Diastereoselective Synthesis of Hydroxylated Dihydrothymidines Resulting from Oxidative Stress

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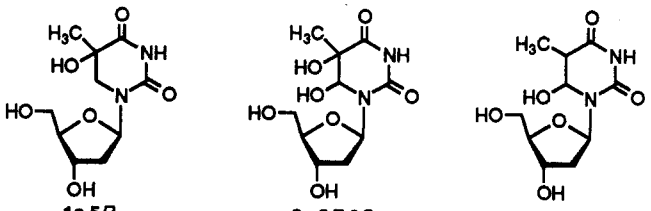
Pyrimidine nucleobases are altered by reaction with oxygen free radicals generated by ionizing radiation, as well as by redox active metal ions and their chelates. 5,6-Dihydro-5-hydroxythymidine (1), *cis*- and *trans*-5,6-dihydro-5,6-dihydroxythymidine (2), and 5,6-dihydro-6-hydroxythymidine (3) are three of the major products formed when thymidine, or biopolymers containing this nucleoside, are exposed to such oxidizing conditions.[1] There is evidence that suggests that one or more of these damaged nucleobases may be mutagenic.[2] Thymidine C6-hydrate 3 is unstable, and reverts to thymidine, perhaps lessening its importance as a mutagen.[1a] Site-specific incorporation of an unknown mixture of diastereomers of 2 into a judiciously designed oligonucleotide was accomplished by reacting an oligonucleotide that contained a single thymidine moiety with osmium tetroxide. This modified oligonucleotide was used to demonstrate that 2 blocks polymerase enzyme activity in vitro. It is uncertain whether individual diastereomers of 2 interact differently from one another with polymerase and/or repair enzymes.[3] However, metabolism studies suggest that this may be the case.[4] The biological effects of thymidine C5-hydrate 1 are even less well understood. This mutated nucleoside has not been selectively incorporated into a biopolymer; nor has it been chemically synthesized. Studies on the biological effects of mutated nucleosides 1 and 2 would be facilitated by their diastereoselective synthesis and subsequent site specific incorporation into oligonucleotides. In this paper, we report on the diastereoselective synthesis of 1 and 2.



A number of chemical syntheses of all four diastereomers of thymidine glycol (2) have been reported. Previous reports have utilized osmium tetroxide or potassium permanganate to oxygenate the nucleobase.[5] Although recently, *cis* and *trans* diastereomeric pairs of 2 were prepared via hydrolysis of their respective bromohydrins.[3a] All of these methods require separation of diastereomeric products via HPLC at one or more points during the synthesis. To our knowledge, (5*R*)- and (5*S*)-thymidine C5-hydrates (1) have only been isolable from the complex mixture of products that results from subjecting thymidine to γ-radiolysis in the presence of cysteine under anoxic conditions.[6] We have achieved diastereoselective syntheses of thymidine glycol (2) and thymidine C5-hydrate (1) using the Sharpless asymmetric dihydroxylation (AD) reaction.[7]

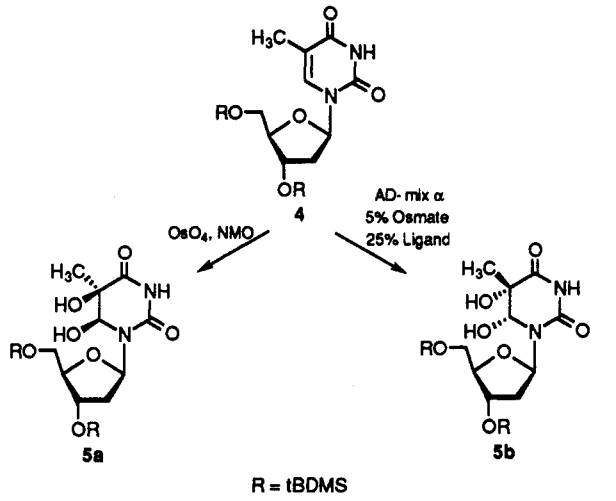


Figure 1. Diastereoselective osmium catalyzed dihydroxylation of 4 (R = *tert*-butyldimethylsilyl).

