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Direct Measurement of Pyrimidine C6-hydrate Stability

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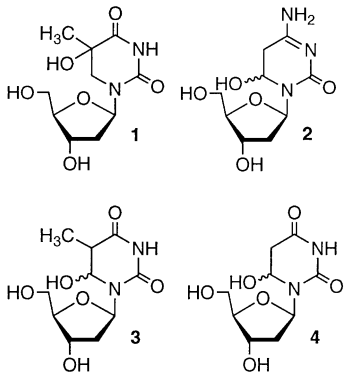
Dedicated to Professor Peter B. Dervan.

Abstract—Pyrimidine C6-hydrates are produced via UV-irradiation and undergo dehydration upon standing. The stability of these compounds has a direct bearing on their genotoxicity. The rate constants for elimination from 5′-benzyoylated derivatives of 5,6-dihydro-5-hydroxythymidine (**6**) and 5,6-dihydro-5-hydroxy-2′-deoxyuridine (**9**) were measured directly via HPLC. The rate constants for dehydration increase from pH 6.0 to 8.0. The half-lives for **6** and **9** at pH 7.4 and 37 °C are 46.5 and 24.4 h, respectively. Deglycosylation is not observed, even upon heating at 90 °C. These observations reinforce proposals that pyrimidine hydrates are sufficiently long-lived that they can exert significant effects on biological systems.

Pyrimidine hydrates are produced by a variety of reaction pathways that are populated when nucleic acids are exposed to ionizing radiation.[1] Thymidine C5-hydrate (**1**) is produced by hydroxyl radical addition under O_2 deficient conditions.[1,2] The C6-hydrates of deoxycytidine (**2**) and thymidine (**3**) are the major non-dimeric lesions produced upon UV-irradiation.[3,4] 5,6-Dihydro-6-hydroxy-2′-deoxyuridine (**4**) is produced in DNA from hydrolysis of the C6-hydrate of dC (**2**).[5–7] The C6-hydrates may also be produced upon hydration of nucleobase cation radicals.[8] The biological relevance of pyrimidine hydrates is underscored by their excision by base excision repair enzymes, such as endonuclease III.[5–7,9–12] Furthermore, thymidine C5-hydrate (**1**) is a potent inhibitor of DNA polymerase I (Klenow exo$^-$ fragment) and **2** is a premutagenic lesion in vitro.[13] The pyrimidine C6-hydrates undergo dehydration, and their chemical instability potentiates their ability to influence the fidelity of replication and transcription. We wish to report on our studies concerning the direct measurement of dehydration in 5′-protected pyrimidine C6-hydrates.

Our interest in the chemical stability of pyrimidine C6-hydrates was cultivated by our studies on 5,6-dihydropyrimidin-6-yl radicals (e.g., **5**) from which pyrimdine C6-hydrates are formed under aerobic conditions. Investigations of radical mediated DNA damage necessitated that we understand the lability of **3** and **4**. Direct measurement of dehydration rates of thymine and uracil C6-hydrates have been reported.[3] However, we were uncertain if one could safely extrapolate from studies on free bases where N-1 is not alkylated to nucleosides, and in turn, DNA. Dehydration rates of thymidine and deoxyuridine C6-hydrates have been measured in DNA using a combined enzymatic and HPLC assay.[6,7,11] In this assay, the amount of modified free base that is released by endonuclease III is measured by HPLC as a function of time. However, this approach would obscure any deglycosylation, which may compete with dehydration. Consequently, we set out to determine if deglycosylation competes with dehydration in nucleoside C6-hydrates, and to measure rates of dehydration directly in **3** and **4**.



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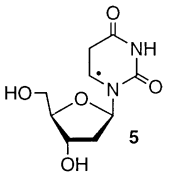
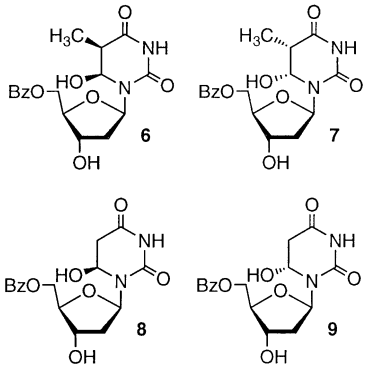


