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Independent Generation and Reactivity of Thymidine Radical Cations

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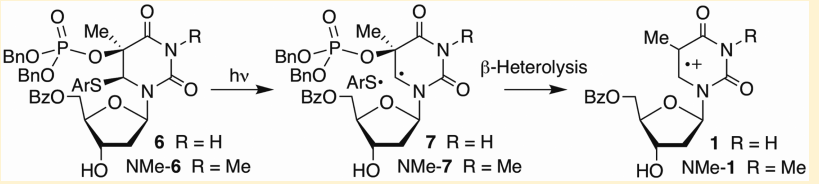
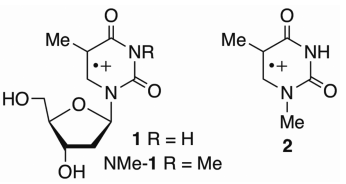
Supporting Information



ABSTRACT: Thymidine radical cation (**1**) is produced by ionizing radiation and has been invoked as an intermediate in electron transfer in DNA. Previous studies on its structure and reactivity have utilized thymidine as a precursor, which limits quantitative product analysis because thymidine is readily reformed from **1**. In this investigation, radical cation **1** is independently generated via *β*-heterolysis of a pyrimidine radical generated photochemically from an aryl sulfide. Thymidine is the major product (33%) from **1** at pH 7.2. Diastereomeric mixtures of thymidine glycol and the corresponding 5-hydroxperoxides resulting from water trapping of **1** are formed. Significantly lower yields of products such as 5-formyl-2′-deoxyuridine that are ascribable to deprotonation from the C5-methyl group of **1** are observed. Independent generation of the N3-methyl analogue of **1** (NMe-**1**) produces considerably higher yields of products derived from water trapping, and these products are formed in much higher yields than those attributable to the C5-methyl group deprotonation in NMe-**1**. N3-Methyl-thymidine is, however, the major product and is produced in as high as 70% yield when the radical cation is produced in the presence of excess thiol. The effects of exogenous reagents on product distributions are consistent with the formation of diffusively free radical cations (**1**, NMe-**1**). This method should be compatible with producing radical cations at defined positions within DNA.

INTRODUCTION

Nucleoside oxidation is a chemical process with important consequences on human health and is a useful biotechnology tool. One electron oxidation of the nucleobase is a general pathway, and much attention has been paid to 2′-deoxyguanosine reactivity in this manner because it is the most readily oxidized of the four native nucleosides.[1,2] However, some of the most common oxidizing agents such as *γ*-radiolysis and photoionization are unselective and ionize pyrimidines in addition to purines.[3] Thymidine radical cation (**1**) is formed by ionizing radiation and chemical oxidants (e.g., sulfate radical anion and photosensitization).[4−8] Radical cation **1** has also been invoked as an important intermediate in DNA electron transfer within A·T rich substrates.[9,10] The structure and reactivity of **1**, its N3-methyl analogue (NMe-**1**), and **2** have been examined via EPR, product analysis, and pulse radiolysis.[11] We wish to report a method for generating **1** and NMe-**1** for the first time from a photochemical precursor other than a pyrimidine. This approach is compatible with biologically relevant conditions and, in contrast to when using pyrimidines themselves as precursors, enables us to quantify the products from all major reaction pathways.



Thymidine (**1**) and N1-methylthymine (**2**) radical cations have been generated by two photon photoionization (248 nm) or *γ*-radiolysis in solid matrices.[7,8,11] The products characterized from *γ*-radiolysis in solid matrices are indicative of the O_2 deficient conditions in which the experiments were carried out. Although it is not mentioned, product mixtures formed from photolysis at 248 nm may be influenced by the short irradiation wavelength. This issue is avoided by using excited 2-methyl-1,4-napthoquinone to generate **1** via photoinduced single electron transfer.[6] In yet another approach, pulse radiolysis yields the radical cations following the generation of a sulfate radical anion from persulfate.[4] The sulfate radical anion yields the radical cation(s) via addition, followed by sulfate elimination. These pulse radiolysis experiments reveal that the radical cations react on the microsecond time scale.[4] Four major reaction pathways are open to monomeric **1** (Scheme 1). Hydration, C5-methyl deprotonation, and single electron transfer are possible from NMe-**1**, but not N3-deprotonation. Spectroscopic measurements indicating that N3-deprotonation of **1** was more rapid than hydration or C5-methyl deprotonation was inconsistent with the aforementioned photoionization study in which the latter reaction was the major process detected.[4,6] Furthermore, N3-methyl-deprotonation, but not C5-methyl-deprotonation, was detected in a time-resolved EPR investigation in which **2** was generated

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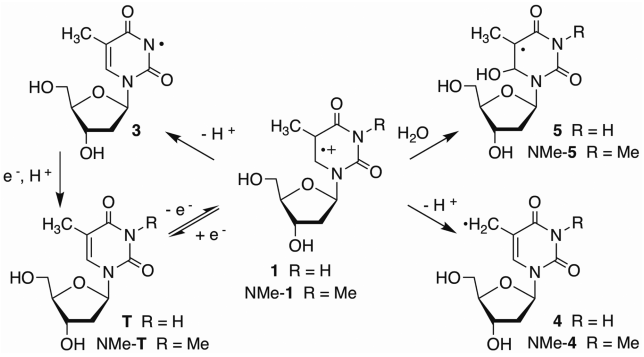
Scheme 1. Formation and Reactivity of the Thymidine Radical Cation



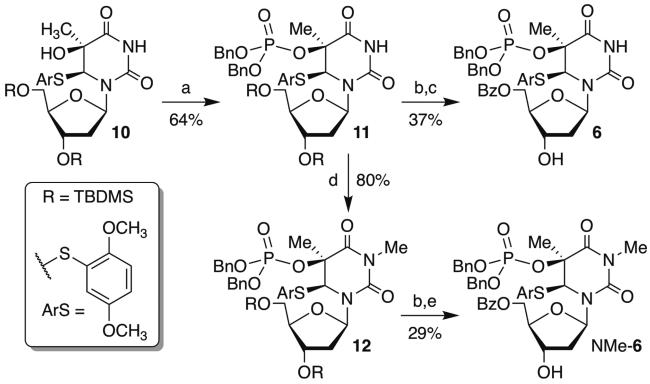
from anthraquinone-2,6-disulfonic acid sensitized ionization.[5] However, in a different matrix isolation investigation Adhikary and Sevilla observed that C5-deprotonation occurred preferentially over hydration.[11] In addition to these discrepancies, product studies are compromised because the radical cations are generated from the nucleobase in each of these investigations. Radical cation generation from thymidine prevents detecting one electron reduction, which regenerates the starting material. The method described herein for independently generating **1** and NMe-**1** from dihydropyrimidine precursors does not face this limitation.

RESULTS AND DISCUSSION

A number of photochemical precursors have been reported for nucleobase radicals resulting from a hydroxyl radical (HO•) or hydrogen atom addition to pyrimidine nucleosides.[12] Aryl sulfides have served as useful precursors to HO• radical adducts.[13−16] We envisioned capitalizing on these molecules by conjoining them with the chemistry largely developed by Crich and Newcomb to generate thymidine radical cations (**1**, NMe-**1**, Scheme 2).[17−20] These investigators established that appropriately substituted alkyl radicals (**7**) generate diffusively free alkene radical cations (**1**) via *β*-heterolysis of a phosphatoxyl group. Formation of the diffusively free radical cation from the ion pair (**8**) is facilitated by polar solvents and strongly influenced by the leaving groups. Crich and Newcomb made extensive use of phosphate diesters as leaving groups. Nishimoto and co-workers also employed this approach to produce thymi(di)ne radical cations.[21] However, these researchers used an alkoxyl group as the leaving group, and radical cation yields were low. This strategy was also attractive to us because of our long-term aspiration of applying this chemistry to oligonucleotides. Phosphate triesters, such as those present in **6**, should be compatible with solid-phase oligonucleotide synthesis. We postulated that **6** and NMe-**6** would serve as precursors to **1** and NMe-**1**, although the respective carbocations (**9**) may also be produced to some extent upon 350 nm photolysis.[14] A benzoyl group was incorporated at the 5′-position to provide a suitable chromophore for UPLC detection of photolysis products by UV−vis absorption. (Please note that for convenience benzoylated molecules (e.g., **1**) are referred to by the same number as the corresponding nonbenzoylated molecules.)

Radical Cation Precursor Synthesis and Stability. The phosphorylated aryl sulfides were synthesized in a direct manner from the previously reported sulfide (**10**, Scheme 3).[14]

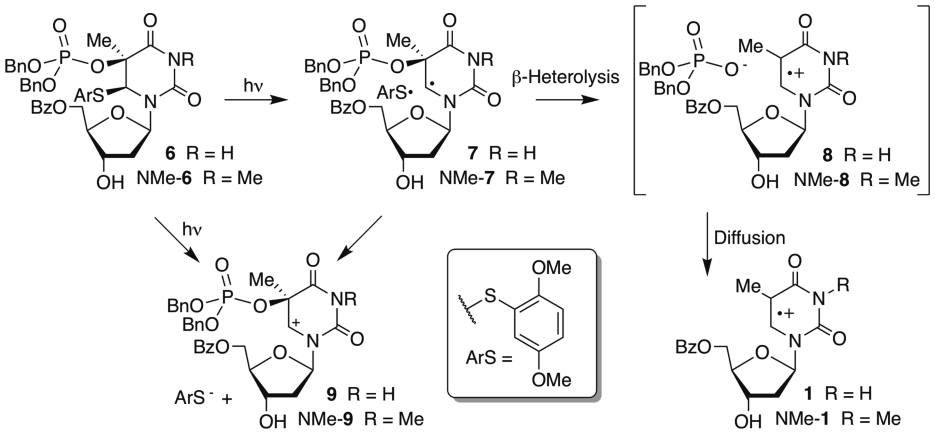
Scheme 3. Syntheses of Radical Cation Precursors[a]



[a]Key: (a) $(BnO)_2P(NiPr_2)$ and *S*-ethyltetrazole then *t*-BuOOH, (b) $Et_3N{\cdot}3HF$, (c) BzCN, (d) MeI, (e) BzCl.

Phosphorylation using chlorophosphate reagents was unsuccessful. However, more electrophilic P(III) reagents reacted with the tertiary alcohol, and in situ oxidation with *t*-BuOOH provided the desired phosphorylated protected nucleoside (**11**). Phosphate triester **11** was carried on to radical cation precursors for **1** and NMe-**1**. Methylation was carried out under mild alkaline conditions using iodomethane. Both **11** and **12** were desilylated and 5′-benzyoylated. Selectivity for the primary alcohol was not very good. Consequently, benzoyla-

Scheme 2. Photochemical Radical Cation Generation



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tion reactions were carried out to ~40% conversion to minimize benzoylation at the 3′- or 3′- and 5′-positions. The reported yields are based on unrecovered starting material. Furthermore, in our hands, BzCN was preferred when preparing **6**, but BzCl provided a higher yield of NMe-**6**.

The half-life of desilylated **11** at 25 °C in a 1:1 mixture of phosphate buffered (100 mM, pH 6.6) saline (1 M) and CD_3OD was monitored by ^{31}P NMR (Figure 1) and determined to be 16.2 days. UPLC-MS indicated that it decomposed by losing a single benzyl group (Figure S1).