Results and Discussion

The desired stereochemistry in 1 and 2 at C5 was introduced via diastereoselective dihydroxylation of 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)thymidine (4). Dihydroxylation of 4 using catalytic osmium tetroxide and *N*-methylmorpholine *N*-oxide proceeded with very high diastereoselectivity. Crude ^{1}H NMR indicated that (5*R*,6*S*)-**5** was the major isomer, being formed in a 16:1 ratio (88% diastereomeric excess) over (5*S*,6*R*)-5. The diastereomers were separable by flash chromatography, yielding (5*R*,6*S*)-**5** (**5a**) in 83% yield (Figure 1). The stereochemistry of this addition product was confirmed by comparing the ^{1}H NMR spectrum and HPLC retention times of desilylated material to those previously reported.[3a,5a] Deprotection using TBAF in THF resulted in formation of the expected 4:1 ratio of **2a**/**2c** due to equilibration in the wet solvent under basic conditions.[3a] Further evidence that the stereochemistry at C5 was indeed *R* was obtained by carrying **5a** on to **1a** as described below and comparing the ^{1}H NMR of this material to that obtained from ionizing radiation. The stereochemistry of **1b** was originally determined by Cadet via X-ray crystallography.[8] Previous reports on the

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osmylation of unprotected thymidine also resulted in selective reaction with the pro-5*R*,6*S* face.[5a] This is consistent with the pro-5*R*,6*S* face being less hindered when the nucleoside is present in the more favorable *anti* conformation.[9] When the pyrimidine nucleoside is in the *anti* conformation, the C5′ hydroxyl group preferentially adopts a *gauche*, *gauche* relationship with respect to the tetrahydrofuran ring, effectively blocking the approach of the osmium tetroxide molecule from the pro-5*S*,6*R* face.[9] In our work, the 5′-*O*-*tert*-butyldimethylsilyloxy group provides an even greater steric hindrance to the osmium approaching the pro-5*S*,6*R* face. We believe that it is appropriate to use ground state species to rationalize the stereochemical outcome of this reaction, because the same steric interactions should be present in the respective transition states.

The natural facial predisposition was overcome using the Sharpless asymmetric dihydroxylation (AD) reaction.[7b] The mnemonic device used for predicting facial selectivity developed by Sharpless and co-workers suggested that the bis-dihydroquinine phthalazine ligand ((DHQ)$_2$PHAL) should preferentially produce (5*S*,6*R*)-**5**. No dihydroxylation product was detected upon reaction of **4** with commercially available AD-mix α (containing 0.2 mol % potassium osmate dihydrate). The reaction was still sluggish at room temperature with 2 mol % osmate and 10 mol % ligand. In order to achieve practical reaction rates, 5 mol % osmium and 25 mol % ligand was required. Crude ^{1}H NMR of preparative scale reactions indicated that three products were formed in a ratio of 13:4:1. The major product was shown to be the desired (5*S*,6*R*)-**5** (**5b**) following desilylation and comparison of the ^{1}H NMR spectrum of the material obtained with that reported for **2b**.[5a] Once again, TBAF mediated deprotection of **5b** yielded the thermodynamic mixture of glycols (**2b**/**2d**; 4:1). A similar procedure showed that the minor product corresponded to (5*R*,6*S*)-**5** (**5a**). The compound formed in intermediate amounts was identified as (5*S*,6*S*)-**5** (**5c**) (Figure 2). HPLC analysis of this material revealed that the same ratio of glycols **2b** and **2d** were formed from deprotection of **5b**.[3a,4a] We assume that the equilibration of **5b** and **5c** is catalyzed by the potassium carbonate present in the AD-mix. The ratio of (5*S*,6*R*)-**5** (**5b**) and (5*S*,6*S*)-**5** (**5c**) is close to that reported (and confirmed by us) for **2b** and **2c**[3a] (Figure 2). The three bis-silyl glycols were formed in a combined yield of 83%.