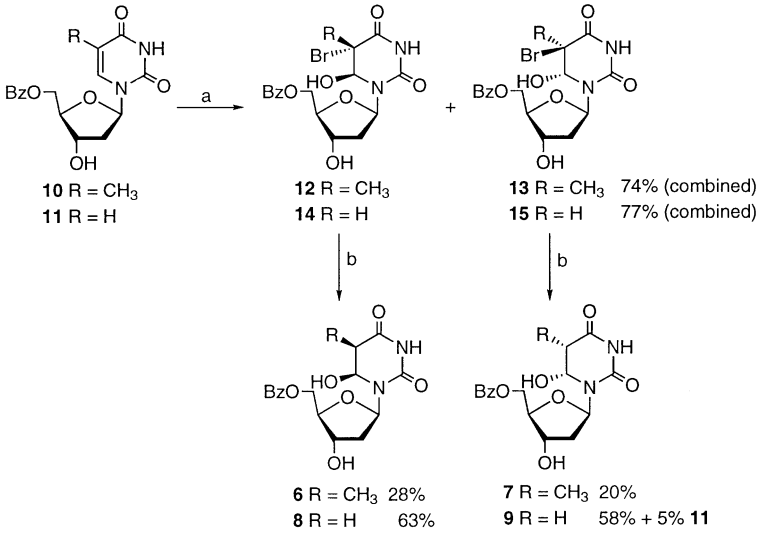
Results and Discussion

Chemical stability studies of single diastereomers of **3** and **4** were carried out using 5′-benzoyl derivatives. The 5′-benzoyl group was incorporated in order to enhance detection by UV-absorption, to facilitate purification, and to remove any possible role that a free 5′-hydroxyl group might play in the chemical stability of the C6-hydrates. There is precedent for involvement of the primary hydroxyl group in the rearrangement of formamidopyrimidine deoxynucleosides at pH 7 and dihydropyrimidines under acidic conditions.[14,15] Such reactions diminish the relevance of monomer studies to DNA where the presence of the 5′-phosphate diester group prohibits rearrangements of this type.



Single diastereomers of benzoylated C6-hydrates of thymidine (**6** and **7**) and 2′-deoxyuridine (**8** and **9**) were synthesized via modification of procedures reported for the free nucleosides.[16] High yields of the bromohydrin precursors were obtained using *N*-bromosuccinimide as a source of Br_2 and $CaCO_3$ to buffer the reactions (Scheme 1). Following separation of the diastereomeric *trans*-bromohydrins, the respective benzoylated C6-hydrates were obtained via Zn^0/AcOH reduction. Isolated yields of 5,6-dihydro-6-hydroxy-2′-deoxyuridines (**8**, **9**) were compromised due to facile elimination under the reaction and purification conditions. Careful chromatography provided the *6S*-diastereomer (**8**) without any contamination of 5′-benzoyl-2′-deoxyuridine (**11**); whereas samples of **9** were contaminated by ~5% of **11.**

Stereochemistry in the dihydropyrimidine rings of the bromohydrins of both pyrimidines and C6-hydrates of thymidine were determined by ^{1}H NMR spectroscopy using analogous compounds as a frame of reference. Examination of the ^{1}H NMR spectra of the bromohydrins and C6-hydrates of deoxyuridine, as well as the 6-hydroperoxy-5-hydroxydihydrothymidines and 5-hydroperoxy-6-hydroxydihydrothymidines consistently indicated a greater difference in the chemical shifts of the diastereotopic $H_{2'}$-protons in the 6*S*-diastereomers than the 6*R*-diastereomers.[17,18] The pro-*S* $H_{2'}$-proton is consistently shifted further downfield relative to the respective pro-*R* proton in the 6*S*-diastereomers than in the 6*R*-diastereomers. Stereochemical assignments of the modified thymidines mentioned above were corroborated by their reduction to the thymidine glycols, for which single crystal X-ray diffraction data is available.[19] This chemical shift pattern was identified in diastereomeric pairs of bromohydrins (**12**–**15**) and 5′-benzoyl C6-hydrates (**6**–**9**) (Table 1). NOE experiments were carried out on **6**, **7**, and **12**–**15** in order to provide additional evidence for these stereochemical assignments. The results of these experiments were consistent with the interpretation of the chemical shift pattern. When comparing pairs of diastereomers (e.g., **6** and **7**) irradiation of H_6 produced a larger NOE at the pro-*S* $H_{2'}$-proton in the compound believed to contain the *R*-configuration at the C6-position (based upon chemical shifts of $H_{2'}$-protons) and a smaller NOE at the $H_{5'}$-protons. These qualitative trends were also observed in the other two pairs of diastereomers that were examined (Table 1).



Scheme 1. (a) NBS, $CaCO_3$, THF/H_2O; (b) Zn/HOAc, THF/H_2O.

