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Synthesis of C3′ Modified Nucleosides for Selective Generation of the C3′-Deoxy-3′-thymidinyl Radical: A Proposed Intermediate in LEE Induced DNA Damage

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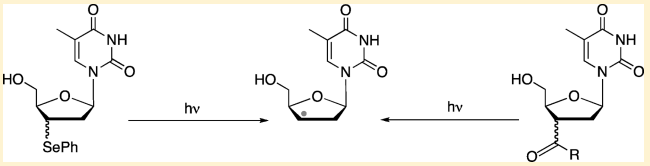
Supporting Information

ABSTRACT: DNA damage pathways induced by low-energy electrons (LEEs) are believed to involve the formation of 2-deoxyribose radicals. These radicals, formed at the C3′ and C5′ positions of nucleotides, are the result of cleavage of the C−O phosphodiester bond through transfer of LEEs to the phosphate group of DNA oligomers from the nucleobases. A considerable amount of information has been obtained to illuminate the identity of the unmodified oligonucleotide products formed through this process. There exists, however, a paucity of information as to the nature of the modified lesions formed from degradation of these sugar radicals. To determine the identity of the damage products formed via the 2′,3′-dideoxy-C3′-thymidinyl radical (C3′$_{dephos}$ sugar radical), phenyl selenide and acyl modified sugar and nucleoside derivatives have been synthesized, and their suitability as photochemical precursors of the radical of interest has been evaluated. Upon photochemical activation of C3′-derivatized nucleosides in the presence of the hydrogen atom donor tributyltin hydride, 2′,3′-dideoxythymidine is formed indicating the selective generation of the C3′$_{dephos}$ sugar radical. These precursors will make the identification and quantification of products of DNA damage derived from radicals generated by LEEs possible.

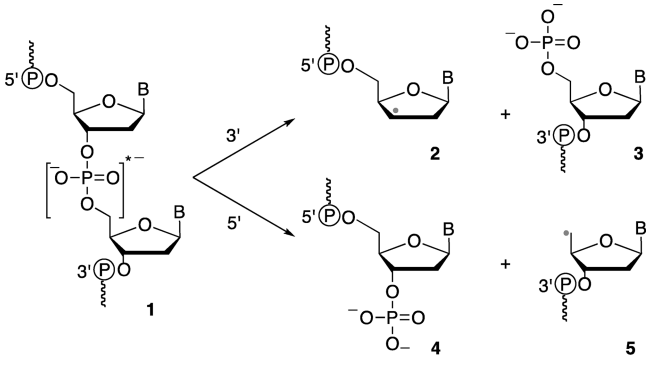


INTRODUCTION

The negative biological effects of ionizing radiation have been largely attributed to damage caused at its most significant biological target, DNA.[1−4] Thus, the genotoxic effect of secondary low-energy electrons (LEEs) on living cells has been a topic of considerable investigation as it relates to radiobiology.[5−7] LEEs (<30 eV) are produced at high levels through the transfer of energy from ionizing radiation to cellular constituents (~3 × 10^4/MeV).[8,9] The elucidation of the mechanistic pathways involved in DNA−LEE interactions has included the use of nucleobases, sugar derivatives, oligonucleotides, and plasmid DNA and has revealed that at a molecular level, LEEs attach to all DNA components (sugar, phosphate, and base) to deliver neutral and anionic radicals.[10−12] It has been established through these investigations that LEEs can induce both single and double strand breaks as well as base release. It is believed that following the capture of these electrons by the nucleobases, they are transferred to the phosphate moiety of the DNA (**1**)[13] generating a transient radical anion leading to C−O σ bond homolytic cleavage at the 3′ and/or 5′ positions. The result is the formation of sugar radicals **2** and **5** along with the corresponding phosphorylated oligonucleotides **3** and **4** (Scheme 1).[10]

The irradiation of short DNA oligomers in the condensed phase, followed by chemical analysis using HPLC, indicated the formation of not only 3′ and 5′-phosphorylated DNA oligomers **3** and **4** but also oligonucleotides terminated with unidentified sugar fragments.[10] Additional evidence for the formation of reactive intermediates **2** and **5** was obtained by the identification of dephosphorylated C3′-sugar radicals (C3′$_{dephos}$)