With **5a** and **5b** in hand, deoxygenation of the secondary alcohol was the next transformation necessary for producing **1** (Scheme I). Realizing that most conventional thionocarbonyl strategies would be ineffective for deoxygenating these vicinal diols, we chose to utilize the photochemical method reported by Saito.[10,11] This method has been shown to be synthetically useful for deoxygenating secondary alcohols.[11a] Stabilization of the incipient radical by the α-nitrogen was expected to make substrates **6a–c** especially amenable for this single electron transfer process.[11b] A variety of photolysis conditions were examined using **6a** as substrate in order to optimize the solvent and hydrogen atom donor combination (Table I). A mixture of tetrahydrofuran and water (9:1 by volume) was found to give the highest reproducible yield of **7a**. The yield of **7a** obtained in the presence of the efficient hydrogen atom donor dithiothreitol was less than expected. This was due at least in part to the difficulty experienced in separating **7a** from dithiothreitol. Photolysis of **6b** under the conditions found most effective for deoxygenation of **6a** yielded a comparable yield of the respective deoxygenated material **7b**.

A variety of desilylation conditions were examined. As expected, tetrabutylammonium fluoride (TBAF) furnished **1** in the highest yield. Acidic methods resulted in de-

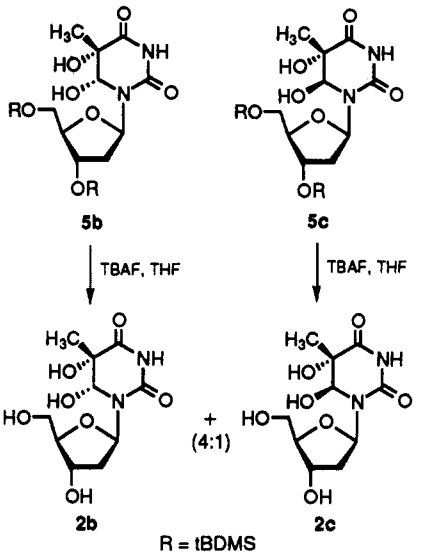
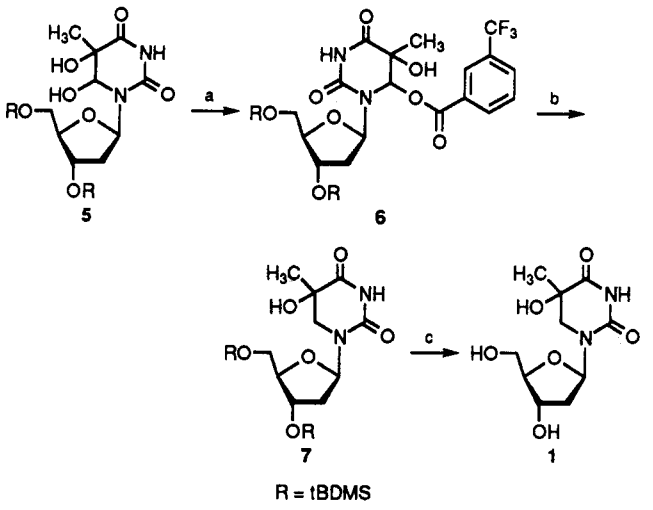


Figure 2. Equilibration of thymidine glycols (**2**) during deprotection of **5** with TBAF.

Scheme I[a]



[a] (a) *m*-(Trifluoromethyl)benzoyl chloride, DMAP, THF, −10 °C; (b) N-methylcarbazole, hν; (c) TBAF, THF.