Epimerization of 5,6-dihydro-6-hydroxy-2′-deoxyuridine

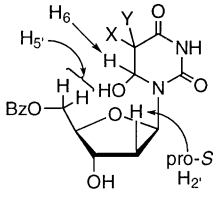
At 37 °C, in the absence of a general base catalyst, hydrates **8** and **9** underwent epimerization with negligible dehydration to form an equilibrium mixture (**9**:**8** = 1.5) of diastereomers. The approach to equilibrium was determined by ^{1}H NMR using the C6-protons to measure the concentrations of each epimer. The mixture of diastereomers was independent of which side of the equilibrium one approaches from, and the absolute rate constants were within 25% of each other (Table 2). Thymidine C6-hydrates (**6** and **7**) also epimerized much more rapidly than they underwent dehydration in the presence or absence of catalyst. Epimerization at C6 was accompanied by isomerization at the C5-position, resulting in four diastereomers from one original isomer. Separation of these isomers by HPLC and resolution of signals in ^{1}H NMR were insufficient to allow analysis of the kinetics of these processes.

Deglycosylation and dehydration in pyrimidine C6-hydrates.

Previous measurements of dehydration in pyrimidine C6-hydrates utilized endonuclease III to hydrolyze the glycosidic bond of the modified nucleotide, followed by HPLC quantitation of the amount of damaged nucleobases released. In some instances these experiments were carried out at high temperatures (up to 80 °C) in order to decrease the time span of the investigation.[5–7,11,12] We considered the possibility that the absolute rate constants determined in these experiments may be affected by deglycosylation. If the rate of dehydration differs in DNA from that of the free base, deglycosylation would result in the measurement of a rate constant for dehydration that is a weighted-average of the two processes. In order to check for deglycosylation from the 5′-benzoylated nucleosides, 5-benzoyl-2-deoxyribose (**16**) was independently prepared as a mixture of α,β-anomers via reduction of the respective 2-deoxyribonolactone. The lactol (**16**) was analyzed for in samples of **6** and **8** that were heated at 90 °C for 1 h in phosphate buffer (20 mM, pH 7.4). Thermolysis of 6 and 8 produces the dehydrated derivatized nucleosides as the sole observed products within 1 h, in 71 and 72% yield, respectively (data not shown). Spiking samples with **16** prior to thermolysis indicates that as little as 4% of the deglycosylation product could have been observed. A lower detection limit is not possible as **16** undergoes significant decomposition under these conditions. Furthermore, based upon the mass balances observed, we cannot exclude the formation of other deoxyribose-derived products.

Dehydration of 5′-benzoylated thymidine C6-hydrate (**6**) was followed by HPLC as a function of pH, water content, and buffer concentration (Table 3, Fig. 1). Epimerization was faster than dehydration, but it was not possible to determine rate constants for elimination from individual stereoisomers. The effects of pH are consistent with previous studies on the stability of pyrimidine hydrates. Maximal stability was observed at pH 6.0, where essentially no decomposition was observed

Table 1. NMR determination of stereochemistry in 5′-benzoyl dihydropyrimidines[a]

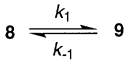


	X	Y
6, **7**	H	CH_3
12, **13**	Br	CH_3
14, **15**	H	Br

Compd			NOE	
	C6-Configuration	$\Delta H_{2'}$-Chemical Shift[b]	$H_6 \rightarrow$pro-*S* $H_{2'}$	$H_6 \rightarrow H_{5'}$
6	*S*	0.34	2.76	0.83
7	*R*	0.10	3.18	0.59
12	*S*	0.33	2.28	0.60
13	*R*	0.26	3.55	0.87[c]
14	*S*	0.26	2.27	0.96
15	*R*	0.11	2.86	0.63

[a]First proton listed is irradiated and second is where effect is measured.
[b]$\Delta H_2' = \delta_{\text{pro-S H2'}} - \delta_{\text{pro-R H2'}}$.
[c]The enhancement could not be distinguished from that at H_3' due to poor chemical shift dispersion.

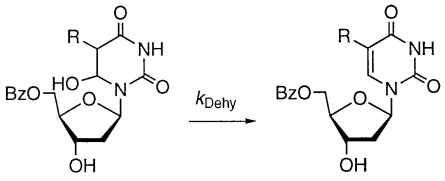
Table 2. The rate of epimerization of 5′-*O*-benzoyl-5,6-dihydro-6-hydroxy-2′-deoxyuridines (**8** and **9**)



Starting diastereomer	$k_1 \times 10^6$ (s^{-1})[a]	$k_{-1} \times 10^6$ (s^{-1})
8	11.2	7.6
9	8.3	5.7

[a]Rate constants are an average of two separate experiments.