Scheme 1. Proposed Pathway of LEE Induced Formation of C3′ and C5′-Deoxyribose Radicals in DNA[10]



B (structures) = DNA bases: G, T, C, A

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in ESR studies of argon ion irradiated and γ-irradiated hydrated DNA.[14] Moreover, dephosphorylated C3′ and C5′ radicals were reported in DNA irradiated with low-LET radiation.[15] These techniques do not, however, lend themselves to the determination of the structure of the final DNA modifications that result from the occurrence of the 2-deoxyribose radicals proposed as a result of DNA exposure to LEEs. Therefore, investigation of the reactivity and fate of these reactive intermediates should further elucidate the mechanisms involved in DNA damage by these low energy electrons.

Organic chemistry has often been used to obtain stable photolabile precursors for the independent generation of reactive intermediates in nucleic acids.[16−20] Acyl and phenyl selenide derivatives of modified nucleosides and oligonucleotides have been utilized as precursors for site-specific generation of several radical-based intermediates, including those originating at the sugar−phosphate backbone of DNA.[21] With the goal to identify LEE induced nucleic acid derived products, as well as to characterize radicals related to **2**, we have synthesized 3-deoxy-C3′-acyl-2-deoxyribose (**6**) and 3′-deoxy-C3′-acyl (**7**) and selenophenyl (**8**) modified thymidines (Figure 1). We have

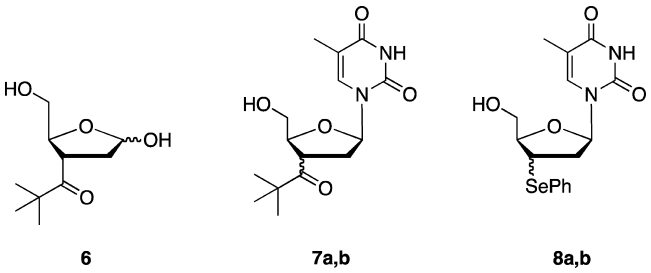


Figure 1. Radical precursors for the site-specific photochemical generation of species related to the dephosphorylated C3′-deoxy-3′-thymidinyl radical.

had an interest for quite some time in reactive species generated at the 2-deoxyribose moiety of nucleic acids.[20,22] In pursuit of our interests in C3′-radical species, we here introduce the synthesis of analogues **6**−**8** as precursors of radical species related to **2**. Our laboratory is the first to investigate this radical utilizing synthetically obtained radical precursors. Comparison of these substrates as well as their overall suitability as precursors of the 3′-radical of interest is presented.

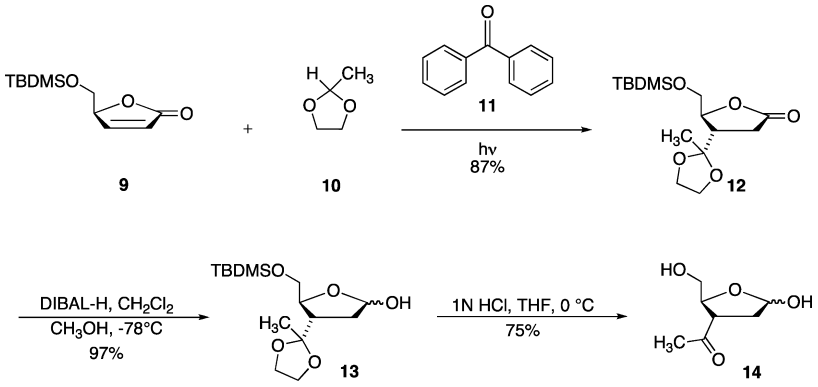
RESULTS AND DISCUSSION

To study the chemistry of sugar radicals related to **2**, several different precursors were considered. Previous studies demonstrated the utility of both acyl modified and phenyl selenide containing substrates in the photochemical generation of nucleoside and nucleotide radicals in a site-specific manner.[21] Acyl-substituted nucleosides have shown their utility as precursors specifically for the site-specific generation of 2-deoxyribose radicals.[23−27] The ketone moiety absorbs light at wavelengths outside the range, which induces other reactions in nucleotides and subsequently undergoes Norrish type I photocleavage. This photochemical process delivers an alkyl radical and an acyl radical that undergo decarbonylation to deliver a second alkyl radical. *t*-Butyl ketones are particularly attractive as radical precursors because of the fact that the *t*-butyl radical diffuses away from the site of radical generation without participating in secondary reactions and is produced in low levels.[28]