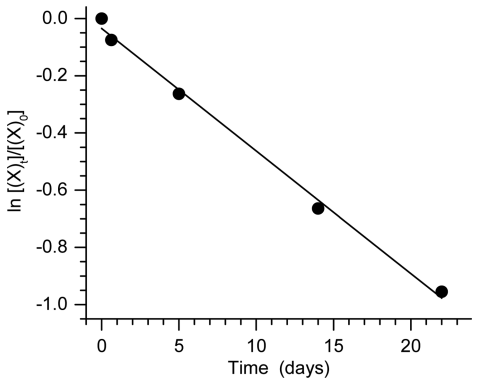
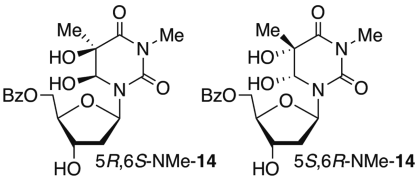
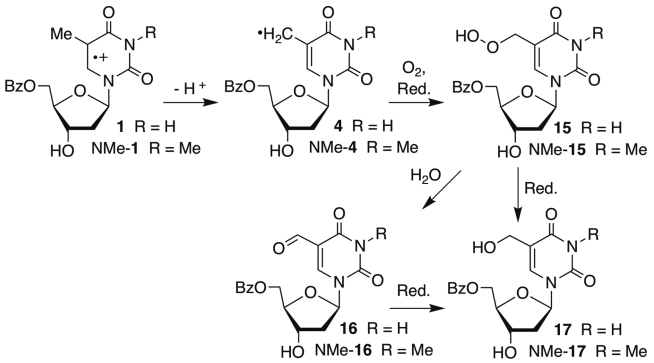


Figure 1. Decomposition of desilylated **11** (*X*) in CD_3OD/phosphate buffered saline (v/v = 1:1) monitored by ^{31}P NMR.

Independent Synthesis and Analysis of Anticipated Photochemical Products. The identities of products (and secondary products) from **1** were established in previous studies.[6,22−24] The syntheses described here are adaptations of known methods to accommodate the N3-methyl group and/or the 5′-benzoyl group. Reduction of the radical cations either directly or following N3-deprotonation of **1** yields the corresponding thymidine derivative.[25] Thymidine glycols are the most stable products resulting from hydration of **1** and NMe-**1** (Scheme 4). Thymidine hydroperoxides (**13**, NMe-**13**) are unstable; consequently, we anticipated characterizing them via LC-MS and by taking advantage of their reduction to thymidine glycols (**14**, NMe-**14**). Thymidine glycol **14** was previously reported, but NMe-**14** was obtained by osmylation of 5′-benzoylated NMe-**T** in 39% yield as a mixture of two isomers in an approximately 92:8 ratio (by ^1H NMR).[14] On the basis of previous work, the major isomer is ascribed to 5*R*,6*S*-NMe-**14** and the minor isomer is believed to be 5*S*,6*R*-NMe-**14**.[14,26,27] For quantitative studies, UPLC response factors were measured for **14** and 5*R*,6*S*-NMe-**14** using 5′-benzoyl-2′-deoxyuridine (**BzU**) as an internal standard. We used these response factors to approximate those of the other thymidine glycol diastereomers, as well as the respective dihydrothymidine hydroperoxides (**13**, NMe-**13**) (Figures S3 and S4).



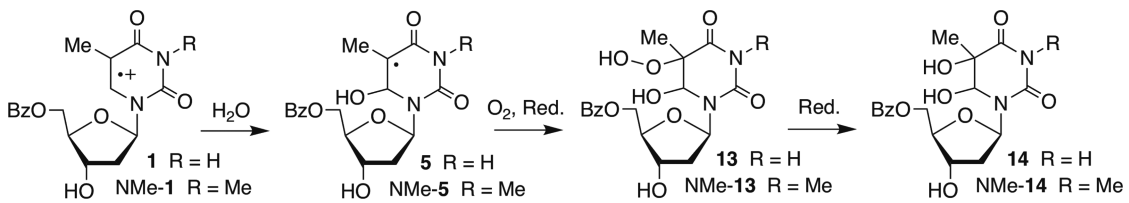
Scheme 5. Anticipated Products following C5-Deprotonation from the Radical Cation



The three products typically associated with C5-methyl deprotonation of **1** are derived from O_2 trapping of **4**. The corresponding hydroperoxides (**15**, NMe-**15**) were not detected by LC-MS, but their hydrolysis products (**16**, NMe-**16**) and reduction product (**17**, NMe-**17**) were observed. We employed an approach developed by Sugiyama and Saito for preparing oligonucleotides containing 5-formyl-2′-deoxyuridine in which the aldehyde group is introduced via Pd(0) coupling to suitably protected 5-iodo-2′-deoxyuridine (Scheme 6).[28] Stille coupling reactions were carried out on 5′-benzoyl-5-iodo-2′-deoxyuridine (**18**)[29] or NMe-**18**. The latter was prepared by benzoylating 5-iodo-3-methyl-2′-doxyuridine.[30] The vinyl substituted nucleosides were converted to the aldehyde products using a one-pot osmylation, periodate oxidation procedure.[31] During the course of the UPLC product analyses discussed below, we determined that **16** was inseparable from the internal standard (5′-benzoyl-2′-deoxyuridine, **BzU**). Consequently, **16** was quantified as its oxime (**20**), which was prepared quantitatively from the aldehyde. The hydroxymethyl nucleosides (**17**, NMe-**17**, Scheme 5) were synthesized from the corresponding protected thymidines, following silyl protection of the 3′-hydroxyl groups (**21**, NMe-**21**, Scheme 7). The hydroxymethyl groups (**22**, NMe-**22**) were introduced via allylic bromination, followed by hydrolysis of the crude material.[32,33]

Abasic sites have been reported to be produced from the 2′-deoxycytidine radical cation but not **1** or NMe-**1**.[34] However, carbocations formed upon photolysis of the aryl sulfide precursors are potential alternative sources of abasic sites (Scheme 2).[15] 5-Benzoyl-2-deoxyribose (**25**) was synthesized

Scheme 4. Anticipated Products following Radical Cation Hydration



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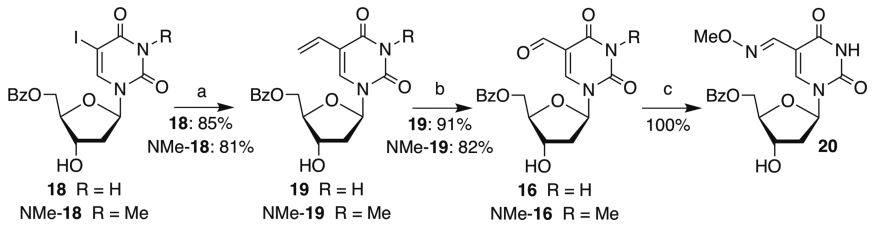
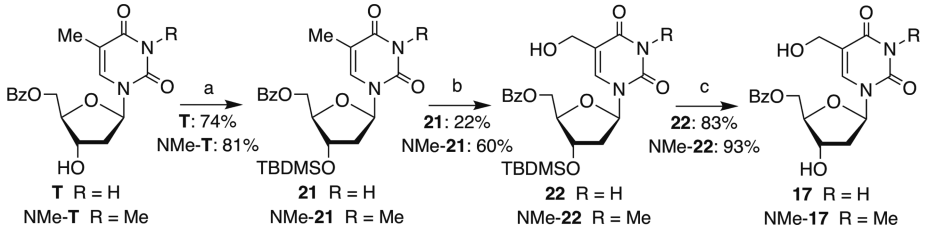
Scheme 6. Syntheses of the Aldehyde Products and Oxime Derivative[a]



[a]Key: (a) Tributyl(vinyl)stannane and Pd(0), (b) OsO_4 and $NaIO_4$, (c) $MeONH_2$·HCl.

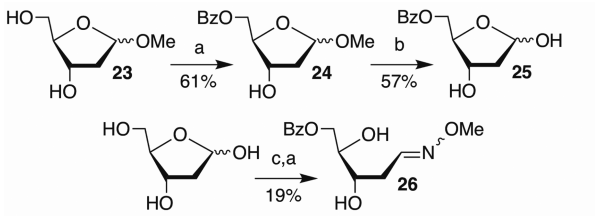
Scheme 7. Syntheses of the 5-Hydroxymethyl-2′-deoxyuridine Products[a]



[a]Key: (a) TBDMSCl, (b) NBS and AIBN then aq $NaHCO_3$, (c) Et_3N·3HF.

from **23** (Scheme 8) but was inseparable from other products in the photolysates in which **1** was generated.[35] Consequently,

Scheme 8. Syntheses of 5-Benzoyl-2-deozyribose and Its *O*-Methyl Oxime[a]



[a]Key: (a) BzCl, (b) AcCl, H_2O, and CH_3CN, (c) $MeONH_2$·HCl.

the respective *O*-methyl oxime (**26**) obtained upon reaction of **25** was used to quantify the abasic site formation when **6** was photolyzed. Methoxyamine is commonly used to functionalize abasic sites in DNA and prevent their repair.[36,37] UPLC analysis indicated that the reaction of **25** with methoxyamine hydrochloride yielded **26** (which was independently synthesized from 2-deoxyribose) in 98% yield, indicating that this method would be suitable for quantifying abasic sites formed in the photolysis experiments.

Photochemical Generation and Reactivity of 1. Precursor **6** was photolyzed under aerobic conditions at 350 nm for 0.5 h in three different solvent mixtures (Table 1). All photolysis mixtures contained 50% CH_3CN by volume due to solubility limitations of **6**. Consequently, photolyses were carried out in H_2O/CH_3CN (1:1 v/v), aqueous phosphate buffer (pH 7.2, 20 mM)/CH_3CN (1:1 v/v), or Chelex treated citrate buffer (pH 5.0, 40 mM)/CH_3CN (1:1 v/v). Chelex treatment was carried out to test for possible trace metal effects, and the purpose of the lower pH was to carry out reactions closer to the pK_a (3.9) of radical cation **1**. Mass balances ranged from 48−63%. AP sites (**26**) were formed in minor amounts. Although it is uncertain, we suggest that they result from the carbocation (**9**) that is formed either directly via heterolysis or as a result of electron transfer within the radical cage. Products (**13**, **14**, and **27**) resulting from hydration of the pyrimidine π-bond were detected in ∼10% combined yield in H_2O and pH 7.2 buffer. Hydroperoxide **13** was identified by LC-MS, and its reactivity was previously well characterized by Wagner and Cadet (Figure S2).[22] For UPLC quantitation of **13**, the response factor of independently synthesized **14** versus **BzU** was used (Figure S4). Product **27** was identified by LC-MS and was consistent with the known degradation products of thymidine glycol hydroperoxides.[22] The response factor of structurally similar **26** was used for quantifying this molecule by UPLC (Figure S3). Higher yields of hydration products were detected at a pH of 7.2 than at 5.0, consistent with a greater concentration of the hydroxide ion. Products consistent with methyl group deprotonation (**16**, **17**) of **1** were also detected, but their yields were consistently lower than those of hydration products.

