and the suspension was stirred at room temperature for 30 min at which time the hexanes were decanted off. The residual hexane was removed under reduced pressure; anhydrous DMSO (20 mL) and Me_3SOI (1.2 g, 5.88 mmol) were added. The reaction mixture was stirred at 25 °C for 30 min, diluted with THF (20 mL), and cooled to −10 °C. A solution of **3** (690 mg, 1.63 mmol) in THF (20 mL) was added dropwise, and the mixture was stirred at 0 °C for 1.5 h. The reaction mixture was diluted with H_2O (50 mL) and extracted with EtOAc (3 × 60 mL). The combined organic layers were washed with brine (60 mL), dried (Na_2SO_4), filtered, and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica column (25 × 3 cm). Elution with 2:3 → 1:1 ethyl acetate−hexanes gave **14** as a colorless oil: yield 380 mg (53%, with slight impurity which was used directly in the next step); silica gel TLC R_f 0.25 (1:1 ethyl acetate−hexanes); ^{1}H NMR ($CDCl_3$) δ 9.40 (s, 1H), 7.63 (d, J = 8.1 Hz, 1H), 7.42−7.27 (m, 10H), 6.37 (s, 1H), 5.49 (dd, J = 8.1, 2.1 Hz, 1H), 4.63−4.51 (m, 2H), 4.48 (dd, J = 11.6, 4.6 Hz, 2H), 4.26 (d, J = 6.4 Hz, 1H), 4.24−4.18 (m, 1H), 3.82 (dt, J = 9.3, 3.1 Hz, 1H), 3.61 (dd, J = 10.8, 2.9 Hz, 1H), 3.30 (d, J = 4.9 Hz, 1H), 2.99 (d, J = 4.9 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 162.9, 150.6, 141.3, 137.4, 137.1, 129.0, 128.7, 128.7, 128.6, 128.4, 128.27, 128.3, 128.1, 128.0, 102.3, 82.0, 80.7, 77.0, 73.7, 73.2, 68.6, 65.7, 48.7; IR (KBr plate) 3069, 1691, 1455, 1272, 1095, 696 cm^{-1}; HRMS (ESI-TOF) m/z calcd for $C_{24}H_{25}N_2O_6^+$ (M + H)$^+$, 437.1713, obsd m/z = 437.1713.

Preparation of 15. A mixture of epoxide **14** (380 mg, 0.87 mmol) and NaOAc (675 mg, 8.23 mmol) in glacial acetic acid (12 mL) was stirred at reflux for 3 h. The reaction mixture was cooled to room temperature, concentrated under diminished pressure, and diluted with EtOAc (80 mL) and satd aq $NaHCO_3$ (50 mL). The organic layer was separated, washed with sat aq $NaHCO_3$ (30 mL) and brine (40 mL), dried (Na_2SO_4), filtered, and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica column (25 × 3 cm). Elution with 1:1 → 3:2 ethyl acetate−hexanes gave **15** as a colorless oil: yield 350 mg (81%); silica gel TLC R_f 0.09 (1:1 ethyl acetate−hexanes); ^{1}H NMR ($CDCl_3$) δ 9.76 (s, 1H), 7.70 (d, J = 8.2 Hz, 1H), 7.42−7.21 (m, 10H), 6.12 (s, 1H), 5.41 (dd, J = 8.2, 1.7 Hz, 1H), 4.68 (d, J = 11.9 Hz, 1H), 4.62−4.35 (m, 6H), 4.17−4.09 (m, 1H), 4.06 (d, J = 4.8 Hz, 1H), 3.79 (dd, J = 10.6, 3.0 Hz, 1H), 3.54 (dd, J = 10.6, 2.5 Hz, 1H), 2.06 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 171.4, 163.9, 150.9, 141.8, 137.0, 136.8, 128.7, 128.6, 128.4, 128.3, 128.1, 101.2, 86.4, 81.9, 80.8, 80.4, 73.8, 72.7, 68.6, 64.3, 20.9; IR (KBr plate) 3064,1742, 1690, 1455, 1275, 1099 cm^{-1}; HRMS (ESI-TOF) m/z calcd for $C_{26}H_{29}N_2O_8^+$ (M + H)$^+$, 497.1924, obsd m/z = 497.1923.