Table I. Photolytic Deoxygenation of 6a (5 mM)

solvent	photolysis time (h)	additional trap (mM)	% yield **7a**
i-PrOH/water (9:1)	3.5	dithiothreitol (100 mM)	35
i-PrOH/water (9:1)	4.0	*tert*-butyl mercaptan (25 mM)	62
THF/water (9:1)	5.0	–	75
CH_3CN/water (9:1)	5.5	*tert*-butyl mercaptan (25 mM)	58

(9) Terms regarding conformations of nucleosides and their relative energies are described in: (a) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; Chapters 2 and 3. (b) Blackburn, G. M.; Gait, M. J. *Nucleic Acids in Chemistry and Biology*; IRL Press: Oxford, 1990; Chapter 2.

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composition of significant amounts of thymidine C5-hydrate product. Unfortunately, TBAF complicated purification of 1, because of difficulties experienced in removing traces of tetraalkylammonium salts. Furthermore, the high solubility of 1 in water prohibited separation of the ammonium salt from the nucleoside by simple extraction. Addition of stoichiometric amounts of TBAF and careful chromatography minimized this problem. Salt-free 1 was obtained following subsequent reverse phase HPLC purification.

Conclusions. We have achieved the first chemical synthesis of 5,6-dihydro-5-hydroxythymidine (1), which is a major product resulting from the interaction between ionizing radiation and thymidine under hypoxic conditions. Our synthesis also provides convenient access to either cis-diastereomer of 5,6-dihydro-5,6-dihydroxythymidine (2). Chemically synthesized oligonucleotides containing these nucleosides should be preparable using reported protecting groups and solid phase supports which eliminate the use of concentrated ammonium hydroxide.[12,13] When combined with the site specific incorporation of 1 and 2 into oligonucleotides, the diastereoselective synthesis of these products will be useful in ascertaining their effects on biopolymer structure with respect to the action of polymerase enzymes.[3b]

Experimental Section

General Methods. All reactions were carried out in oven dried glassware under positive nitrogen pressure. ^{1}H NMR spectra were recorded in $CDCl_3$ or D_2O at 300 or 270 MHz. ^{13}C NMR spectra were recorded in $CDCl_3$ or D_2O (CH_3OH as internal standard) at 75.5 or 67.9 MHz. Infrared spectra were recorded in either KBr pellets or thin films on KBr plates. Tetrahydrofuran (THF) was distilled from benzophenone ketyl. CH_2Cl_2 was distilled from CaH_2. Melting points are uncorrected. Microanalysis was obtained from MHW Laboratories (Phoenix, AZ).

(5*R*,6*S*)-Bis-TBDMS Glycol (5a). 3',5'-Bis-O-(*tert*-butyldimethylsilyl)thymidine (1g, 2.13 mmol) was added to *N*-methylmorpholine *N*-oxide (498 mg, 4.25 mmol) and OsO_4 (20 mg, 0.08 mmol) in a mixture of THF (5 mL), *t*-butyl alcohol (5 mL), and water (2 mL). The mixture was stirred and heated in an oil bath at 45°C for 24 h, at which time TLC (1:3; ethyl acetate (EtOAc)/CH_2Cl_2) revealed that no starting material remained. The solution was cooled in an ice bath, and sodium sulfite (530 mg, 4.25 mmol) was added. After stirring for 30 min, the mixture was poured into water (25 mL) and extracted three times with CH_2Cl_2 (50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Glycol **5a** (890 mg, 83%) was purified by flash chromatography[14] on silica gel (1:4; EtOAc/CH_2Cl_2): mp 178–181°C; ^{1}H NMR ($CDCl_3$) δ 7.42 (bd s, 1H), 6.19 (dd, 1H, J = 6, 8 Hz), 5.22 (s, 1H), 4.38 (m, 1H), 3.74 (m, 3H), 3.60 (s, 1H), 3.19 (s, 1H), 2.26 (m, 1H), 2.14 (m, 1H), 1.42 (s, 3H), 0.90 (s, 9H), 0.87 (s, 9H), 0.06 (s, 6H), 0.05 (s, 6H); IR (KBr) 3406 (bd), 2956, 2930, 1719, 1686, 1463, 1254, 1229, 1022 cm^{-1}. Anal. Calcd for $C_{22}H_{44}N_2O_7Si_2$: C, 52.35; H, 8.72; N, 5.55. Found: C, 52.49; H, 8.71; N, 5.49.