Table 1. Product Yields from Photolysis of Radical Cation Precursor 6

	% yield[a]								
conditions[b]	**13**	**14**	**27**	**16**	**17**	**26**	**T**	**6**	mass balance
H_2O	3.4 ± 0.6	3.7 ± 0.1	3.2 ± 0.1	2.0 ± 0.1	1.2 ± 0.0	4.4 ± 0.1	30.1 ± 0.5	6.8 ± 0.9	54.8 ± 0.7
BME[c]	0.6 ± 0.1	6.7 ± 0.9			3.9 ± 0.1		42.5 ± 0.2	9.2 ± 3.5	63.0 ± 4.8
pH 5.0 (Chelex)	1.5 ± 0.3	4.3 ± 1.1	2.1 ± 0.5	2.1 ± 0.2	1.7 ± 0.3	3.6 ± 0.0	27.3 ± 0.3	5.2 ± 0.5	47.9 ± 2.3
pH 7.2	4.8 ± 0.1	3.6 ± 0.1	1.9 ± 0.1	1.1 ± 0.1	4.0 ± 0.1	1.0 ± 0.1	36.1 ± 0.8	5.6 ± 0.5	58.1 ± 0.5

[a]Yields and mass balances are the average ± standard deviation of at least 3 measurements. [b]All experiments were carried out in a 1:1 (v/v) mixture of CH_3CN and the solvent listed. [c][BME] = 10 mM.

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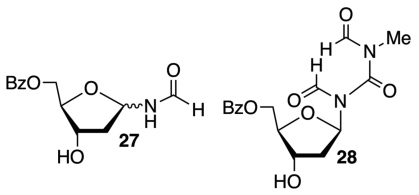

Thymidine was the major product under all conditions, and its yield increased from 30 to >42% when β-mercaptoethanol (BME, 10 mM) was included in the photolysis mixture. The presence of BME also resulted in an increase in yields of thymidine glycol(s) (**14**) and 5-hydroxymethyl-2′-deoxyuridine (**17**) at the expense of the corresponding thymidine glycol hydroperoxides (**13**) and 5-formyl-2′-deoxyuridine (**16**), a hydrolysis product of hydroperoxide **15**. However, the overall yields of these products were unaffected by 10 mM BME, indicating that the thiol did not compete with H_2O, deprotonation, or alkyl radical trapping by O_2.

Photochemical Generation and Reactivity of NMe-1. Mass balances from photolysis of NMe-**6** (Table 2) were typically higher than those determined from **6** and approached 100% when carried out in the presence of BME (10 mM) (Figure S5). In the absence of thiol (BME), higher yields of products attributable to H_2O trapping of NMe-**1** (NMe-**13**, NMe-**14**, **27**, **28**) accounted for most of the increased mass balance. In contrast to trapping studies on **1**, the combined yield of water trapping products of NMe-**1** were greater than that of NMe-**T** under all photolysis conditions except pH 5.0. Increases in the yields of water trapping products of NMe-**1** account for higher mass balances at pH 7.2 and 8.0 than at pH 5.0. We attribute higher relative yields of NMe-**1** hydration products relative to NMe-**T** to the inability of the radical cation to yield thymidine via N3-deprotonation, which produces **3** from **1** (Scheme 1). The elimination of an intramolecular pathway for the radical cation also explains why the presence of BME (10 mM) during photolysis has a greater effect on the thymidine yield from NMe-**1** than from **1**.

Although the thymidine glycol (NMe-**14**) analogue was independently synthesized, the respective hydroperoxides (NMe-**13**) and fragmentation products (**27**, **28**) were initially identified by LC-MS and comparison to the thorough reports by Wagner and Cadet on the hydroperoxides (Figure S2).[22,24] The hydroperoxide assignment is also consistent with observations made upon treatment of the photolysate with BME, which decreased the amount of the product assigned to NMe-**13** and increased the yield of NMe-**14** (Table 2). ^{18}O-Isotopic labeling and MS/MS analysis provided additional structural support and mechanistic information regarding product formation. Hydration of NMe-**1** is expected to occur at C6 (Scheme 1), followed by O_2 trapping of the C5-radical (NMe-**5**, Scheme 4). Photolysis in $H_2{}^{16}O/{}^{16}O_2$ produced products with m/z = 433.1207 and 417.1273 corresponding to the molecular ions for NMe-**13** (Figure 2A) and NMe-**14** (Figure 3), respectively. Fragmentation within the MS supported the regioisomeric assignment shown for NMe-**13** (Figure 2). The molecular ion fragments released the C5-carbon containing the methyl group and, importantly, the hydroperoxide component. When $H_2{}^{18}O$ is present during photolysis (Figure 2B), the observed parent ion increases by 2 Da and the ^{18}O is retained upon fragmentation. In contrast, when $^{18}O_2$ is employed (Figure 2C), the parent ion of NMe-**13** increases by 4 Da, as expected for trapping of NMe-**5** (Scheme 4), but both ^{18}O atoms are lost upon fragmentation. The labeling pattern is consistent with the anticipated C6-hydration, followed by O_2 trapping.

Similar analyses were carried out for the thymidine glycol (NMe-**14**) formed upon photolysis of NMe-**6** (Figure 3), which was expected to result from reduction of the hydroperoxide (Scheme 4). The glycol molecular ion increases by 2 Da when either $H_2{}^{18}O$ (Figure 3B) is substituted for H_2O or $^{18}O_2$ (Figure 3C) is bubbled through the solution, prior to sealing and photolysis. These observations are consistent with the aforementioned observations concerning the hydroperoxide (NMe-**13**, Figure 2).

5-Formyl-2′-deoxyuridine (NMe-**16**) was attributed to C5-deprotonation from NMe-**1**, followed by O_2 trapping of NMe-**4** to form the peroxyl radical, which is ultimately transformed into the aldehyde via hydrolysis of the hydroperoxide (NMe-**15**, Scheme 5). However, LC-MS analysis of ^{18}O-labeling experiments (Figure 4) suggests that the formal reduction product, 5-hydroxymethyl-2′-deoxyuridine (NMe-**17**), is formed by two pathways. Isotopic incorporation (m/z ~ 401.12) is observed when either $H_2{}^{18}O$ (Figure 4B) or $^{18}O_2$ (Figure 4C) is employed. The latter is consistent with the radical pathway, but the incorporation of ^{18}O from $H_2{}^{18}O$ is indicative of nucleophilic substitution. One explanation for the formation of NMe-**17** (Scheme 9) that is consistent with literature precedent on related molecules involves initial formation of the carbocation NMe-**9** (Scheme 2).[14,15,38−42] Phosphate rearrangement, followed by deprotonation, produces an allylic species analogous to others that undergo nucleophilic substitution by H_2O.

Overall, the competition (Scheme 1) between the C5-methyl deprotonation of NMe-**1** to yield NMe-**4** and hydration products (NMe-**5**) is strongly in favor of the latter, consistent with what is observed for **1**. Hydration of NMe-**1** is favored

Table 2. Product Yields from Photolysis of Radical Cation Precursor NMe-6

	% yield[a]								
conditions[b]	NMe-**13**	NMe-**14**	**27**	**28**	NMe-**16**	NMe-**17**	NMe-**T**	NMe-**6**	mass balance
H_2O	15.5 ± 1.7	7.7 ± 1.1	7.2 ± 0.2	4.8 ± 1.1	5.4 ± 0.5	3.5 ± 0.3	33.4 ± 0.3	7.3 ± 6.5	84.8 ± 3.0
BME[c]	3.8 ± 2.6	13.2 ± 3.5	2.6 ± 0.5	1.8 ± 0.6	1.6 ± 0.4	1.5 ± 0.2	71.4 ± 1.1	6.5 ± 6.7	102.4 ± 7.2
BME[d](after hν)	4.4 ± 3.7	17.8 ± 0.6	8.4 ± 0.8	5.3 ± 0.6	7.0 ± 0.6	4.5 ± 0.6	33.4 ± 0.3	2.8 ± 0.4	83.7 ± 1.4
pH 5.0 (Chelex)	9.0 ± 0.1	6.5 ± 0.3	3.1 ± 0.2	6.1 ± 0.1	3.8 ± 0.1	3.2 ± 0.2	28.1 ± 0.1	7.6 ± 6.9	67.3 ± 6.3
pH 5.0	9.5 ± 0.4	6.8 ± 0.1	3.3 ± 0.1	6.4 ± 0.2	3.9 ± 0.1	3.6 ± 0.4	28.6 ± 0.5	7.4 ± 7.1	69.3 ± 6.0
pH 7.2	20.8 ± 1.9	10.6 ± 1.1	3.3 ± 0.4	3.0 ± 0.5	4.3 ± 0.3	5.9 ± 0.1	32.5 ± 0.4	8.1 ± 7.0	88.5 ± 5.8
pH 8.0	18.0 ± 0.3	11.2 ± 0.4	3.4 ± 0.3	2.8 ± 0.2	2.4 ± 0.1	6.2 ± 0.5	32.7 ± 0.2	8.8 ± 7.7	85.5 ± 7.0

[a]Yields and mass balances are the average ± standard deviation of at least 3 measurements. [b]All experiments were carried out in a 1:1 (v/v) mixture of CH_3CN and the solvent listed. [c][BME] = 10 mM. [d][BME] = 100 mM at 37 °C for 1 h.

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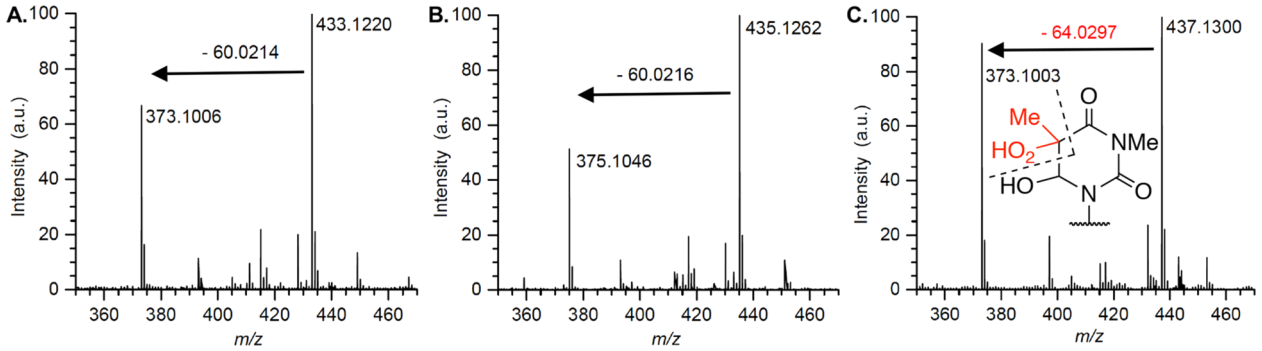


Figure 2. ESI-MS spectra of obtained NMe-**13** from photolysis of NMe-**6** in the presence of (A) H_2O/O_2, (B) $H_2{}^{18}O/O_2$, and $H_2O/{}^{18}O_2$.