Preparation of 16. Triethylsilyl triflate (0.7 mL, 0.97 g, 3.10 mmol) and 2,6-lutidine (0.45 mL, 0.41 g, 3.85 mmol) were added to a solution of alcohol **15** (350 mg, 0.71 mmol) in anhydrous CH_2Cl_2 (5 mL), and the mixture was stirred at 25 °C for 12 h. More triethylsilyl triflate (0.7 mL) and 2,6-lutidine (0.45 mL) were added, and the mixture was stirred at 25 °C for another 12 h. The reaction mixture was diluted with 5% aq $NaHCO_3$ (25 mL) at 0 °C and extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine (40 mL), dried (Na_2SO_4), filtered, and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica column (20 × 3 cm). Elution with 1:2 → 2:3 ethyl acetate−hexanes gave **16** as a colorless oil: yield 280 mg (65%); silica gel TLC R_f 0.44 (1:1 ethyl acetate−hexanes); ^{1}H NMR ($CDCl_3$) δ 9.46 (d, J = 1.8 Hz, 1H), 7.50 (d, J = 8.2 Hz, 1H), 7.43−7.19 (m, 10H), 6.17 (s, 1H), 5.56 (dd, J = 8.2, 2.2 Hz, 1H), 4.65−4.52 (m, 3H), 4.43 (d, J = 12.0 Hz, 1H), 4.28 (q, J = 12.4 Hz, 2H), 4.20 (d, J = 3.2 Hz, 1H), 3.88 (d, J = 3.2 Hz, 1H), 3.62 (dd, J = 10.4, 5.8 Hz, 2H), 1.96 (s, 3H), 0.78 (dd, J = 9.7, 6.2 Hz, 9H), 0.42 (qd, J = 7.9, 2.1 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 170.1, 163.5, 150.4, 142.6, 137.5, 136.8, 128.67, 128.66, 128.6, 128.4, 128.2, 128.22, 128.22, 128.18, 100.5, 86.8, 83.5, 82.8, 81.4, 73.7, 71.7, 69.7, 64.8, 20.9, 6.9, 6.0; IR (KBr plate) 2877, 1747, 1693, 1455, 1380, 1276, 1099 cm^{-1}; HRMS (ESI-TOF) m/z calcd for $C_{32}H_{43}N_2O_8Si^+$ (M + H)$^+$, 611.2789, obsd m/z = 611.2786.

Preparation of 17. EtMgBr (1.0 M soln in THF, 2.2 mL, 2.2 mmol) was added to a solution of **16** (280 mg, 0.46 mmol) in anhydrous Et_2O (8 mL) at −10 °C over 10 min, and the resulting mixture was stirred at 0 °C for 2 h. The reaction mixture was diluted with wet ether (50 mL) and 5% aq $NaHCO_3$ (20 mL). The ether layer was separated, and the aqueous layer was extracted with ether (30 mL). The combined ether layers were washed with brine (20 mL), dried (Na_2SO_4), filtered, and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica column (25 × 3 cm). Elution with 2:3 → 1:1 ethyl acetate−hexanes gave **17** as a colorless oil: yield 220 mg (84%); silica gel TLC R_f 0.32 (1:1 ethyl acetate−hexanes); ^{1}H NMR ($CDCl_3$) δ 8.89 (s, 1H), 7.49 (d, J = 8.2 Hz, 1H), 7.43−7.30 (m, 8H), 7.29−7.25 (m, 2H), 6.14 (s, 1H), 5.58 (dd, J = 8.2, 2.1 Hz, 1H), 4.60 (dt, J = 13.0, 11.8 Hz, 3H), 4.42 (d, J = 11.8 Hz, 1H), 4.20 (ddd, J = 6.1, 5.2, 3.4 Hz, 1H), 3.87 (dd, J = 18.9, 7.9 Hz, 2H), 3.80−3.57 (m, 3H), 0.76 (td, J = 7.9, 4.0 Hz, 9H), 0.48−0.35 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 163.1, 150.6, 142.4, 137.5, 136.7, 128.8, 128.6, 128.4, 128.3, 128.2, 128.1, 100.7, 87.1, 84.70, 84.65, 81.6, 73.6, 71.8, 69.7, 63.4, 6.9, 5.9; IR (KBr plate) 2955, 1693, 1455, 1275, 1101, 737, 698 cm^{-1}; HRMS (ESI-TOF) m/z calcd for $C_{30}H_{41}N_2O_7Si^+$ (M + H)$^+$, 569.2683, obsd m/z = 569.2685.