(5*S*,6*R*)-Bis-TBDMS Glycol (5b). 3',5'-Bis-O-(*tert*-butyldimethylsilyl)thymidine (153 mg, 0.33 mmol) was added to a solution of *t*-butyl alcohol and water (4 mL, 1:1 by volume) containing potassium osmate dihydrate (6 mg, 0.016 mmol), $(DHQ)_2PHAL$ (63 mg, 0.08 mmol), $K_3Fe(CN)_6$ (321 mg, 0.98 mmol), K_2CO_3 (135 mg, 0.98 mmol), and methanesulfonamide (31 mg, 0.33 mmol). The mixture was stirred at room temperature for 72 h, at which time TLC (1:3; EtOAc/CH_2Cl_2) revealed that no starting material remained. Workup was performed as that above for **5a**. Flash chromatographic separation of the three products was accomplished using a solvent gradient (1:6; EtOAc/CH_2Cl_2 to 1:3; EtOAc/CH_2Cl_2). Glycol **5b** (103 mg, 63%) eluted first (R_f = 0.42, 1:3; EtOAc/CH_2Cl_2): mp 179°C; ^{1}H NMR ($CDCl_3$) δ 7.67 (bd s, 1 H), 6.42 (t, 1H, J = 7 Hz), 4.87 (s, 1H), 4.39 (m, 1H), 4.29 (s, 1H), 3.76 (m, 3H), 3.26 (s, 1H), 2.15 (m, 1H), 1.96 (m, 1H), 1.42 (s, 3H), 0.89 (s, 9H), 0.87 (s, 9H), 0.09 (s, 6H), 0.06 (s, 6H); IR (thin film) 3419 (bd), 3236 (bd), 3088, 2953, 2929, 2857, 1713, 1468, 1463, 1388, 1254, 1097, 1061 cm^{-1}. Anal. Calcd for $C_{22}H_{44}N_2O_7Si_2$: C, 52.35; H, 8.72; N, 5.55. Found: C, 52.17; H, 8.61; N, 5.34. Glycol **5a** (7 mg, 4%) eluted next (R_f = 0.27, 1:3 EtOAc/CH_2Cl_2). This was followed by (5*S*,6*S*)-glycol **5c**: yield of 26 mg, 16%; R_f = 0.18 (1:3, EtOAc/CH_2Cl_2); mp 76–78 °C; ^{1}H NMR ($CDCl_3$) δ 7.88 (bd s, 1H), 6.09 (dd, 1H, J = 7, 7 Hz), 5.14 (s, 1H), 4.41 (m, 1H), 3.86 (m, 2H), 3.72 (m, 1H), 2.32 (m, 1H), 2.21 (m, 1H), 1.48 (s, 3H), 0.1 (s, 9H). 0.08 (s, 9H), 0.06 (s, 6H), 0.05 (s, 6H); ^{13}C ($CDCl_3$) δ 171, 151, 86.7, 85.2, 80.1, 71.4, 70.9, 62.9, 40.1, 26, 25.7, 19, 18.5, 17.9, -4.6, -4.9, -5.4, -5.5; IR (film) 3550 (bd), 3400 (bd), 2990, 2970, 2840, 1695, 1680, 1490, 1230 cm^{-1}; exact mass (FAB) calcd for $C_{22}H_{44}N_2O_7Si_2Na$ (M^+ + Na) 527.2585, found 527.2583. The stereochemistry of **5c** was further established via desilylation as described below for **5a** and **5b**.