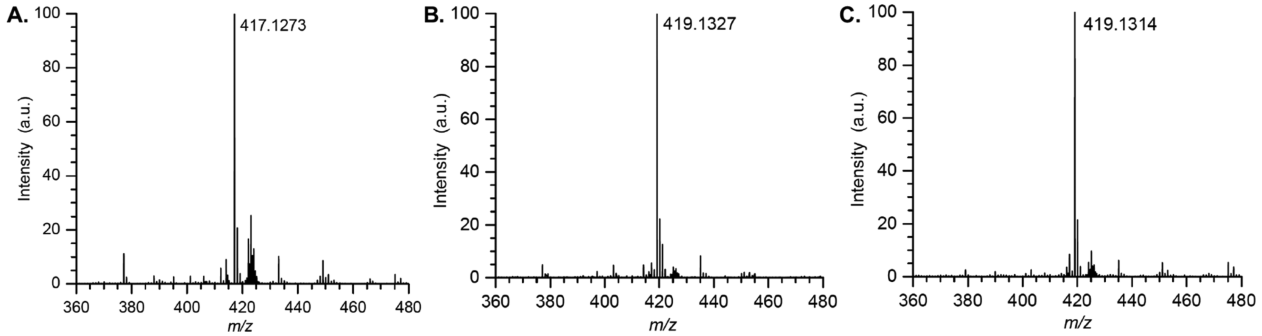


Figure 3. ESI-MS spectra of obtained NMe-**14** from photolysis of NMe-**6** in the presence of (A) H_2O/O_2, (B) $H_2{}^{18}O/O_2$, and $H_2O/{}^{18}O_2$.

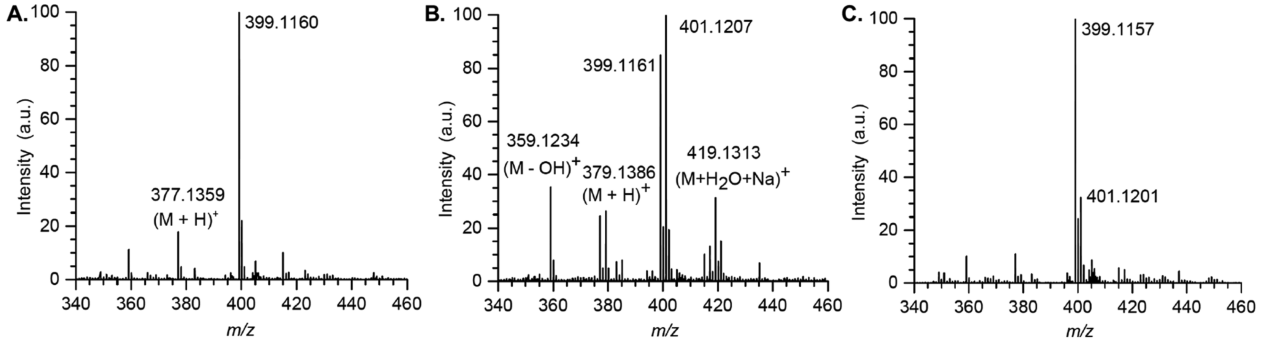
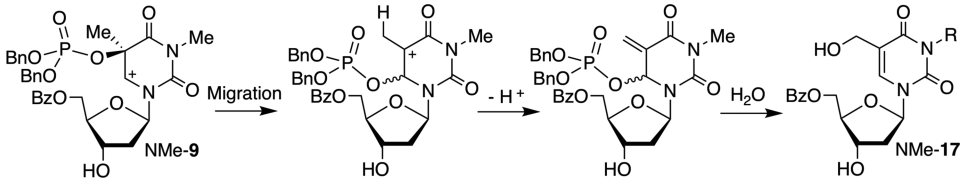


Figure 4. ESI-MS spectra of NMe-**17** from photolysis of NMe-**6** in the presence of $H_2O/H_2{}^{18}O$ and $O_2/{}^{18}O_2$.

Scheme 9. Possible Non-Radical Cation Mechanism for the Formation of NMe-17



over methyl group deprotonation by at least 5-fold over a range of pHs and almost 15-fold at pH 8.

Radical Cation Reduction. A variety of reductants (10 mM) were screened for their ability to reduce NMe-**1** (Figure 5 and Table S1). The reaction of NMe-**1** with each of these molecules was expected to be thermodynamically favorable. Increased yields of the thymidine analogue (NMe-**T**) were observed when any one of the reductants in Figure 5 was present during photolysis. Of the reductants screened, BME and KI were the most effective at reducing NMe-**1**.[43] The yields of NMe-**T** were greater than 60% in the presence of either BME or KI and approached 70% when using the latter.

The varying effects of different reductants on the NMe-**T** yield support the proposal that freely diffusible NMe-**1** is produced in these reactions. In previous studies, the formation of freely diffusible olefin radical cations by β-heterolysis of radicals was dependent upon the radical substituents, the leaving group in the β-position, and solvent polarity.[17−20] The ability of exogenous reductants to increase the NMe-**T** yields suggests that they are reacting with freely diffusible NMe-**1**. Further evidence for trapping freely diffusible NMe-**1** was

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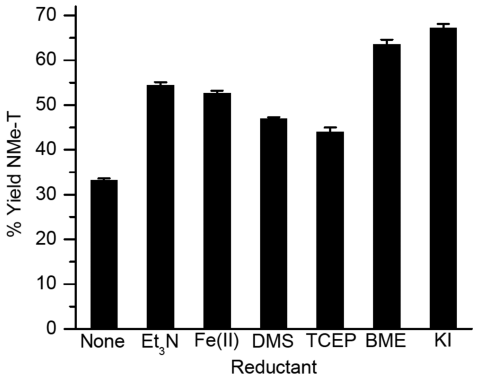

Figure 5. Effect of reductant (10 mM) on the yield of NMe-**T** from photolysis of NMe-**6**. DMS = dimethyl sulfide. TCEP = tris-(carboxyethyl)phosphine.

gleaned from the examination of the competition of the reducing agent for NMe-**1** with hydration (NMe-**13**, NMe-**14**, **27**, **28**) and deprotonation pathways (NMe-**16**). NMe-**17** is not included in this analysis because we believe it is produced from two pathways (per above). Furthermore, given that NMe-**17** is formed in a low yield, we do not believe that it significantly affects the conclusions drawn from Figure 6. The two most effective nucleophiles, BME (Figure 6A) and KI (Figure 6B), were employed. In each instance, the combined yields of trapping products attributable to hydration and C5-methyl group deprotonation approached similar limiting values, 12.3 ± 0.4% for BME and 9.0 ± 0.6% for KI (Supporting Information, Tables S2 and S3). These values

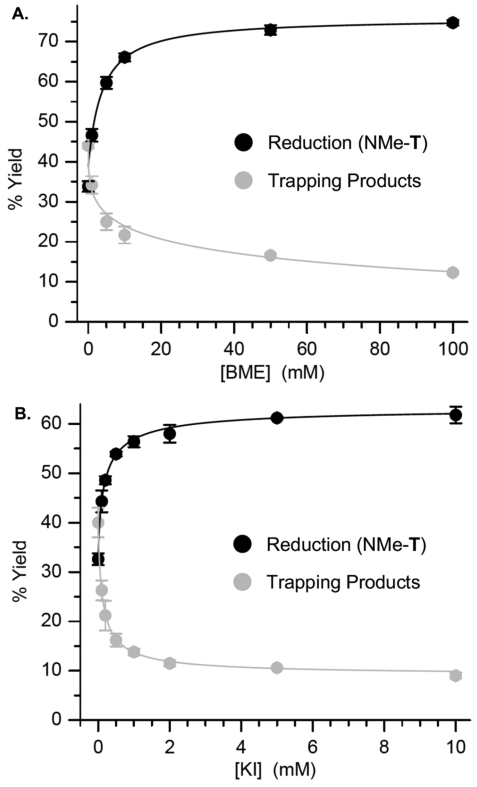

Figure 6. Effect of reductant on product yields from photolysis of NMe-**6**. (A) Reductant = BME. (B) Reductant = KI. "Trapping products" are the summation of yields of NMe-**13**, NMe-**14**, NMe-**16**, **27**, and **28**.

could represent product formation that occurs within the ion pair (NMe-**8**, Scheme 2).

CONCLUSIONS

Thymidine (**1**) and N3-methyl thymidine (NMe-**1**) radical cations were independently generated from precursors other than (N3-methyl) thymidine for the first time. This enabled us to more completely determine the product distributions from the radical cations because their facile reduction produces thymidine. (N3-Methyl) Thymidine can be produced via direct reduction (e.g., by thiol) of the corresponding radical cation. An additional pathway is available to the radical cation derived from the native nucleoside (**1**). N3-Deprotonation (**3**, Scheme 1), followed by formal one electron reduction and protonation, also yields thymidine. Products resulting from hydration are formed in the greatest yields from the N3-methylated radical cation. 5-Formyl-2′-deoxyuridine (NMe-**16**) resulting from deprotonation of the methyl group of NMe-**1** is formed in a considerably lower yield over the entire pH range (5−8) examined. ^{18}O-Labeling studies indicate that the corresponding 5-hydroxymethyl product (NMe-**17**), which is also formed in a relatively low yield, arises from the radical cation and via a second pathway, possibly the carbocation (Schemes 2 and 9). We find that N3-methyl thymidine is a major product from NMe-**1** and is the major product when suitable reducing agents, including the biologically relevant thiol or ferrous agent, are present.

Thymidine is the major product formed upon generation of **1**, even in the absence of excess exogenous reducing agent, presumably via deprotonation from N3 of the radical cation (Scheme 1). The resulting N3-radical (**3**) could yield thymidine by either hydrogen atom abstraction or sequential reduction and protonation. Product analysis indicates that deprotonation from N3 is faster than hydration or deprotonation from the C5-methyl group. Although pulse radiolysis and anthraquinone photosensitization experiments indicate that N3-deprotonation of **1** is rapid,[4,5] the pathway was not detectable in product studies where thymidine was the source of the radical cation.[6] Generation of **1** (and NMe-**1**) in our hands also indicates that hydration is more rapid than methyl group deprotonation. ^{18}O-Labeling studies on the NMe-**1** reactivity are consistent with hydration at C6, followed by O_2 trapping (Scheme 4). This mechanism is consistent with other product and spectroscopic studies but is possibly inconsistent with the work of Sevilla.[4−6,11] However, the latter was carried out at a greater pH (as high as pH 12) in 7.5 M LiCl and in the absence of O_2. This observation is contrasted by studies concerning electron transfer in dA·T rich DNA sequences, where deprotonation from the C5-methyl group of the thymidine radical cation (**1**) has been suggested to predominate.[9,44] It is possible that the reactivity of **1** is different when paired with dA in duplex DNA than when present as a monomeric nucleoside in solution. Certainly, one can envision how base pairing would affect the pK_a of the radical cation and base stacking in duplex DNA could influence the rate of hydration. The independent generation of **1** from a photochemical precursor (**6**) will enable us to address this issue in synthetic oligonucleotides.