Table 3. Rate constants for dehydration of 5′-benzoylated pyrimidine C6-hydrates at 37 °C



6 R = CH_3 **10** R = CH_3
9 R = H **11** R = H

Compound	pH	$[KH_2PO_4]$ (mM)	% CH_3CN[a]	$k_{Dehy} \times 10^6$ (s^{-1})[a]	$t_{1/2}$ (h)
6	6.0	20	5	< 0.4	> 785
6	7.4	20	5	4.1	46.5
6	8.0	20	5	11.3	17.0
6	7.4	40	5	4.7	40.6
6	6.0	20	50	< 0.2	> 1046
6	7.4	20	50	2.9	66.3
6	8.0	20	50	5.9	32.6
9	6.0	20	5	~0.5	~385
9	7.4	20	5	7.9	24.4
9	8.0	20	5	26.8	7.2

[a]Rate constants are the composite of four separate experiments.

over the course of 5 days. The rate constant for dehydration increased significantly as the pH was increased to 8.0. The dehydration reaction also showed a dependence on buffer concentration, which is consistent with general base catalysis. Finally, the decrease in half-life for **6** as the solvent polarity increased from 50% H_2O to 95% H_2O is also consistent with the anticipated negative charge buildup in the transition state.

Similar behavior was observed for the C6-hydrate of 2′-deoxyuridine (**9**). As predicted based upon previous experiments in biopolymers, dehydration from **9** was significantly faster than from the thymidine analogue (Table 3). The higher rate of dehydration observed in the 2′-deoxyuridine analogue compared to **6** is consistent with the anticipated higher acidity of the C5-proton in these compounds. The half-life for elimination at 37 °C was 24.4 and 7.2 h at pH 7.4 and 8.0, respectively. This is very close to that measured for 5,6-dihydro-6-hydroxy-2′-deoxyuridine in DNA when it is base-paired to 2′-deoxyadenosine.[6]

Conclusion

These studies support previous investigations regarding the stability of pyrimidine C6-hydrates in DNA, indicating that the repair of such lesions is important in order to avoid their potential deleterious biological effects. In addition, the facile and quantitative dehydration of **6**–**9** at higher temperatures indicates that this reaction will be a useful tool for manipulating tandem nucleic acids containing these lesions.

Experimental

All reactions were carried out in oven-dried glassware under an atmosphere of argon or nitrogen unless otherwise noted. THF was freshly distilled from Na/benzophenone ketyl. Dichloromethane, DMF, and triethylamine were distilled from CaH_2. Acetonitrile was passed through $CuSO_4$ and then distilled from CaH_2. *N*-Bromosuccinimide (NBS) was recrystallized from H_2O.

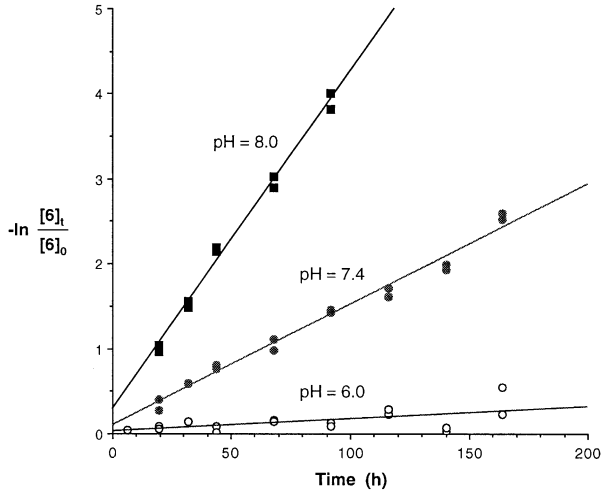


Figure 1. Disappearance of **6** as a function of time at 37 °C.