(5*R*,6*S*)-5,6-Dihydro-5,6-dihydroxythymidine (2a) and (5*R*,6*R*)-5,6-Dihydro-5,6-dihydroxythymidine (2c). A 1 M solution of tetrabutylammonium fluoride (440 λ) was added via syringe to a solution of **5a** (100 mg, 0.198 mmol) in THF (1.5 mL) at 0 °C. The mixture was allowed to warm to room temperature and stir for 18 h. TLC (1:19; CH_3OH/CH_2Cl_2) indicated that the reaction was complete after 8 h. Glycols **2a** and **2c** (31 mg, 57%) were obtained following removal of the THF in vacuo, and chromatography of the crude material (1:19; CH_3OH/CH_2Cl_2). The ^{1}H NMR and ratio of **2a**/**2c** (4:1) were identical to that reported in the literature.[5a] The mixture of glycols was contaminated with a small amount of tetrabutylammonium hydroxide. Salt free **2a** and **2c** (16 mg, 61%) were obtained separately via reverse-phase HPLC (μBondpak C18; water, t_R: **2c**, 4.6 min; **2a**, 7.3 min).

(5*S*,6*R*)-5,6-Dihydro-5,6-dihydroxythymidine (2b) and (5*S*,6*S*)-5,6-Dihydro-5,6-dihydroxythymidine (2d). A 1 M solution of tetrabutylammonium fluoride (200 λ) was added via syringe to a solution of **5b** (48 mg, 0.095 mmol) in THF (1.5 mL) at 0 °C. The mixture was allowed to warm to room temperature and stir for 18 h. TLC (1:19; CH_3OH/CH_2Cl_2) indicated that the reaction was complete after 8 h. Glycols **2b** and **2d** (21 mg, 80%) were obtained following removal of the THF in vacuo, and chromatography of the crude material (1:19; CH_3OH/CH_2Cl_2). The ^{1}H NMR and ratio of **2b**/**2d** (4:1) were identical to that reported in the literature.[5a] The mixture of glycols was contaminated with a small amount of tetrabutylammonium hydroxide. Salt free **2b** and **2d** (16 mg, 61%) were obtained via reverse phase HPLC (μBondpak C18; water, t_R: **2d**, 4.5 min; **2b**, 6.9 min).

(5*R*,6*S*)-*m*-(Trifluoromethyl)benzoate Ester 6a. *m*-(Trifluoromethyl)benzoyl chloride (43 mg, 0.205 mmol) was added via syringe to a solution of **5a** (94 mg, 0.186 mmol) and DMAP (27 mg, 0.223 mmol) in THF (2 mL) at −10 °C. The reaction was quenched via the addition of water (1 mL). The mixture was poured into water (10 mL) and extracted three times with dichloromethane (20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated in vacuo. Ester **6a** (102 mg, 81%) was purified by column chromatography on silica gel (1:9; EtOAc/CH_2Cl_2): mp 86°C; ^{1}H NMR ($CDCl_3$) δ 8.35 (bd s, 1H), 8.19 (s, 1H), 8.09 (d, 1H, J= 8 Hz), 7.82 (d, 1H, J = 8 Hz), 7.56 (dd, 1H, J = 7, 8 Hz), 6.50 (s, 1H), 6.24 (dd, 1H, J = 6, 7 Hz), 4.35 (m, 1H), 3.68 (m, 3H), 3.54 (bd s, 1H), 1.91 (m, 2H), 1.57 (s, 3H), 0.92 (s, 9H), 0.83 (s, 9H), 0.11 (s, 6H), 0.02 (s, 3H), −0.02 (s, 3H). IR (KBr) 3425, 2953, 2921, 2848, 1722, 1455, 1335, 1235, 1131 cm^{-1}. Anal. Calcd for $C_{30}H_{47}N_2F_3O_8Si_2$: C, 53.22; H, 7.01; N, 4.09. Found: C, 53.47; H, 7.10; N, 4.15.