Preparation of 18. A suspension of Dess−Martin reagent (300 mg, 0.71 mmol) in anhydrous CH_2Cl_2 (3 mL) was added dropwise to a solution of alcohol **17** (220 mg, 0.39 mmol) in anhydrous CH_2Cl_2 (11 mL) at 0 °C, and the mixture was stirred at 25 °C for 1 h. The reaction mixture was diluted with satd aq $NaHCO_3$ (15 mL), satd aq $Na_2S_2O_3$ (15 mL), and Et_2O (50 mL) and stirred at 0 °C for 30 min. The ether layer was separated, washed with brine (10 mL), dried (Na_2SO_4), filtered, and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica column (20 × 3 cm). Elution with 2:3 → 1:1 ethyl acetate−hexanes gave **18** as a colorless oil: yield 172 mg (78%); silica gel TLC R_f 0.57 (1:1 ethyl acetate−hexanes); ^{1}H NMR ($CDCl_3$) δ 9.92 (s, 1H), 8.81 (s, 1H), 7.48−7.27 (m, 9H), 7.20 (dd, J = 6.7, 3.0 Hz, 2H), 6.51 (s, 1H), 5.60 (dd, J = 8.2, 2.1 Hz, 1H), 4.65−4.50 (m, 3H), 4.47−4.33 (m, 2H), 3.98 (d, J = 2.7 Hz, 1H), 3.65 (dd, J = 9.5, 5.7 Hz, 1H), 3.53 (dd, J = 9.5, 6.7 Hz, 1H), 0.79−0.68 (m, 9H), 0.55−0.33 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 198.3, 163.5, 150.4, 141.3, 137.4, 136.2, 128.7, 128.7, 128.4, 128.3, 128.2, 128.1, 101.1, 88.2, 86.9, 86.7, 82.5, 73.6, 72.0, 69.3, 7.0, 6.6; IR (KBr plate) 2876, 1736, 1693, 1455, 1278, 1096 cm^{-1}; HRMS (ESI-TOF) m/z calcd for $C_{30}H_{39}N_2O_7Si^+$ (M + H)$^+$, 567.2527, obsd m/z = 567.2523.

Preparation of 19. Benzylmagnesium chloride (0.15 mL, 1.0 M, 0.150 mmol) was added to dropwise to a solution of aldehyde **18** (24 mg, 0.042 mmol) in THF (1 mL) at 0 °C. The reaction mixture was stirred at the same temperature for 30 min and then at 25 °C for 2 h. The reaction was diluted with satd aq NH_4Cl (10 mL) and extracted with EtOAc (2 × 20 mL). The combined organic layer was washed with brine (10 mL), dried (Na_2SO_4), filtered, and concentrated under diminished pressure. The residue was purified by flash chromatography on a silica column (15 × 1 cm). The desired product (**19**) was usually isolated contaminated with **23** and other side products. Consequently, it was typically used directly in the next reaction. Elution with 15 → 25% ethyl acetate in dichloromethane gave **19** as a colorless oil: yield 5 mg (18%, single diastereomer); silica gel TLC R_f 0.15 (3:7 ethyl acetate−hexanes); ^{1}H NMR ($CDCl_3$) δ 8.04 (d, J = 1.3 Hz, 1H), 7.75 (d, J = 8.2 Hz, 1H), 7.33 (m, 11H), 7.23 (dd, J = 6.8, 2.1 Hz, 4H), 6.50 (s, 1H), 5.42 (dd, J = 8.2, 2.3 Hz, 1H), 4.63−4.49 (m, 4H), 4.40 (d, J = 7.6 Hz, 1H), 4.30−4.17 (m, 1H), 4.14 (d, J = 7.5 Hz, 1H), 3.83 (d, J = 2.3 Hz, 1H), 3.63 (d, J = 2.7 Hz, 1H), 2.90 (dd, J = 22.3, 6.6 Hz, 2H), 2.44−2.31 (m, 1H), 0.91−0.84 (m, 9H), 0.59 (dt, J = 8.7, 4.8 Hz, 6H); IR (KBr plate) 2876, 1682, 1455, 1270, 1083, 738, 697 cm^{-1}; HRMS (ESI-TOF) m/z calcd for $C_{37}H_{47}N_2O_7Si^+$ (M + H)$^+$, 659.3153, obsd m/z = 659.3148. TES-migrated side product (**23**); ^{1}H NMR ($CDCl_3$) δ 8.71 (s, 1H), 7.84 (d, J = 8.2 Hz, 1H), 7.38−7.27 (m, 10H), 7.26−7.18 (m, 5H), 6.33 (s, 1H), 5.45 (d, J = 8.2