Alkyl phenyl selenides undergo facile homolytic cleavage under similar conditions to deliver alkyl radicals with dissociation energies of the Se−alkyl bond in the range of ∼60 kcal/mol as demonstrated by formation of the ethyl radical from diethylselenide.[29] The synthesis and purification of these highly stable substrates is also straightforward. In addition to these factors, the use of this moiety as a photochemical radical precursor has facilitated the study of several DNA sugar and base radicals with radical generation occurring at suitable wavelengths.[23]

Synthesis of a Photochemical Precursor for ESR Studies. Our initial synthetic approach to acyl modified nucleic acid monomers involved the synthesis of modified ribose derivatives as glycosyl donors to be utilized in Vorbrüggen type couplings to nucleobases.[30] Even though this approach did not successfully lead to the nucleoside radical precursors desired, it did allow us to develop a model substrate to facilitate the identification of species related to **2** in argon-ion beam irradiated DNA using ESR spectroscopy.[14] For these investigations 3-acetyl-2,3-dideoxyribose (**14**) was synthesized (Scheme 2). Studies performed by Mann et al.[31] proved that the photoaddition of alcohols to unsaturated cyclic esters occurs regiospecifically as well as face-selectively at the β-carbon of butenolides such as **9**. Subsequent work demonstrated that these conditions could also successfully be utilized for the introduction of cyclic acetals at the same position. On the basis of these observations, we decided to introduce a methyl ketone, in its protected ketal form, at the β-position of