(5*S*,6*R*)-*m*-(Trifluoromethyl)benzoate Ester 6b. Esterification was carried out as described for **6a**. Reaction of **5b** (47 mg, 0.093 mmol), followed by chromatographic purification yielded 50 mg (79%) of **6b**: mp 88–91 °C; ^{1}H NMR ($CDCl_3$) δ

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8.24 (bd s, 1H), 8.18 (s, 1H), 8.09 (d, 1H, J = 8 Hz), 7.79 (d, 1H, J = 8 Hz), 7.54 (1H, dd, J = 7.8 Hz), 6.51 (s, 1H), 6.19 (dd, 1H, J = 5, 9 Hz), 4.34 (m, 1H), 3.75 (m, 1H), 3.45 (m, 3 H), 2.05 (m, 2H), 1.56 (s, 3H), 0.86 (s, 9H), 0.85 (s, 9H), 0.06 (s, 3h), 0.05 (s, 3H), 0.02 (s, 3H), 0.00 (s, 3H); IR (film) 3424 (bd), 3223 (bd), 3117 (bd), 2955, 2930, 2856, 1733, 1712, 1337, 1251, 1137, 836 cm^{-1}. Anal. Calcd for $C_{30}H_{47}N_2F_3O_8Si_2$: C, 53.22; H, 7.01; N, 4.09. Found: C, 53.01; H, 7.06; N, 4.04.

General Procedure for Photochemical Deoxygenation of 6a. Solutions of **6a** (5 mM) and *N*-methylcarbazole (5 mM)were placed in Pyrex tubes fitted with Teflon vacuum stopcocks after adding the appropriate solvent(s) and exogenous hydrogen atom donor. The solutions were degassed using three freeze-pump-thaw cycles and photolyzed in a Rayonnet photoreactor using UV lamps (λ_{max} = 350 nm) for 5 h. Following photolysis the solvents were removed in vacuo, and the crude mixture was chromatographed (1:9; EtOAc/CH_2Cl_2).

(5*R*)-3′,5′-Bis-O-(*tert*-butyldimethylsilyl)-5,6-dihydro-5-hydroxythymidine (7a). *m*-(Trifluoromethyl)benzoate **6a** (69 mg, 0.1 mmol) and N-methylcarbazole (18 mg, 0.1 mmol) were dissolved in 20 mL of THF/water (9:1 by volume). The solution was degassed and photolyzed as described above. Chromatography yielded **7a** (34 mg, 71%). mp 112–115 °C; ^{1}H NMR ($CDCl_3$) δ 7.60 (bd s, 1H), 6.26 (dd, 1H, J = 7, 7 Hz), 4.34 (m, 1H), 3.80 (m, 1H), 3.70 (m, 2H), 3.65 (d, 1H, J = 12 Hz), 3.17 (d, 1H, J= 12 Hz), 1.95 (m, 2H), 1.45 (s, 3H), 0.93 (s, 9H), 0.90 (s, 9H), 0.1 (s, 6H), 0.05 (s, 6H); IR (thin film) 3400 (bd), 2946, 2924, 2891, 2852, 1699, 1468, 1325, 1138 cm^{-1}. Anal. Calcd for $C_{22}H_{44}N_2O_6Si_2$: C, 54.07; H, 9.08; N, 5.74. Found: C, 53.93; H, 8.94; N, 5.66.