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Hz, 1H), 4.55 (d, J = 11.4 Hz, 1H), 4.50−4.41 (m, 4H), 4.10 (dt, J = 5.6, 2.9 Hz, 1H), 3.90 (d, J = 5.1 Hz, 1H), 3.74 (dd, J = 10.5, 3.1 Hz, 1H), 3.50−3.44 (m, 1H), 3.33 (dd, J = 14.4, 6.1 Hz, 1H), 3.13 (s, 1H), 2.99 (dd, J = 14.3, 6.8 Hz, 1H), 0.86 (t, J = 8.0 Hz, 9H), 0.44 (m, 6H); ESI m/z calcd for $C_{37}H_{47}N_2O_7Si^+$ $(M + H)^+$, 659.3, obsd m/z = 658.9; m/z calcd for $C_{37}H_{48}N_2O_7SiNa^+$ $(M + Na)^+$, 681.3, obsd m/z = 681.1.

Preparation of 20. Dess−Martin reagent (0.16 g, 0.38 mmol) was added to a solution of alcohol **19** (42 mg, 0.064 mmol) in anhydrous CH_2Cl_2 (2 mL) at 0 °C, and the mixture was stirred at 25 °C overnight. The reaction mixture was diluted with satd aq $NaHCO_3$ (15 mL) and satd aq $Na_2S_2O_3$ (15 mL) and stirred vigorously at 0 °C for 30 min. The mixture was then extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with brine (30 mL), dried (Na_2SO_4), filtered, and concentrated under diminished pressure. The residue was resuspended in ether (3 × 50 mL) and filtered through a pad (3 × 3 cm) of 1:1 silica gel−anhydrous $MgSO_4$. The filtrate was concentrated under diminished pressure. The residue was purified by flash chromatography on a silica column (15 × 1 cm). Elution with 1:3 ethyl acetate−hexanes gave **20** as a colorless oil: yield 31 mg (23% from **18**); silica gel TLC R_f 0.26 (3:7 ethyl acetate−hexanes); ^{1}H NMR ($CDCl_3$) δ 8.74 (d, J = 1.9 Hz, 1H), 7.57 (d, J = 8.2 Hz, 1H), 7.42−7.28 (m, 9H), 7.23 (d, J = 1.6 Hz, 3H), 7.17−7.12 (m, 2H), 7.06 (dd, J = 7.3, 2.3 Hz, 2H), 6.61 (s, 1H), 5.58 (dd, J = 8.2, 2.3 Hz, 1H), 4.59−4.41 (m, 4H), 4.35 (q, J = 4.8 Hz, 1H), 4.27−4.12 (m, 2H), 4.08 (d, J = 5.2 Hz, 1H), 3.73−3.60 (m, 2H), 0.80−0.74 (m, 9H), 0.62−0.43 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 204.7, 163.1, 150.2, 142.2, 137.4, 136.4, 133.8, 130.3, 128.7, 128.7, 128.4, 128.4, 128.3, 128.2, 126.9, 101.1, 89.7, 88.1, 87.2, 81.5, 73.7, 73.0, 69.1, 46.7, 7.1, 6.6; IR (KBr plate) 2927, 1713, 1693, 1455, 1275, 1099, 738, 698 cm^{-1}; HRMS (ESI-TOF) m/z calcd for $C_{37}H_{45}N_2O_7Si^+$ $(M + H)^+$, 657.2996, obsd m/z = 657.2990.