5,6-Dihydro-5-bromo-6-hydroxy-2′-deoxyuridine (14 and 15). $CaCO_3$ (407 mg, 4.06 mmol) and NBS (578 mg, 3.25 mmol) were added to solution of 5′-benzoyl-2′-deoxyuridine (**11**, 900 mg, 2.71 mmol) in 3:1 THF/H_2O (21 mL) at 0 °C. The solution was allowed to warm to ambient temperature and stirred for 6 h. The reaction mixture was filtered through Celite and concentrated. The crude mixture was subjected to silica gel flash chromatography (2–10% MeOH/$CHCl_3$) to yield **15** (489 mg, 42%) and **14** (407 mg, 35%) as white foams. **15**: ^{1}H NMR (MeOH-d_4) δ 8.07–8.03 (m, 2H), 7.67–7.61 (m, 1H), 7.53–7.48 (m, 2H), 6.25 (t, J = 6.6 Hz, 1H), 5.31 (d, J = 2.4 Hz, 1H), 4.53 (d, J = 4.2 Hz, 2H), 4.46–4.42 (m, 1H), 4.21 (d, J = 2.4 Hz, 1H), 4.14 (dd, J = 8.1, 4.2 Hz), 2.31–2.27 (m, 1H), 2.20–2.16 (m, 1H); ^{13}C NMR (MeOH-d_4) δ 168.1, 167.9, 152.4, 134.6, 131.2, 130.7, 129.9, 86.1, 85.4, 77.9, 72.5, 66.0, 41.8, 40.2; IR (film) 3431, 1700, 1465, 1273, 1087 cm^{-1}; HR-MS (FAB) calcd (M + H) 431.0277, found 431.0285. 14: ^{1}H NMR (MeOH-d_4) δ 8.07–8.05 (m, 2H), 7.65–7.60 (m, 1H), 7.52–7.47 (m, 2H), 6.24 (t, J = 6.9 Hz, 1H), 5.28 (d, J = 2.1 Hz, 1H) 4.54–4.41 (m, 3H), 4.17 (d, J = 2.1 Hz, 1H), 4.14–4.10 (m, 1H), 2.47–2.38 (m, 1H), 2.19–2.14 (m, 1H); ^{13}C NMR (MeOH-d_4) δ 168.2, 167.9, 152.3, 134.6, 131.3, 130.8, 129.8, 85.6, 84.7, 77.6, 72.4, 65.8, 41.4, 38.9; IR (film) 3396, 1698, 1452, 1276, 1090 cm^{-1}; HR-MS (FAB) calcd (M + H) 431.0277, found 431.0279.

5,6-Dihydro-5-bromo-6-hydroxythymidine (12 and 13). To a solution of 5′-benzoyl thymidine (**10**, 1.00 g, 2.89 mmol) in 3:1 THF/H_2O (28 mL) at 0 °C was added $CaCO_3$ (434 mg, 4.33 mmol) and NBS (616 mg, 3.46 mmol) as described above for the preparation of **14** and **15**. The reaction mixture was stirred for 0.5 h at 0 °C and then allowed to warm to ambient temperature where it was stirred for an additional 2 h. The reaction mixture was filtered through Celite and concentrated. The crude reaction mixture was purified via silica gel flash chromatography (2–10% MeOH/$CHCl_3$) to afford **12** (701 mg, 55%) and **13** (247 mg, 19%) as a partially separable mixture of diastereomers. **12**: ^{1}H NMR (MeOH-d_4) δ 8.09–8.07 (m, 2H), 7.65–7.61 (m, 1H), 7.52–7.48 (m, 2H), 6.25 (t, J = 5.5 Hz, 1H), 5.15 (s, 1H), 4.56 (dd, J = 9.0, 3.0 Hz, 1H), 4.13–4.10 (m, 1H), 2.48–2.42 (m, 1H), 2.19–2.14 (m, 1H), 1.69 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.7, 165.7, 150.7, 133.5, 129.6, 129.2, 128.8, 84.0, 83.1, 78.9, 70.6, 65.3, 55.3, 38.1, 22.5; IR (film) 3446, 1701, 1466, 1273, 1070 cm^{-1}; HR-MS (FAB) calcd 445.0433 (M + H), found 445.0447. 13: ^{1}H NMR (MeOH-d_4) δ 8.09–8.07 (m, 2H), 7.67–7.63 (m, 1H), 7.54–7.50 (m, 2H), 6.29 (t, J = 5.2 Hz, 1H), 5.17 (s, 1H), 4.62 (dd, J = 9.0, 2.4 Hz, 1H), 4.51–4.47 (m, 2H), 4.18–4.15 (m, 1H), 2.45–2.39 (m, 1H), 2.22–2.16 (m, 1H), 1.64 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 167.8, 165.6, 150.4, 133.5, 129.6, 129.4, 128.8, 83.2, 82.5, 78.8, 70.2, 64.3, 54.3, 37.1, 22.5; IR (film) 3396, 1700, 1452, 1275, 1087 cm^{-1}, HR-MS (FAB) calcd 445.0433 (M + H), found 445.0438.