Scheme 2. Synthesis of Precursor 14



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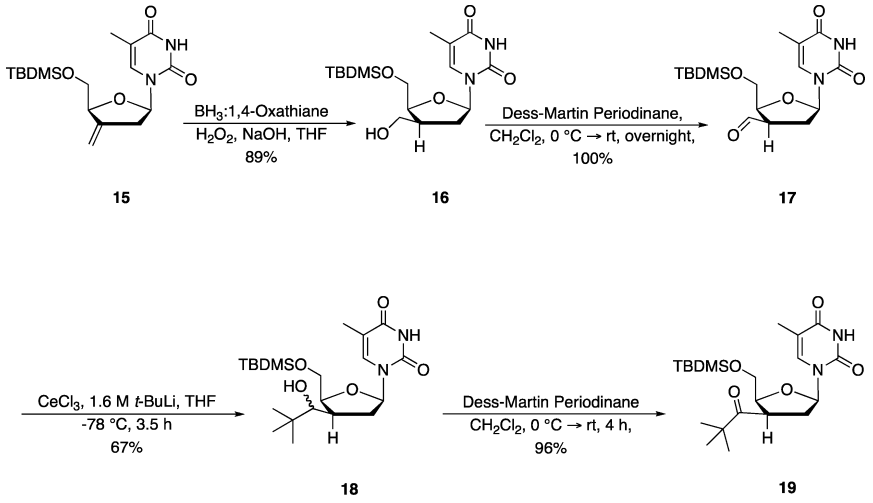
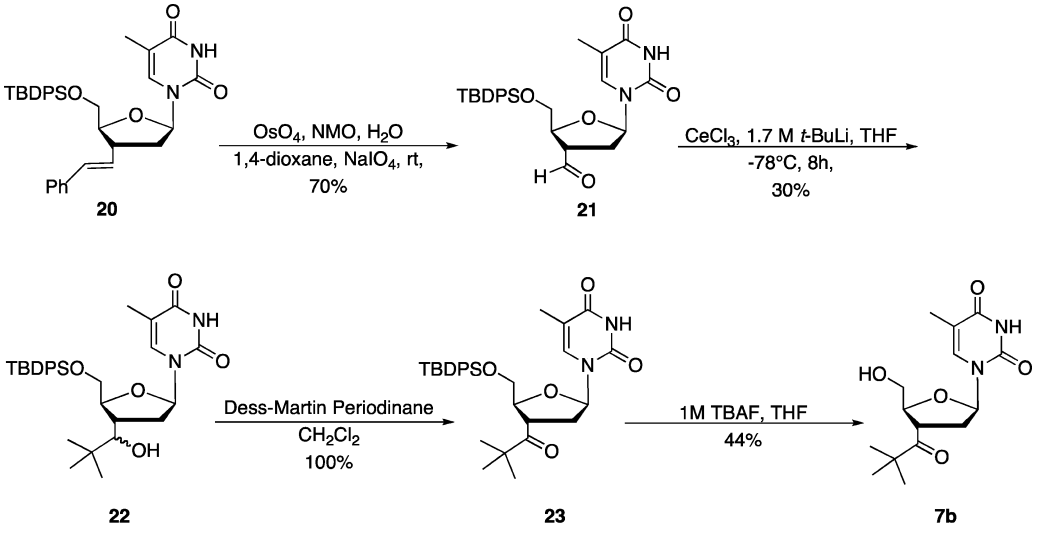
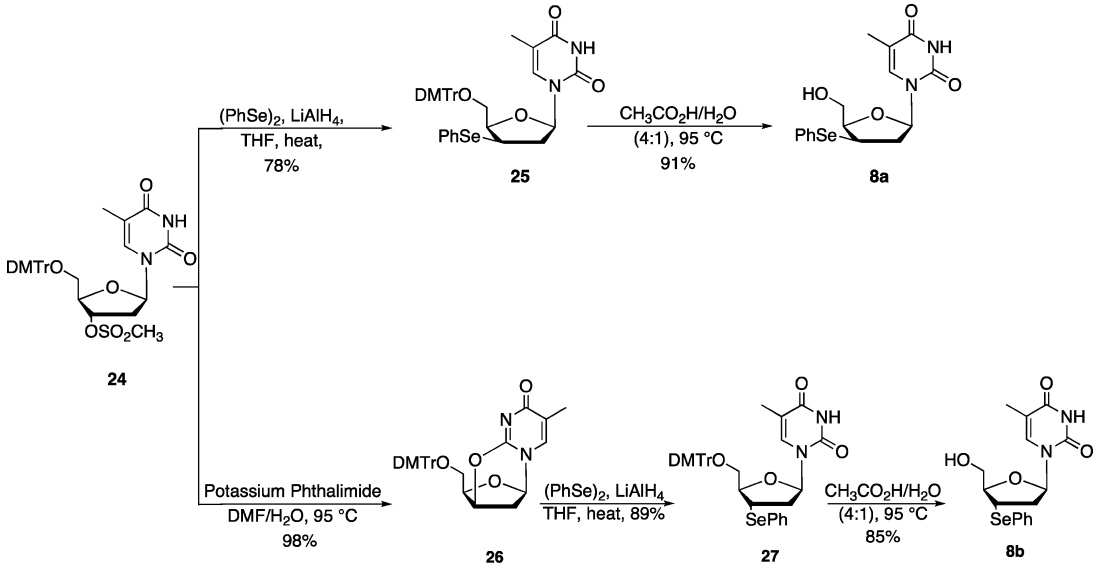
Scheme 3. Synthesis of Ketone 19