Preparation of 21. A solution of TBAF·3H_2O (16 mg, 50.7 μmol) in THF (0.2 mL) was added dropwise to a solution of **20** (16 mg, 29.5 μmol) in THF (1 mL), and the mixture was stirred at 25 °C for 1 h (at which time silica TLC analysis indicated complete consumption of **20**). The reaction mixture was concentrated under diminished pressure. The residue was purified by flash column chromatography on a silica gel column (5 × 1 cm). Elution with 1:19 → 1:9 methanol−dichloromethane gave **21** as a colorless solid: yield 15 mg (60%); silica gel TLC R_f 0.05 (3:7 ethyl acetate−hexanes); ^{1}H NMR ($CDCl_3$) δ 8.96 (s, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.41−7.27 (m, 10H), 7.26−7.21 (m, 3H), 7.18−7.11 (m, 2H), 6.33 (s, 1H), 5.42−5.31 (m, 1H), 4.61 (s, 2H), 4.51 (d, J = 20.3 Hz, 2H), 4.42 (s, 1H), 4.23 (d, J = 24.4 Hz, 4H), 3.87−3.75 (m, 1H), 3.56 (d, J = 11.0 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 205.9, 163.2, 150.6, 141.5, 137.2, 136.5, 133.0, 130.1, 128.6, 128.6, 128.41, 128.39, 128.2, 127.9, 127.1, 101.4, 87.0, 86.7, 84.8, 80.3, 73.6, 73.2, 67.9, 45.8; IR (KBr plate) 2928, 1723, 1706, 1681, 1274, 1093, 697 cm^{-1}; HRMS (ESI-TOF) m/z calcd for $C_{31}H_{31}N_2O_7^+$ $(M + H)^+$, 543.2131, obsd m/z = 543.2131.

Preparation of 2a. Hydrogen gas was bubbled through a mixture of compound **21** (10 mg, 18.4 μmol) and a catalytic amount of 10% palladium on carbon (20 mg) in THF (5 mL) for 10 min and the mixture stirred under H_2 atmosphere for another 45 min. The suspension was filtered through a pad (2 × 3 cm) of Celite and concentrated under diminished pressure, and the residue was purified by flash column chromatography on a silica gel column (8 × 1 cm). Elution with 1:19 → 2:25 methanol−dichloromethane gave **2a** as a colorless foam: yield 4.5 mg (67%); silica gel TLC R_f 0.37 (1:9 methanol−dichloromethane); ^{1}H NMR (CD_3OD) δ 7.89 (d, J = 8.1 Hz, 1H), 7.33−7.13 (m, 5H), 6.28 (s, 1H), 5.69 (d, J = 8.1 Hz, 1H), 4.29 (d, J = 8.4 Hz, 1H), 4.09 (d, J = 3.1 Hz, 2H), 3.91 (dd, J = 12.3, 2.4 Hz, 1H), 3.83 (ddd, J = 8.4, 3.5, 2.3 Hz, 1H), 3.71 (dd, J = 12.4, 3.5 Hz, 1H); ^{13}C NMR (CD_3OD) δ 210.9, 166.3, 152.2, 143.8, 135.8, 131.3, 129.1, 127.5, 101.6, 88.3, 88.3, 83.0, 79.9, 60.8; IR (KBr plate) 3305, 1691, 1468, 1395, 1274, 1082 cm^{-1}; HRMS (ESI-TOF) m/z calcd for $C_{17}H_{19}N_2O_7^+$ $(M + H)^+$, 363.1192, obsd m/z = 363.1187.

Photolysis of 2a and Subsequent HPLC Analysis. Photolyses were carried out in Pyrex tubes using a Rayonet photochemical reactor equipped with a merry-go-round apparatus and 16 lamps having a maximum output at 350 nm. Reaction mixtures (50 μL each) containing **2a** (100 μM), thymidine (40 μM), and various amounts of thiol with or without buffer (10 mM phosphate, 100 mM NaCl, pH 7.2) were photolyzed at room temperature for 7 h under aerobic or anaerobic conditions. Samples for anaerobic reactions were degassed by three freeze−pump−thaw cycles at 2 mTorr and sealed under vacuum. The reaction mixtures (including unphotolyzed controls) were evaporated to dryness under vacuum, dissolved in 50 μL of water, and analyzed by reversed-phase HPLC while being monitored at 260 nm. HPLC was performed on an Agilent microsorb-MV C-18 column (250 × 4.6 mm) using water and acetonitrile as eluents from t = 0 to 9 min, from 3% → 28% ACN, and then from 28% → 97% ACN over 5 min. Peaks corresponding to uracil, uridine, arabinouridine (**25**), thymidine, and **2a** eluted at 4.1, 5.6, 7.3, 8.9, and 14.3 min, respectively. The peaks were integrated and quantified against the internal standard thymidine. The response factors calculated for uracil, arabinouridine (**25**), uridine, and **2a** were 1.32, 0.83, 0.96, and 1.13, respectively.

■ ASSOCIATED CONTENT

Supporting Information

NMR spectra of compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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