EXPERIMENTAL PROCEDURES

General Methods. THF was distilled over Na/benzophenone. DCM, TEA, DMF, and pyridine were dried over CaH_2. $^{18}O_2$ gas was 98% enriched, and that of $H_2{}^{18}O$ was 97%. All other reagents were

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purchased from commercial sources and were used without further purification unless noted otherwise. All reactions were carried out under a positive pressure of an argon atmosphere and monitored by TLC on silica gel G-25 UV254 (0.25 mm) unless stated otherwise. Spots were detected under UV light and/or by an ethanolic solution of *p*-anisaldehyde. Column flash chromatography was performed with Silicycle grade 70−230 mesh, 60−200 μm, 60 Å silica. The ratio between silica gel and crude product ranged from 100:1 to 20:1 (w/w). BME, KI, and solutions of other reductants were freshly prepared. Photolysis samples were prepared in a mixture of acetonitrile and water/buffer (phosphate buffer (20 mM) or Chelex-treated pH = 5 citrate buffer (40 mM)) at a ratio of 1:1 (v/v) containing the appropriate precursor (100 μM) and 20 μM internal standard 5′-benzoyl-2′-deoxyuridine (**BzU**). Photolyses were carried out in Pyrex tubes in a Rayonet photoreactor fitted with 16 lamps with maximum output at 350 nm. Samples containing **6** were photolyzed for 30 min, while samples containing NMe-**6** were photolyzed for 45 min. Photolyzed samples were analyzed using UPLC with an Acquity 1.8 μm C18 UPLC HSS column (100 × 2.1 mm). Detection was carried out at 230 nm, following separation using water (solvent A) and acetonitrile (solvent B) with the following linear gradient (0.2 mL/min): (time (min), % B) 0, 5; 30, 40; 35, 70; 40, 97; 42, 5; 45, 5. (UP)LC/MS/MS analysis was performed on a UPLC system equipped with a Q-TOF ESI mass spectrometer using the same UPLC column and gradient conditions. Response factors (R_f) for each compound (**X**) versus 5′-benzoyl-2′-deoxyuridine (**BzU**) was calculated using the following formula: ([**X**]/[**BzU**]) = R_f(*A*(**X**)/*A*(**BzU**)), where [**X**] is the concentration of compound **X** and [**BzU**] is the concentration of **BzU**. *A*(**X**) and *A*(**BzU**) are the areas under the peaks corresponding to **X** and **BzU**.

^{31}P NMR/UPLC-MS Monitoring of Desilylated-11 Decomposition. Desilylated **11** (5 mg, 7.3 mmol) was dissolved in a mixture of Chelex-treated PBS buffer (400 μL, pH 6.6, 100 mM sodium phosphate, 1 M NaCl) and CD_3OD (400 μL) at room temperature. The ^{31}P NMR spectrum was collected at various times. After 22 days, the sample was dried under reduced pressure and redissolved in CH_3CN for UPLC-MS analysis.

General Procedure for the $MeONH_2$·HCl Trapping Study. Photolyzed samples were incubated at 37 °C in the presence of $MeONH_2$·HCl (10 mM) and NaOAc (10 mM) for 1 h. The samples were neutralized using pH 8.0 PBS buffer (100 mM, 12% volume of samples) prior to the UPLC analysis.

General Procedure for the ^{18}O Labeling Study. Photolyses in H_2O/O_2, $H_2{}^{18}O/O_2$, or $H_2O/{}^{18}O_2$ were carried out side-by-side. For experiments with $H_2{}^{18}O$, the isotopically labeled solvent was substituted for H_2O, otherwise all other conditions were the same. For experiments with $^{18}O_2$, the gas was bubbled through the solution in an ice bath at a rate of 1 mL/min for 5 min and sealed before photolysis. The photolyzed samples were evaporated to dryness and then redissolved in a mixture of acetonitrile and water at a ratio of 1:1 (v/v) with the same volume before LC-MS/MS analysis.

Preparation of 11. Dibenzyl *N*,*N*-diethyl phosphoramidite (76 mg, 0.24 mmol) was added to a solution of **10**[14] (131 mg, 0.20 mmol) in CH_2Cl_2 (2 mL), followed by 5-ethylthiotetrazole (0.25 M in acetonitrile, 0.24 mmol). The solution was stirred at room temperature overnight and then cooled to 0 °C with an ice bath. *t*-BuOOH (5−6 M in decane, 0.3 mL) was added, and the mixture was stirred for 1 h at 0 °C. After warming to room temperature, the mixture was directly subjected to flash column chromatography (EtOAc/CH_2Cl_2 = 1:3) to provide **11** (101 mg, 0.13 mmol, 64%) as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42−7.28 (m, 10H), 7.07 (d, *J* = 3.1 Hz, 2H), 6.85 (dd, *J* = 9.0, 3.1 Hz, 1H), 6.75 (d, *J* = 9.0 Hz, 1H), 6.17 (dd, *J* = 9.4, 5.4 Hz, 1H), 5.34 (s, 1H), 5.28−5.03 (m, 4H), 4.29 (dd, *J* = 3.8, 1.9 Hz, 1H), 3.87−3.76 (m, 1H), 3.74 (s, 3H), 3.69 (dd, *J* = 10.6, 4.6 Hz, 1H), 3.62 (s, 3H), 3.53 (dd, *J* = 10.6, 6.8 Hz, 1H), 2.46 (ddd, *J* = 13.5, 9.5, 5.7 Hz, 1H), 1.86 (s, 3H), 1.72 (ddd, *J* = 13.4, 5.4, 1.8 Hz, 1H), 0.89 (s, 16H), 0.07 (s, 6H), 0.07 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.1, 153.2, 150.0, 136.0, 128.49, 128.46, 128.37, 128.31, 128.0, 127.8, 123.1, 117.6, 117.2, 111.7, 86.3, 84.6, 79.7, 79.6, 72.7, 69.9, 69.4, 63.4, 56.0, 55.7, 36.2, 26.0, 25.8, 22.9, 18.4, 18.0, −4.66, −4.74, −5.3, −5.4; ^{31}P NMR (162 MHz, $CDCl_3$) δ −5.88; IR (NaCl) 1642, 1492, 1454 cm^{-1}; HRMS (ESI-TOF) *m*/*z* [M + H]$^+$ calcd for $C_{44}H_{66}N_2O_{11}PSSi_2$ 917.3663, found 917.3657.

Preparation of 12. K_2CO_3 (69 mg, 0.5 mmol) and MeI (71 mg, 0.5 mmol) were added to a solution of **11** (198 mg, 0.22 mmol) in DMF (10 mL). The mixture was stirred at room temperature for 7 h. After quenching with water (40 mL), the mixture was extracted with EtOAc (3 × 30 mL). The combined organic phases were washed with water (50 mL), brine (50 mL), dried over Na_2SO_4, filtered, and then concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel (ethyl acetate/dicholoromethane = 1:9), affording **12** (160 mg, 0.17 mmol, 80%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30−7.39 (m, 10H), 7.05 (d, *J* = 3.0 Hz, 1H), 6.86−6.78 (m, 2H), 6.76 (d, *J* = 9.0 Hz, 1H), 6.33−6.23 (m, 1H), 5.27 (s, 1H), 5.26−5.07 (m, 4H), 4.32 (dd, *J* = 3.5, 2.0 Hz, 1H), 3.89−3.78 (m, 1H), 3.78−3.66 (m, 4H), 3.63 (s, 3H), 3.60−3.49 (m, 1H), 2.79 (s, 3H), 2.67−2.48 (m, 1H), 1.86 (ddd, *J* = 13.3, 5.4, 1.9 Hz, 1H), 1.82 (s, 3H), 0.97−0.82 (m, 18H), 0.17−0.03 (m, 12H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.6, 154.9, 153.2, 151.3, 136.2, 136.0, 128.47, 128.45, 128.31, 128.26, 128.0, 127.7, 123.2, 117.5, 117.2, 111.8, 86.3, 85.1, 79.5, 79.4, 72.7, 69.9, 69.3, 63.8, 62.6, 62.5, 56.0, 55.7, 36.3, 27.9, 26.0, 25.8, 23.2, 18.4, 18.0, −4.65, −4.72, −5.3, −5.4; ^{31}P NMR (162 MHz, $CDCl_3$) δ −5.85; IR (NaCl) 2951, 2857, 1734, 1689, 1458 cm^{-1}; HRMS (ESI-TOF) *m*/*z* [M + H]$^+$ calcd for $C_{45}H_{68}N_2O_{11}PSSi_2$ 931.3820, found 931.3810.

Preparation of Desilylated 11. Et_3N·3HF (290 mg, 1.8 mmol) was added to a solution of **11** (92 mg, 0.1 mmol) in THF (3.0 mL), and the mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography (methanol/CH_2Cl_2 = 1:9) to afford the product desilylated **11** (66 mg, 0.096 mmol, 96%) as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.44−7.28 (m, 10H), 7.23 (s, 1H), 7.05 (d, *J* = 3.1 Hz, 1H), 6.89 (dd, *J* = 9.0, 3.1 Hz, 1H), 6.76 (d, *J* = 9.0 Hz, 1H), 6.19 (dd, *J* = 9.0, 5.8 Hz, 1H), 6.03 (s, 1H), 5.40 (dd, *J* = 11.9, 7.4 Hz, 1H), 5.31 (dd, *J* = 11.9, 6.6 Hz, 1H), 5.11 (dd, *J* = 7.7, 1.1 Hz, 2H), 4.45 (d, *J* = 5.1 Hz, 1H), 4.00 (s, 1H), 3.84 (d, *J* = 13.7 Hz, 1H), 3.74 (s, 3H), 3.68 (s, 3H), 2.44 (ddd, *J* = 14.6, 9.1, 5.6 Hz, 1H), 2.05 (s, 1H), 1.83 (s, 3H), 1.67 (ddd, *J* = 13.7, 5.8, 1.7 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.5, 155.6, 153.1, 150.4, 135.73, 135.65, 135.23, 135.15, 128.7, 128.6, 128.58, 128.53, 128.1, 127.9, 124.0, 117.9, 116.8, 111.7, 86.9, 85.6, 79.34, 72.9, 70.2, 70.1, 62.8, 62.3, 56.0, 55.8, 37.0, 23.0; ^{31}P NMR (162 MHz, $CDCl_3$) δ −6.33; IR (NaCl) 1698, 1541 cm^{-1}; HRMS (ESI-TOF) *m*/*z* [M + H]$^+$ calcd for $C_{32}H_{38}N_2O_{11}PS$ 689.1934, found 689.1928.

Preparation of Desilylated 12. Et_3N·3HF (725.4 mg, 4.5 mmol) was added to a solution of **12** (233 mg, 0.25 mmol) in THF (3.0 mL). The mixture was stirred at room temperature overnight, and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (methanol/CH_2Cl_2 = 1:9) to afford desilylated **12** (146 mg, 0.21 mmol, 83%) as a white foam: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39−7.31 (m, 10H), 7.01 (d, *J* = 3.1 Hz, 1H), 6.85 (dd, *J* = 9.0, 3.1 Hz, 1H), 6.77 (d, *J* = 9.0 Hz, 1H), 6.30 (dd, *J* = 8.7, 6.0 Hz, 1H), 5.96 (s, 1H), 5.44 (dd, *J* = 11.9, 7.3 Hz, 1H), 5.32 (dd, *J* = 11.9, 6.5 Hz, 1H), 5.13 (dd, *J* = 7.6, 1.4 Hz, 2H), 4.53 (dd, *J* = 3.9, 1.8 Hz, 1H), 3.87 (dd, *J* = 15.1, 3.1 Hz, 2H), 3.74 (s, 3H), 3.72−3.70 (m, 1H), 3.69 (s, 3H), 2.74 (s, 3H), 2.73−2.63 (m, 1H), 1.87 (ddd, *J* = 13.8, 6.0, 2.0 Hz, 1H), 1.78 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.3, 155.5, 153.1, 151.5, 135.8, 135.3, 128.6, 128.59, 128.57, 128.5, 128.1, 127.9, 124.0, 117.8, 116.7, 111.8, 86.9, 86.1, 79.0, 72.8, 70.2, 70.1, 62.8, 61.4, 56.0, 55.9, 37.1, 27.8, 23.4; ^{31}P NMR (162 MHz, $CDCl_3$) δ −6.35; IR (NaCl) 3424, 1732, 1685, 1457 cm^{-1}; HRMS (ESI-TOF) *m*/*z* [M + H]$^+$ calcd for $C_{33}H_{40}N_2O_{11}PS$ 702.2090, found 702.2092.