(5*S*)-3′,5′-Bis-O-(*tert*-butyldimethylsilyl)-5,6-dihydro-5-hydroxythymidine (7b). *m*-(Trifluoromethyl)benzoate **6b** (50 mg, 0.073 mmol) was subjected to the photolysis conditions described above for **7a**. Chromatography yielded **7b** (26 mg, 73%): mp 158°C; ^{1}H NMR ($CDCl_3$) δ 7.45 (bd s, 1H), 6.32 (dd, 1H, J = 7, 7 Hz), 4.35 (m, 1H), 3.81 (m, 1H), 3.67 (m, 1H), 3.64 (d, 1H, J = 13.5 Hz), 3.38 (d, 1H, J = 13.5 Hz), 1.96 (m, 2H), 1.39 (s, 3H), 0.90 (s, 9H), 0.88 (s, 9H), 0.09 (s, 6H), 0.06 (s, 6H). IR (film) 3220 (bd), 2953, 2928, 2857, 1712, 1472, 1434, 1253, 1134, 1095, 1029 cm^{-1}; HRMS (FAB) calcd 511.2635 (M + Na), found 511.2621.

(5*R*)-5,6-Dihydro-5-hydroxythymidine (1a). A 1 M solution of tetrabutylammonium fluoride (200 λ) was added via syringe to a solution of **7a** (45 mg, 0.09 mmol) in THF (1.5 mL) at 0 °C. The mixture was allowed to stir and warm to room temperature. TLC (1:19 CH_3OH/CH_2Cl_2) indicated that the reaction was complete after 8 h. Thymidine C5-hydrate **1a** (22 mg, 94%) was obtained following removal of the THF in vacuo and chromatography of the crude material (1:9; $CH_3OH/CHCl_3$), mp 216°C. The ^{1}H NMR spectrum was identical to the material obtained from ionizing radiation following purification by reverse phase HPLC. ^{1}H NMR (D_2O) δ6.15 (dd, 1H, J = 7, 7 Hz), 4.25 (m, 1H), 3.66 (m, 1H), 3.61 (m, 2H), 3.35 (s, 2H), 2.15 (m, 1H), 2.01 (m, 1H), 1.34 (s, 3H); ^{13}C (D_2O, CH_3OH internal reference) 174.8, 153.5, 85.4, 84.0, 71.0, 68.5, 61.7, 47.1, 35.4, 21.5. IR (film) 3314 (bd), 2929, 1696, 1479, 1436, 1297, 1222, 1089, 1048 cm^{-1}; HRMS (FAB) calcd 261.1087 (M + H), found 261.1093. The material was contaminated with a small amount of tetrabutylammonium hydroxide. Salt free **1a** (17 mg, 71%) was obtained via reverse phase HPLC (μBondpak C18; 1:9; methanol/water) purification (t_R: 10.5 min).

(5*S*)-5,6-Dihydro-5-hydroxythymidine (1b). Bis-silyl thymidine C5-hydrate **7b** (10 mg, 20 μmol) was subjected to identical reaction conditions as **7a** above, yielding **1b** (5.25 mg, 94%): mp 192–194°C. As for **1a**, the ^{1}H NMR of thymidine C5-hydrate **1b** was identical to that recorded for material obtained via ionizing radiation: ^{1}H NMR (D_2O) δ 6.15 (dd, 1H, J = 7, 7 Hz), 4.24 (m, 1H), 3.78 (m, 1H), 3.64 (m, 2H), 3.40 (d, 1H, J = 13.2 Hz), 3.36 (d, 1H, J = 13.2 Hz), 2.13 (m, 1H), 2.06 (m, 1H), 1.34 (s, 3H); ^{13}C (D_2O, CH_3OH internal standard) δ 174.5, 153.7, 85.4, 83.9, 70.8, 68.4, 61.0, 47.2, 35.6, 21.0; IR (film) 3327 (bd), 2929, 1700, 1479, 1297, 1233, 1048, 768 cm^{-1}; HRMS (FAB) calcd 261.1087 (M + H), found 261.1083. Removal of the tetrabutylammonium hydroxide impurity by reverse-phase HPLC (t_R: 9.4 min) yielded 4 mg (75%) of clean **1b**.

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