5*R*,6*S*-Dihydro-6-hydroxy thymidine (6). To a solution of 5,6-dihydro-5-bromo-6-hydroxy thymidine **12** (100 mg, 0.226 mmol) in 3:1 THF/H_2O at 0 °C was added zinc dust (46 mg, 1.31 mmol) in acetic acid (24 mg, 0.41 mmol). The reaction was stirred at 0 °C for 20 min, filtered through Celite and concentrated at ambient temperature. The crude reaction mixture was purified via silica gel flash chromatography (2% MeOH/$CHCl_3$) to afford **6** (23 mg, 28%) as a white residue; ^{1}H NMR (MeOH-d_4) δ 8.08–8.05 (m, 2H), 7.66–7.61 (m, 1H), 7.53–7.48 (m, 2H), 6.27 (t, J = 6.3 Hz, 1H), 5.10 (d, J = 3.0 Hz, 1H), 4.56–4.43 (m, 3H), 4.06 (dd, J = 8.7, 3.9 Hz, 1H), 2.73–2.65 (m, 1H), 2.53–2.43 (m, 1H), 2.18–2.10 (m, 1H), 1.07 (d, J = 6.9 Hz, 3H); ^{13}C NMR (MeOH-d_4) δ 173.8, 167.9, 154.0, 134.6, 131.2, 130.8, 129.9, 85.5, 84.5, 76.8, 72.5, 65.7, 43.1, 38.5, 10.5; IR (film) 3445, 3231, 2929, 2857, 1709, 1462, 1256, 1215, 1125, 1028, 835; HR-MS (FAB) calcd (M + H) 365.1349, found 365.1352.

5*S*,6*R*-Dihydro-6-hydroxy thymidine (7). Using the same general procedure for the preparation of **7**, **13** (100 mg, 0.226 mmol) was treated with zinc dust (46 mg, 1.31 mmol) and acetic acid (24 mg, 0.41 mmol). Purification of the crude product by silica gel flash chromatography (2% MeOH/$CHCl_3$) afforded **7** (16 mg, 20%) as a white solid; ^{1}H NMR (MeOH-d_4) δ 8.07–8.04 (m, 2H), 7.66–7.61 (m, 1H), 7.52–7.48 (m, 2H), 6.19 (t, J = 6.6 Hz, 1H), 5.10 (d, J = 3.3 Hz, 1H), 4.62–4.44 (m, 3H), 4.13–4.11 (m, 1H), 2.76–2.72 (m, 1H), 2.32–2.28 (m, 1H), 2.22–2.19 (m, 1H), 1.04 (d, J = 7.2 Hz, 3H); ^{13}C NMR (MeOH-d_4) 173.9, 168.0, 154.4, 134.7, 131.3, 130.8, 129.9, 86.7, 85.3, 78.0, 72.6, 65.9, 42.6, 40.2, 10.6; IR (film) 3417, 2924, 1714, 1472, 1276, 1251, 711; HR-MS (FAB) calcd (M + H) 365.1349, found 365.1347.

6*S*-5,6-Dihydro-6-hydroxy-2′-deoxyuridine (8). Using the same procedure for the reduction of **15** (see below), 5,6-dihydro-5-bromo-6-hydroxy-2′-deoxyuridine (**14**, 75 mg, 0.17 mmol) was treated with zinc dust (66 mg, 1.0 mmol) in acetic acid (19 mg, 0.31 mmol). The crude product was purified via silica gel flash chromatography (1–6% MeOH/$CHCl_3$) to afford **8** (42 mg, 63% overall) as a white foam; ^{1}H NMR (MeOH-d_4) δ 8.06–8.04 (m, 2H), 7.65–7.60 (m, 1H), 7.52–7.47 (m, 2H), 6.23 (t, J = 6.0 Hz, 1H), 5.31 (dd, J = 3.9, 2.1 Hz, 1H), 4.56–4.41 (m, 3H), 4.07 (dd, J = 9.3, 4.2 Hz, 1H), 2.77 (dd, J = 16.8, 3.9 Hz, 1H), 2.54–2.44 (m, 2H), 2.18–2.10 (m, 1H); ^{13}C NMR (MeOH-d_4) δ 174.3, 171.3, 167.9, 153.7, 134.6, 131.2, 130.8, 129.9, 85.7, 84.5, 73.1, 72.4, 65.7, 40.7, 38.7; IR (film) cm^{-1} 3392, 1700, 1472, 1274, 1067, 711 cm^{-1}.