Preparation of 6. BzCN (8.8 mg, 0.07 mmol) and Et_3N (8.5 mg, 0.084 mmol) were added to desilylated **11** (31.6 mg, 0.056 mmol) in THF (1.0 mL) at −78 °C. The mixture was stirred at −78 °C for 3 h. After quenching the reaction with MeOH (0.3 mL), the solution was warmed to room temperature, and the solvent was removed under reduced pressure. The residue was purified by flash column

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chromatography ($MeOH/CH_2Cl_2$ = 1:15) to afford the starting material (20 mg, 0.0354 mmol) and **6** (6.4 mg, 0.008 mmol, 39% based on unrecovered starting material) as a white foam: ^1H NMR (400 MHz, $CDCl_3$) δ 8.05 (dd, J = 8.4, 1.4 Hz, 2H), 7.56−7.46 (m, 1H), 7.45−7.36 (m, 2H), 7.36−7.27 (m, 10H), 7.16 (s, 1H), 7.06 (d, J = 3.1 Hz, 1H), 6.85 (dd, J = 9.0, 3.1 Hz, 1H), 6.73 (d, J = 9.1 Hz, 1H), 6.26 (dd, J = 8.1, 6.2 Hz, 1H), 5.44 (s, 1H), 5.21−4.88 (m, 4H), 4.52 (dd, J = 11.8, 6.0 Hz, 1H), 4.42 (dd, J = 11.8, 4.8 Hz, 2H), 4.10 (ddd, J = 5.9, 4.7, 3.5 Hz, 1H), 3.74 (s, 3H), 3.60 (s, 3H), 2.80 (ddd, J = 14.5, 8.2, 6.7 Hz, 1H), 2.26 (s, 1H), 2.01 (ddd, J = 14.0, 6.2, 3.4 Hz, 1H), 1.84 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.3, 166.5, 155.1, 153.2, 150.0, 135.9, 133.3, 129.8, 129.6, 128.50, 128.48, 128.41, 128.35, 128.0, 127.8, 123.1, 117.5, 117.3, 111.9, 84.5, 82.9, 79.6, 72.3, 69.9, 69.4, 64.7, 63.4, 56.1, 55.7, 36.0, 22.8; ^{31}P NMR (162 MHz, $CDCl_3$) δ −5.77; IR (NaCl) 3397, 1699, 1650, 1541 cm^{-1}; HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{39}H_{42}N_2O_{12}PS$ 793.2196, found 793.2183.

Preparation of NMe-6. Desilylated **12** (84.24 mg, 0.12 mmol) was azeotropically dried with pyridine (3 × 2.0 mL) and redissolved in pyridine (2.0 mL) at 0 °C. Benzoyl chloride (18.6 mg, 0.13 mmol) was added. The mixture was allowed to warm to room temperature and stirred for 6 h. After quenching the reaction with MeOH (0.3 mL), the solvent was removed under reduced pressure. The residue was purified by flash column chromatography ($EtOAc/CH_2Cl_2$ = 1:1) to afford NMe-**6** (34 mg, 0.042 mmol, 35%) as a colorless oil: ^1H NMR (400 MHz, $CDCl_3$) δ 8.11−7.98 (m, 2H), 7.48 (d, J = 7.4 Hz, 1H), 7.42−7.27 (m, 12H), 7.04 (d, J = 3.1 Hz, 1H), 6.82 (dd, J = 9.0, 3.1 Hz, 1H), 6.75 (d, J = 9.0 Hz, 1H), 6.35 (dd, J = 7.9, 6.3 Hz, 1H), 5.36 (s, 1H), 5.13 (ddd, J = 30.5, 15.0, 5.0 Hz, 4H), 4.53 (dd, J = 11.8, 5.9 Hz, 1H), 4.48−4.32 (m, 2H), 4.11 (dd, J = 3.7, 1.4 Hz, 1H), 3.73 (s, 3H), 3.61 (s, 3H), 2.93 (ddd, J = 14.4, 7.9, 6.8 Hz, 1H), 2.77 (s, 3H), 2.12 (ddd, J = 14.0, 6.3, 3.6 Hz, 1H), 1.79 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.4, 166.5, 155.1, 153.2, 151.3, 133.2, 129.8, 129.7, 128.5, 128.34, 128.28, 128.0, 127.72, 127.71, 123.3, 117.5, 117.2, 111.9, 85.0, 82.9, 79.4, 79.3, 76.7, 72.3, 69.9, 69.4, 69.3, 64.8, 56.1, 55.7, 36.2, 27.9, 23.2; ^{31}P NMR (162 MHz, $CDCl_3$) δ −5.82; IR (NaCl) 3407, 1688, 1455 cm^{-1}; HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{40}H_{44}N_2O_{12}PS$ 807.2353, found 807.2340.

Preparation of NMe-14. To a solution of NMe-**T** (180 mg, 0.5 mmol) in a mixture of THF (4 mL) were added *t*-butyl alcohol (4 mL), water (1 mL), *N*-methylmorpholine *N*-oxide (117 mg, 1 mmol), and OsO_4 (5.1 mg, 0.02 mmol). The yellowish solution was stirred at 45 °C for 36 h. After cooling the solution to 0 °C, sodium sulfite (252 mg, 2 mmol) was added. The mixture was stirred at 0 °C for 30 min and warmed to room temperature. The solvent was removed, and the residue was dissolved in ethyl acetate (100 mL). The organic phase was washed with water (50 mL) and brine (50 mL) and then dried over with Na_2SO_4. After concentrating under reduced pressure, the residue was purified by flash chromatography on silica gel (ethyl acetate/hexane = 1:1), affording NMe-**14** as a mixture of diastereomers (92:8) in 39% (76 mg) yield as a white foam. Major stereoisomer of NMe-**14**: ^1H NMR (400 MHz, $CDCl_3$) δ 8.10−8.00 (m, 2H), 7.63−7.56 (m, 1H), 7.50−7.43 (m, 2H), 6.34 (dd, J = 7.2, 6.6 Hz, 1H), 5.01 (s, 1H), 4.60 (dd, J = 12.1, 4.4 Hz, 1H), 4.56−4.45 (m, 2H), 4.14 (dd, J = 7.9, 3.7 Hz, 1H), 3.21 (s, 3H), 2.42 (ddd, J = 17.7, 7.3, 6.6 Hz, 1H), 2.27 (ddd, J = 13.9, 6.3, 3.9 Hz, 1H), 1.35 (s, 3H). Minor stereoisomer of NMe-**14**: ^1H NMR (400 MHz, $CDCl_3$) δ 8.10−8.00 (m, 2H), 7.63−7.56 (m, 1H), 7.50−7.43 (m, 2H), 6.44 (t, J = 6.8 Hz, 1H), 4.85 (s, 1H), 4.70 (dd, J = 12.2, 5.0 Hz, 1H), 4.45 (d, J = 4.3 Hz, 2H), 4.10 (d, J = 7.2 Hz, 1H), 3.21 (s, 3H), 2.62 (s, 1H), 2.14 (dd, J = 14.1, 6.9 Hz, 1H), 1.32 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.5, 166.5, 151.1, 133.5, 129.7, 129.4, 128.6, 84.6, 83.3, 77.3, 77.0, 76.7, 71.7, 71.5, 64.1, 38.5, 28.3, 22.8. IR (NaCl) 3425 (bd), 1679, 1464 cm^{-1}; HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{18}H_{22}N_2NaO_8$ 417.1274, found 417.1265.

Preparation of 21. To a solution of 5′-benzoyl-thymidine (692 mg, 2.0 mmol) in anhydrous DMF (12 mL) were added imidazole (409 mg, 6.0 mmol) and TBDMSCl (452 mg, 3.0 mmol). The mixture was stirred at room temperature overnight, and methanol (0.5 mL), followed by water (30 mL), was added to quench the reaction. The mixture was extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with H_2O (30 mL) and brine (30 mL) and then dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel (ethyl acetate/hexane = 1:1), providing **21** (680 mg, 1.48 mmol, 74%) as a white foam: ^1H NMR (400 MHz, $CDCl_3$) δ 9.28 (s, 1H), 8.09−7.96 (m, 2H), 7.59 (ddd, J = 8.8, 5.4, 1.3 Hz, 1H), 7.46 (td, J = 7.6, 1.7 Hz, 2H), 7.24 (d, J = 1.2 Hz, 1H), 6.30 (t, J = 6.6 Hz, 1H), 4.62 (dd, J = 12.3, 3.3 Hz, 1H), 4.51−4.43 (m, 2H), 4.19 (q, J = 3.6 Hz, 1H), 2.37 (ddd, J = 13.4, 6.2, 3.7 Hz, 1H), 2.13 (dt, J = 13.5, 6.7 Hz, 1H), 1.66 (d, J = 1.1 Hz, 3H), 0.89 (d, J = 0.9 Hz, 9H), 0.09 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 134.9, 133.6, 129.5, 129.4, 128.7, 111.2, 85.2, 84.9, 72.0, 63.8, 41.1, 25.70, 25.69, 18.0, 12.3, −4.7, −4.9; IR (NaCl) 1697, 1465 cm^{-1}; HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{23}H_{32}N_2NaO_6Si$ 483.1927, found 483.1922.

Preparation of NMe-21. 5′-Benzoyl-3-methyl-thymidine (432 mg, 1.2 mmol) was reacted with imidazole (245 mg, 3.6 mmol) and TBDMSCl (271 mg, 1.8 mmol) in DMF (12 mL) as described above for the preparation of **21**. Following flash column chromatography (ethyl acetate/hexane = 1:2), NMe-**21** (460 mg, 0.97 mmol, 81%) was obtained as a colorless oil: ^1H NMR (400 MHz, $CDCl_3$) δ 8.05−7.94 (m, 2H), 7.61−7.53 (m, 1H), 7.48−7.39 (m, 2H), 7.23 (d, J = 1.2 Hz, 1H), 6.29 (t, J = 6.6 Hz, 1H), 4.60 (dd, J = 12.3, 3.3 Hz, 1H), 4.50−4.41 (m, 2H), 4.18 (q, J = 3.6 Hz, 1H), 3.30−3.27 (m, 3H), 2.36 (ddd, J = 13.5, 6.2, 3.7 Hz, 1H), 2.10 (dt, J = 13.4, 6.6 Hz, 1H), 1.66 (d, J = 0.9 Hz, 3H), 0.91−0.84 (m, 9H), 0.07 (d, J = 0.7 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.1, 163.5, 150.9, 133.5, 132.7, 129.5, 129.4, 128.7, 110.1, 85.9, 84.8, 71.9, 63.7, 41.2, 27.8, 25.69, 25.67, 17.9, 13.0, −4.7, −4.9; IR (NaCl) 1708, 1466 cm^{-1}. HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{24}H_{34}N_2NaO_6Si$ 497.2084, found 497.2083.

Preparation of 22. *N*-Bromosuccinimide (107 mg, 0.6 mmol) and AIBN (8.2 mg, 0.05 mmol) were added to a solution of **21** (230 mg, 0.5 mmol) in benzene (5 mL). The mixture was refluxed for 3 h, at which time additional *N*-bromosuccinimide and AIBN (half the amount of the first addition) were added. The mixture was refluxed for another 3 h and then cooled to room temperature. After removing the solvent under a vacuum, aq 5% $NaHCO_3$ (15 mL) was added. The mixture was stirred at room temperature overnight and then extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with H_2O (30 mL) and brine (30 mL) and then dried over anhydrous Na_2SO_4. After removing the solvent under a vacuum, the residue was purified by chromatography (ethyl acetate/hexane = 1:1 to 2:1), yielding **22** (52 mg, 0.11 mmol, 22%) as a colorless oil: ^1H NMR (400 MHz, $CDCl_3$) δ 9.44 (s, 1H), 8.02 (dt, J = 8.4, 1.6 Hz, 2H), 7.60 (t, J = 7.4 Hz, 1H), 7.52 (s, 1H), 7.51−7.41 (m, 2H), 6.34−6.21 (m, 1H), 4.54 (qd, J = 12.3, 3.9 Hz, 2H), 4.48−4.42 (m, 1H), 4.26−4.17 (m, 2H), 4.12 (dd, J = 10.1, 4.3 Hz, 1H), 2.74 (s, 1H), 2.46−2.34 (m, 1H), 2.22−2.09 (m, 1H), 0.92−0.87 (m, 9H), 0.13−0.06 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.0, 166.3, 163.7, 150.0, 136.8, 133.6, 129.6, 129.4, 128.7, 114.1, 85.6, 85.0, 71.8, 63.7, 58.4, 41.2, 29.6, 25.7, 17.9, −4.7, −4.9; IR (NaCl) 3482, 1712, 1468 cm^{-1}; HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{23}H_{32}N_2NaO_7Si$ 499.1876, found 499.1876.

Preparation of NMe-22. Protected nucleoside NMe-**21** (237 mg, 0.5 mmol) was reacted as described above for **21** using the same amount of reagents, with one exception. Acetonitrile (5 mL) was added during hydrolysis to help solubilize the bromide intermediate. Following flash chromatography (ethyl acetate/hexane = 1:1 to 2:1), NMe-**22** (146 mg, 0.30 mmol, 60%) was obtained as a colorless oil: ^1H NMR (400 MHz, $CDCl_3$) δ 8.05−7.97 (m, 2H), 7.64−7.56 (m, 1H), 7.50 (s, 1H), 7.49−7.42 (m, 2H), 6.28 (t, J = 6.5 Hz, 1H), 4.54 (qd, J = 12.3, 3.9 Hz, 2H), 4.48−4.40 (m, 1H), 4.28−4.17 (m, 2H), 4.11 (ddd, J = 14.2, 11.2, 6.3 Hz, 1H), 3.30 (s, 3H), 2.76 (s, 1H), 2.41 (ddd, J = 13.5, 6.2, 3.9 Hz, 1H), 2.12 (dt, J = 13.4, 6.6 Hz, 1H), 0.91−0.86 (m, 9H), 0.10−0.07 (m, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 163.2, 150.6, 134.2, 133.6, 129.5, 129.4, 128.7, 113.0, 86.2, 85.0, 71.8, 63.6, 59.3, 41.3, 27.6, 25.7, 17.9, −4.7, −4.9; IR

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(NaCl) 3474, 1713, 1469 cm^{-1}; HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{24}H_{34}N_2NaO_7Si$ 513.2033, found 513.2031.

Preparation of 17. $Et_3N{\cdot}3HF$ (102 mg, 0.63 mmol) was added to a solution of **22** (33 mg, 70 μmol) in THF (2 mL). The solution was stirred at room temperature overnight. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography (methanol in CH_2Cl_2 5−10%) to afford **17** (21 mg, 58 μmol, 83%) as a white foam: ^1H NMR (400 MHz, MeOD) δ 8.06−8.04 (m, 1H), 8.04−8.02 (m, 1H), 7.66 (t, J = 1.0 Hz, 1H), 7.64−7.59 (m, 1H), 7.52−7.46 (m, 2H), 6.30 (t, J = 6.8 Hz, 1H), 4.62−4.52 (m, 2H), 4.52−4.44 (m, 1H), 4.26−4.19 (m, 1H), 4.20−4.08 (m, 2H), 2.40 (dtd, J = 9.9, 6.5, 3.8 Hz, 1H), 2.29 (ddd, J = 13.8, 7.1, 6.6 Hz, 1H); ^{13}C NMR (101 MHz, MeOD) δ 166.3, 163.6, 150.7, 137.2, 133.1, 129.6, 129.2, 128.3, 114.1, 85.4, 84.6, 71.0, 64.2, 56.4, 39.6; IR (NaCl) 3355, 1657, 1557 cm^{-1}; HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{17}H_{18}N_2NaO_7$ 385.1012, found 385.1004.

Preparation of NMe-17. The reaction of NMe-**22** (128 mg, 0.26 mmol) was carried out as described above for **22**, with the exception that 377 mg (2.34 mmol) of $Et_3N{\cdot}3HF$ was employed. Following flash chromatography, 91 mg (0.24 mmol, 93%) of NMe-**17** was obtained as a white foam: ^1H NMR (400 MHz, DMSO) δ 8.03−7.94 (m, 2H), 7.72−7.61 (m, 1H), 7.60−7.48 (m, 2H), 6.26 (t, J = 6.7 Hz, 1H), 5.51 (d, J = 4.5 Hz, 1H), 4.97 (t, J = 5.4 Hz, 1H), 4.47 (ddd, J = 17.5, 12.0, 4.7 Hz, 2H), 4.40−4.30 (m, 1H), 4.19−4.01 (m, 3H), 3.34 (d, J = 6.1 Hz, 2H), 3.15 (s, 3H), 2.34−2.17 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 166.1, 162.0, 150.9, 135.0, 134.0, 129.8, 129.7, 129.3, 113.9, 85.8, 84.3, 70.6, 64.9, 56.8, 27.8; IR (NaCl) 3622, 1698, 1557 cm^{-1}; HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{18}H_{20}N_2NaO_7$ 399.1168, found 399.1160.

Preparation of NMe-18. 5-Iodo-3-methyl-2′-deoxyuridine[4] (736 mg, 2 mmol) was azeotropically dried with pyridine (5 mL × 3) and redissolved in pyridine (8 mL). The solution was cooled to 0 °C, and benzoyl chloride (309 mg, 2.2 mmol) was added. The mixture was stirred at 0 °C for 3 h, at which time methanol (1 mL) was added to quench the reaction, and the mixture was warmed to room temperature. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography (dry loading, EtOAc/hexane = 1:3 to 2:3 to remove the remaining pyridine; then methanol in dicholoromethane 2−10%) to yield NMe-**18** (0.78 g, 1.65 mmol, 83%) as a white foam: ^1H NMR (400 MHz, DMSO) δ 8.03 (s, 1H), 8.02−7.96 (m, 2H), 7.73−7.64 (m, 1H), 7.54 (t, J = 7.7 Hz, 2H), 6.15 (t, J = 6.7 Hz, 1H), 5.49 (d, J = 4.3 Hz, 1H), 4.50 (ddd, J = 17.6, 12.1, 4.6 Hz, 2H), 4.37 (td, J = 7.5, 3.7 Hz, 1H), 4.13 (dt, J = 5.4, 3.6 Hz, 1H), 3.19 (s, 3H), 2.37−2.18 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 166.1, 160.1, 150.6, 143.2, 136.6, 134.0, 129.7, 129.4, 124.4, 86.7, 84.8, 70.7, 69.0, 64.8, 29.2; IR (NaCl) 3461, 1708, 1452 cm^{-1}; HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{17}H_{18}IN_2O_6$ 473.0210, found 473.0208.

Preparation of 19. Tributyl(vinyl) stannane (419 mg, 1.32 mmol) was added to a solution of **18**[5] (504 mg, 1.1 mmol) and $Pd(Ph_3P)_2Cl_2$ (39 mg, 55 μmol) in anhydrous THF (5 mL). The solution was refluxed overnight. After cooling the dark mixture to room temperature, the solvent was removed under reduced pressure and then dissolved in EtOAc (100 mL). The solution was washed with H_2O (30 mL) and brine (30 mL) and then dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the residue was purified by chromatography (methanol in CH_2Cl_2 2−5%), yielding **19** (336 mg, 85%) as a white foam: ^1H NMR (400 MHz, DMSO) δ 11.46 (s, 1H), 8.02−7.92 (m, 2H), 7.72−7.62 (m, 2H), 7.58−7.48 (m, 2H), 6.25−6.12 (m, 2H), 5.89 (dd, J = 17.6, 2.1 Hz, 1H), 5.49 (d, J = 4.4 Hz, 1H), 5.01 (dd, J = 11.5, 2.1 Hz, 1H), 4.55 (dd, J = 12.0, 3.7 Hz, 1H), 4.50−4.36 (m, 2H), 4.09 (dt, J = 5.4, 3.7 Hz, 1H), 2.32 (dt, J = 13.5, 6.8 Hz, 1H), 2.21 (ddd, J = 13.6, 6.5, 3.8 Hz, 1H); ^{13}C NMR (101 MHz, DMSO) δ 166.1, 162.4, 149.9, 138.2, 134.0, 129.8, 129.7, 129.3, 129.2, 115.0, 111.6, 85.1, 84.4, 70.7, 64.9; IR (NaCl) 3417, 1699, 1458 cm^{-1}; HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{18}H_{18}N_2NaO_6$ 381.1063, found 381.1056.

Preparation of NMe-19. 5′-Benzoyl-5-iodo-3-methyl-2′-deoxyuridine (NMe-**18**, 519 mg, 1.1 mmol) was subjected to the reaction conditions described above for the preparation of **19**, resulting in NMe-**19** (330 mg, 81%) as a white foam: ^1H NMR (400 MHz, $CDCl_3$) δ 8.04−7.97 (m, 2H), 7.63−7.57 (m, 1H), 7.53 (s, 1H), 7.44 (td, J = 7.6, 1.6 Hz, 2H), 6.32 (t, J = 6.5 Hz, 1H), 6.26−6.15 (m, 1H), 5.77 (dd, J = 17.6, 1.4 Hz, 1H), 5.05 (dd, J = 11.4, 1.3 Hz, 1H), 4.63 (t, J = 3.5 Hz, 2H), 4.55−4.49 (m, 1H), 4.30 (dd, J = 7.1, 3.7 Hz, 1H), 3.32 (s, 3H), 2.71 (s, 1H), 2.55 (ddd, J = 13.9, 6.2, 4.0 Hz, 1H), 2.27−2.20 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.5, 161.7, 150.2, 133.7, 133.6, 129.6, 129.2, 128.7, 128.2, 115.5, 112.0, 86.2, 84.7, 71.4, 63.9, 41.1, 27.9; IR (NaCl) 3429, 1708, 1464 cm^{-1}; HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{19}H_{21}N_2O_6$ 373.1400, found 373.1387.