***6R*-5,6-Dihydro-6-hydroxy-2′-deoxyuridine (9).** To a solution of 5,6-dihydro-5-bromo-6-hydroxy-2′-deoxyuridine (**15**, 75 mg, 0.17 mmol) in 3:1 THF/H_2O at 0 °C was added zinc dust (66 mg, 1.0 mmol) and acetic acid (19 mg, 0.31 mmol). The reaction was stirred 20 min at 0 °C, filtered through Celite and concentrated at ambient temperature. The crude reaction mixture was purified via silica gel flash chromatography (1–6% MeOH/$CHCl_3$) to afford an 11.5:1 mixture of **9** and 5′-benzoyl-2′-deoxyuridine (**11**, 42 mg, 63% overall) as a white foam; ^{1}H NMR (MeOH-d_4) δ 8.05–8.01 (m, 2H), 7.65–7.59 (m, 1H), 7.51–7.47 (m, 2H), 6.17 (t, J = 6.9 Hz, 1H), 3.52 (dd, J = 4.1, 2.1 Hz, 1H), 4.53–4.41 (m, 3H), 4.10 (dd, J = 9.0, 4.5 Hz), 2.82 (dd, J = 17, 4.1 Hz, 1H), 2.53 (dd, J = 17, 2.1 Hz, 1H) 2.35–2.28 (m, 1H), 2.21–2.16 (m, 1H); ^{13}C NMR (MeOH-d_4) δ 171.4, 168.0, 153.9, 142.1, 134.6, 131.2, 130.7, 129.8, 86.9, 85.3, 74.5, 72.6, 65.9, 39.8; IR (film) 3446, 1700, 1472, 1275, 1069, 757 cm^{-1}.

5-Benzoyl-2-deoxyribonolactone (17). To a solution of 2-deoxyribonolactone[20] (230 mg, 1.74 mmol) and Et_3N (229 mg, 2.26 mmol) in DMF (15 mL) at −40 °C was added benzoyl cyanide (274 mg, 2.09 mmol). The reaction was stirred overnight and allowed to warm to ambient temperature. The reaction was quenched with H_2O (4 mL), diluted with EtOAc (50 mL), washed with H_2O (3×50 mL), brine (50 mL) dried over Na_2SO_4, and concentrated. The crude product was purified via silica gel flash chromatography (1:2–1:1, EtOAc/hexanes) to afford **17** (127 mg, 31%) as a clear oil. ^{1}H NMR ($CDCl_3$) δ 7.99–7.96 (m, 2H), 7.62–7.57 (m, 1H), 7.48–7.45 (m, 2H), 4.70–4.68 (m, 1H), 4.61–4.55 (m, 3H), 3.08 (s, J = 3 Hz, 1H), 2.95 (dd, J = 18, 6.9 Hz, 1H), 2.62 (dd, J = 18 Hz, 3.9 Hz, 1H), IR (film) 3461, 2950, 1601, 1451, 1781, 1720, 1176, 1164, 1026, 944 cm^{-1}.

5′-Benzoyl-2′-deoxyribose (16). A toluene solution of DIBAL (2.0 mL, 1.0 M.) was added via syringe pump overnight to a solution of **17** (496 mg, 2.09 mmol) in CH_2Cl_2 (20 mL) maintained at −78 °C. The reaction was quenched while cold with MeOH (1 mL) and allowed to warm to ambient temperature. The reaction mixture was diluted with CH_2Cl_2 (80 mL) and washed with saturated Rochelle salt solution (100 mL), brine (100 mL), and concentrated in vacuo. The crude residue was purified via silica gel flash chromatography (1:3–1:1 EtOAc/CH_2Cl_2) to afford both anomers of **16** (22 mg, 4.4%) as a clear oil. ^{1}H NMR ($CDCl_3$) δ 8.07–8.00 (m, 2H), 7.60–7.54 (m, 1H), 7.46–7.41 (m, 2H), 5.64 (t, J = 4.5 Hz, 1H), 4.58–4.54 (m, 1H), 4.49 (d, J = 5.1 Hz, 0.6H), 4.37–4.32 (m, 2H), 4.20 (q, J = 5.1 Hz, 0.4H), 3.71 (d, J = 5.1 Hz, 0.7H), 3.48 (s, 0.3 Hz), 3.11 (d, J = 8.1 Hz, 0.7H), 2.47 (s, 0.3H), 2.98–2.14 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 166.9, 166.6, 133.5, 129.9, 129.8, 128.7, 99.6, 98.9, 85.3, 73.5, 72.6, 65.8, 64.8, 42.5, 41.5; IR (film) 3418, 2926, 1715, 1468, 1385, 1277, 1070, 1026 cm^{-1}; ESI-MS, 237.1 (M−H).

Analysis of pyrimidine hydrate decomposition. Aliquots were taken at appropriate times from a 1 mM solution (95/5 20 mM KH_2PO_4/CH_3CN) of the respective pyrimidine hydrate maintained at 37 or 90 °C. An internal standard (10 μL of a 1 mM solution of **10** for 2′-deoxyuridine hydrates, *p*-methoxybenzyl alcohol for thymidine hydrates) was added and the samples were diluted with 1:1 40 mM KH_2PO_4, pH 6.0/CH_3CN to inhibit further decomposition.

^{1}H NMR analysis of 2′-deoxyuridine hydrate epimerization. Hydrate **8** or **9** (10 mg, 0.028 mmol) and internal standard (*i*-PrOH, 0.3 mg, 0.005 mmol) were dissolved in 2.8:1 D_2O/CD_3CN (0.95 mL). Samples were placed in

NMR tubes and incubated in a 37 °C bath. ^{1}H NMR spectra were collected at appropriate time intervals. The rate of epimerization was determined by observing the change in integration of the C-6 hydrogen peaks (δ 5.29 for 9, δ 5.22 for 8).

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References and Notes

1. von Sonntag, C. *The Chemical Basis of Radiation Biology*; Taylor & Francis: New York, 1987.
2. Barvian, M. R.; Barkley, R. M.; Greenberg, M. M. *J. Am. Chem. Soc*. **1995**, *117*, 4894.
3. Fisher, G. J.; Johns, H. E. In *Photochemistry & Photobiology of Nucleic Acids*; Wang, S. Y., Ed.; Academic: New York, 1976; Vol. 1, pp 169–224.
4. Cadet, J.; Vigny, P. In *Bioorganic Photochemistry*; Morrison, H., Ed.; Wiley Interscience: New York, 1990; Vol. 1, pp 1–272.
5. Boorstein, R. J.; Hilbert, T. P.; Cadet, J.; Cunningham, R. P.; Teebor, G. W. *Biochemistry* **1989**, *28*, 6164.
6. Boorstein, R. J.; Hilbert, T. P.; Cunningham, R. P.; Teebor, G. W. *Biochemistry* **1990**, *29*, 10455.
7. O'Donnell, R. E.; Boorstein, R. J.; Cunningham, R. P.; Teebor, G. W. *Biochemistry* **1994**, *33*, 9875.
8. Shaw, A. A.; Voituriez, L.; Cadet, J.; Gregoli, S.; Symons, M. C. R.. *J. Chem. Soc., Perkin Trans*. **1988**, *2*, 1303.
9. David, S. S.; Williams, S. D. *Chem. Rev*. **1998**, *99*, 1221.
10. Dizdaroglu, M.; Bauche, C.; Rodriguez, H.; Laval, J. *Biochemistry* **2000**, *39*, 5586.
11. Ganguly, T.; Duker, N. J. *Nucleic Acids Res*. **1991**, *19*, 3319.
12. Ganguly, T.; Weems, K. M.; Duker, N. J. *Biochemistry* **1990**, *29*, 7222.
13. Greenberg, M. M.; Matray, T. J. *Biochemistry* **1997**, *36*, 14071.
14. Raoul, S.; Bardet, M.; Cadet, J. *Chem. Res. Toxicol*. **1995**, *8*, 924.
15. Cadet, J.; Teoule, R. *Carbohydrate Res*. **1973**, *29*, 345.
16. Cadet, J.; Ducolomb, R.; Teoule, R. *Tetrahedron* **1977**, *33*, 1603.
17. Cadet, J.; Kan, L.-S.; Wang, S. Y. *J. Am. Chem. Soc*. **1978**, *100*, 6715.
18. Wagner, J. R.; van Lier, J. E.; Berger, M.; Cadet, J. *J. Am. Chem. Soc*. **1994**, *116*, 2235.
19. Hruska, F. E.; Sebastian, R.; Grand, A.; Voituriez, L.; Cadet, J. *Can. J. Chem*. **1987**, *65*, 2618.
20. Deriaz, R. E.; Overend, W. G.; Stacey, M.; Teece, E. G.; Wiggins, L. F. *J. Chem. Soc*. **1949**, 1879.