Preparation of 16. OsO_4 (4.0% solution in water, 7.0 mg, 27.5 μmol) and $NaIO_4$ (705.9 mg, 3.3 mmol) were added to a suspension of **19** (197 mg, 0.55 mmol) in a mixture of dioxane and water (8 mL, v/v = 3:1) and 2,6-lutidine (118 mg, 1.1 mmol). The mixture was stirred at room temperature overnight. The solvent was removed, and the residue was dissolved in EtOAc (80 mL). The mixture was washed with water (30 mL) and brine (30 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure. The residue was purified by flash column chromatography (methanol in dichloromethane 2−5%), providing **16** (180 mg, 91%) as a white foam: ^1H NMR (400 MHz, DMSO) δ 11.80 (s, 1H), 9.67 (s, 1H), 8.33 (s, 1H), 8.03−7.93 (m, 2H), 7.69−7.62 (m, 1H), 7.53−7.45 (m, 2H), 6.11 (t, J = 6.4 Hz, 1H), 5.54−5.49 (m, 1H), 4.59−4.51 (m, 1H), 4.48−4.42 (m, 1H), 4.40−4.31 (m, 1H), 4.25−4.18 (m, 1H), 2.39−2.30 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 186.4, 166.1, 162.0, 149.9, 146.8, 134.0, 129.7, 129.3, 129.2, 111.1, 87.1, 85.2, 70.6, 64.7; IR (NaCl) 3390, 1701, 1464 cm^{-1}; HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{17}H_{16}N_2NaO_7$ 383.0855, found 383.0843.

Preparation of NMe-16. Compound NMe-**19** (186 mg, 0.5 mmol), 2,6-lutidine (107 mg, 1 mmol), OsO_4 (6.4 mg, 25 μmol), and $NaIO_4$ (641.7 mg, 3 mmol) were subjected to the reaction conditions described above for the formation of **16** to provide NMe-**16** (153 mg, 82%) as a white foam: ^1H NMR (400 MHz, DMSO) δ 9.72 (s, 1H), 8.37 (s, 1H), 8.02−7.94 (m, 2H), 7.68−7.62 (m, 1H), 7.53−7.46 (m, 2H), 6.14 (t, J = 6.4 Hz, 1H), 5.52 (dd, J = 8.3, 4.4 Hz, 1H), 4.55 (dd, J = 12.2, 3.4 Hz, 1H), 4.44 (dt, J = 11.9, 4.7 Hz, 1H), 4.40−4.34 (m, 1H), 4.23 (dt, J = 5.5, 3.6 Hz, 1H), 3.17 (s, 3H), 2.42−2.34 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 186.7, 166.1, 161.2, 150.1, 144.8, 134.0, 129.7, 129.2, 110.2, 88.1, 85.4, 70.5, 64.7, 27.7; IR (NaCl) 3415, 1719, 1462 cm^{-1}; HRMS (ESI-TOF) m/z $[M + Na]^+$ calcd for $C_{18}H_{18}N_2NaO_7$ 397.1012, found 397.1004.

Preparation of 20. A mixture containing **16** (48 mg, 0.13 mmol), $MeONH_2{\cdot}HCl$ (30.1 mg, 0.36 mmol), NaOAc (48.1 mg, 0.59 mmol), H_2O (3.6 mL), and THF (1.2 mL) was stirred at room temperature for 2 h. The solution was extracted with EtOAc (2 × 30 mL). The combined organic layers were washed with water (30 mL) and brine (30 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to quantitatively yield **20** (56 mg, 100%) as a white foam: ^1H NMR (400 MHz, DMSO) δ 11.70 (s, 1H), 8.01 (s, 1H), 7.98−7.93 (m, 2H), 7.81−7.76 (m, 1H), 7.70−7.63 (m, 1H), 7.54−7.47 (m, 2H), 6.15 (t, J = 6.6 Hz, 1H), 5.50 (t, J = 3.8 Hz, 1H), 4.56−4.47 (m, 1H), 4.48−4.40 (m, 1H), 4.41−4.33 (m, 1H), 4.22−4.14 (m, 1H), 3.55 (s, 3H), 2.33−2.23 (m, 2H); ^{13}C NMR (101 MHz, DMSO) δ 166.1, 161.7, 150.0, 142.0, 137.6, 134.0, 129.7, 129.7, 129.2, 106.2, 86.2, 84.9, 70.8, 65.1, 61.6; IR (NaCl) 3395, 1699, 1460 cm^{-1}; HRMS (ESI-TOF) m/z $[M + H]^+$ calcd for $C_{18}H_{20}N_3O_7$ 390.1301, found 390.1298.

Preparation of 24. BzCl (247 mg, 1.76 mmol) was added to **23**[6] (237 mg, 1.6 mmol) in pyridine (2.0 mL) at 0 °C. The mixture was allowed to warm to room temperature overnight. After quenching the reaction with MeOH (0.3 mL), the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (EtOAc in hexane 10−25%) to afford **24** as a mixture of stereoisomers (86:14) in 61% (246 mg) yield as a colorless oil. Major stereoisomer: ^1H NMR (400 MHz, $CDCl_3$) δ 8.03−7.98 (m, 2H), 7.57−7.52 (m, 1H), 7.47−7.42 (m, 2H), 5.13 (d, J = 4.2 Hz, 1H), 4.37 (ddd, J = 6.2, 4.8, 2.2 Hz, 3H), 4.25 (d, J = 6.2 Hz, 1H), 3.40 (s, 3H), 2.18 (ddd, J = 13.9, 6.2, 4.6 Hz, 1H), 2.06 (ddd, J =

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13.9, 1.4, 0.6 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.4, 133.2, 129.7, 128.5, 105.5, 85.0, 73.1, 64.6, 55.0, 41.0. Minor stereoisomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10−8.04 (m, 2H), 7.58 (t, J = 1.3 Hz, 1H), 7.42−7.40 (m, 2H), 5.09 (dd, J = 5.3, 1.6 Hz, 1H), 4.55 (td, J = 6.9, 4.7 Hz, 1H), 4.47 (dd, J = 11.6, 5.1 Hz, 1H), 4.40 (d, J = 2.3 Hz, 1H), 4.19 (dd, J = 10.2, 5.2 Hz, 1H), 3.31 (s, 3H), 2.35−2.26 (m, 1H), 2.14−2.09 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.5, 133.1, 129.8, 128.4, 105.1, 83.7, 72.4, 65.3, 55.2, 41.7; IR (NaCl) 3426, 1719, 1455 cm^{-1}; HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{13}H_{17}NNaO_5$ 290.1004, found 290.0991.

Preparation of 25. Acetyl chloride (471 mg, 6 mmol) was added to an ice-cold mixture of acetonitrile and water (6 mL, CH_3CN/H_2O = 1:99), and the solution was warmed to room temperature and stirred for 30 min. A solution of **24** (202 mg, 0.8 mmol) in 1,4-dioxane (2 mL) was added, and the mixture was stirred at room temperature overnight. The solution was neutralized with a saturated $NaHCO_3$ solution (15 mL). After extraction with CH_2Cl_2 (3 × 30 mL), the combined organic phases were washed with water (30 mL) and brine (30 mL) and then dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (EtOAc in hexane 30−60%) to afford **25** as a mixture of diastereomers (81:19) in 57% (108 mg) yield as a colorless oil. Major stereoisomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04−8.02 (m, 1H), 8.01−7.98 (m, 1H), 7.61−7.53 (m, 1H), 7.48−7.40 (m, 2H), 5.71−5.58 (m, 1H), 4.60−4.53 (m, 1H), 4.39−4.30 (m, 3H), 3.41 (d, J = 4.5 Hz, 1H), 2.93 (d, J = 8.3 Hz, 1H), 2.19−2.08 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.4, 133.3, 129.7, 128.5, 99.5, 85.2, 73.4, 64.6, 41.4. Minor stereoisomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08−8.07 (m, 1H), 8.05 (d, J = 1.4 Hz, 1H), 7.57 (dddd, J = 8.5, 7.4, 2.8, 1.4 Hz, 1H), 7.47−7.41 (m, 2H), 5.65 (t, J = 4.5 Hz, 1H), 4.63−4.59 (m, 1H), 4.51−4.48 (m, 3H), 4.19 (dd, J = 10.1, 5.1 Hz, 1H), 3.16 (d, J = 2.7 Hz, 1H), 2.31 (ddd, J = 13.5, 6.8, 2.2 Hz, 1H), 2.24−2.19 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.7, 133.3, 129.7, 128.5, 98.8, 83.8, 72.4, 65.6, 42.4; IR (NaCl) 3395 (bd), 1709, 1450 cm^{-1}; HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{12}H_{14}NaO_5$ 261.0739, found 261.0727.

Preparation of 26. 2-Deoxy-D-ribose (268 mg, 2 mmol) and $MeONH_2$·HCl (201 mg, 2.4 mmol) were dissolved in pyridine (2 mL). The mixture was stirred at room temperature for 36 h. The solvent was removed under reduced pressure, and the major product was collected by flash column chromatography (CH_2Cl_2/MeOH = 1:1). This material was dissolved in pyridine (3.0 mL) at 0 °C. Benzoyl chloride (155 mg, 1.1 mmol) was added, and the solution was allowed to warm to room temperature overnight. After quenching the reaction with MeOH (0.3 mL), the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (methanol in CH_2Cl_2 2−8%) to provide **26** as a mixture of stereoisomers (52:48) as a colorless oil in a 19% overall yield (101 mg). Major stereoisomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09−8.03 (m, 2H), 7.62−7.56 (m, 1H), 7.53 (t, J = 5.2 Hz, 1H), 7.49−7.42 (m, 2H), 4.65−4.47 (m, 2H), 4.01−3.91 (m, 1H), 3.90 (s, 3H), 3.88−3.85 (m, 1H), 2.73−2.43 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.3, 148.3, 133.5, 129.8, 129.6, 128.5, 73.1, 69.9, 66.2, 61.8, 32.7. Minor stereoisomer: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09−8.03 (m, 2H), 7.62−7.56 (m, 1H), 7.49−7.42 (m, 2H), 6.90 (dd, J = 7.8, 3.9 Hz, 1H), 4.65−4.47 (m, 2H), 4.01−3.91 (m, 1H), 3.88−3.85 (m, 1H), 3.84 (s, 3H), 2.73−2.43 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.3, 148.3, 133.4, 129.8, 129.5, 128.5, 72.7, 69.5, 66.0, 61.7, 29.3; IR (NaCl) 3421 (bd), 1702, 1457 cm^{-1}; HRMS (ESI-TOF) m/z [M + Na]$^+$ calcd for $C_{13}H_{17}NNaO_5$ 290.1004, found 290.0991.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.7b02017.

NMR and MS spectra of new compounds, structures and response factors of photoproducts or trapping products, and determination of response factors (PDF)

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Notes

The authors declare no competing financial interest.

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