Scheme 4. Synthesis of Precursor 7b



Scheme 5. Synthesis of Precursors 8a and 8b



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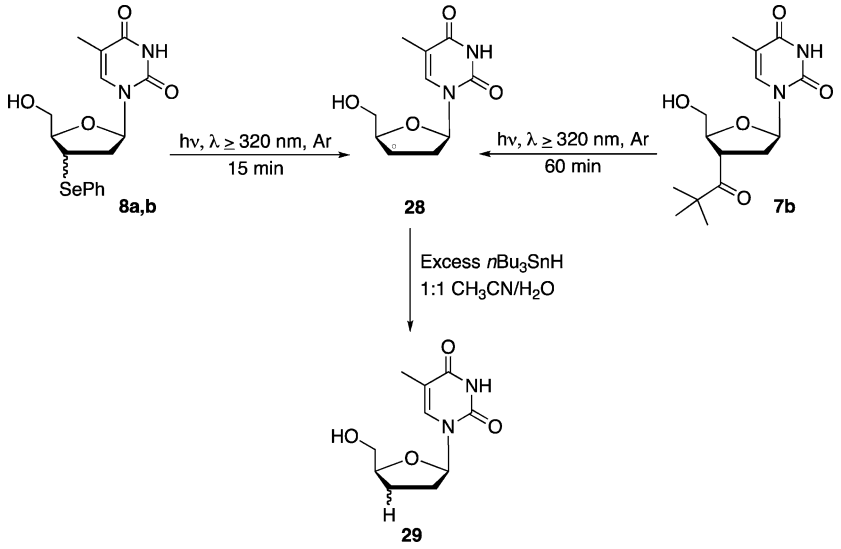
Scheme 6. Photochemical Generation of 28 from Precursors 7b and 8a,b in the Presence of Tributyltin Hydride



butenolide **9** under the above-described conditions. Methyl ketones also participate in Norrish type I photocleavage to generate nucleic acid radicals, however with lower efficiency than their *t*-butyl counterparts.[22] A 2-methyl-1,3-dioxolane substituent was introduced at position 4 of compound **9** through photochemical addition of the corresponding ketal (**10**) in the presence of benzophenone (**11**, 87% yield). Following reduction of the resulting lactone (**12**) with DIBAL-H (97% yield), acidic hydrolysis delivered the desired ketone (**14**) in 75% yield (Scheme 2).

Compound **14** was then utilized for experimental comparison to determine the presence of a proposed $C3'_{dephos}$ sugar radical in argon-ion beam irradiated DNA. The successful use of this substrate in the generation of the radical of interest has been reported elsewhere.[14]

Synthesis of C3′-Modified Thymidines as Photochemical Precursors. As mentioned, our attempts to form C3′-modified nucleosides through the coupling of modified ribose derivatives to nucleobases were unsuccessful. We therefore decided to turn our attention to the use of intact nucleosides as starting materials for our syntheses. Preliminary results, obtained from the above-described ESR studies in which the highly diffusible and extremely reactive methyl radical was generated, as well as the known efficiency of radical generation by *t*-butyl ketones via Norrish type I photocleavage, lead us to the synthesis of pivaloyl ketones and phenyl selenide derivatives as precursors of the C3′-nucleoside radical. The demonstrated efficiency of both classes of substrates in the generation of nucleoside radicals warrants the synthesis of both types of precursors. Additionally, to investigate the relationship between the stereochemistry of the starting material and radical initiated product formation, both the alpha and beta isomers of the modified nucleosides were synthesized (Schemes 3, 4, and 5).

Consequently, commercially available thymidine was converted to compound **15** according to previously published synthetic methods.[32] Through hydroboration−oxidation, alkene **15** was converted to primary alcohol **16** as exclusively the *β*-isomer (Scheme 3). Oxidation of the primary alcohol using the Dess−Martin periodinane at low temperature delivered only the *β*-isomer of aldehyde **17** in 100% yield. Upon treatment of **17** with *t*-butyllithium in the presence of $CeCl_3$, alcohols **18** were obtained in good yield (67%) as a diastereomeric mixture. Subsequent Dess−Martin oxidation delivered the desired ketone (**19**) as the *β*-isomer in 96% yield (Scheme 3). The absolute configuration of these substrates was confirmed through NOESY NMR analysis.

To obtain *α*-isomer **7b**, an alternative synthetic approach was necessary (Scheme 4). Methods developed by Mesmaeker et al.[33] made it possible for us to obtain exclusively **21** from *t*-butyldiphenylsilyl protected C3′-styrylthymidine (**20**) via oxidative cleavage. Using the same synthetic strategy as employed for the *β*-isomer, the desired *t*-butyl alcohol was obtained from **21** as a diastereomeric mixture of isomers (**22**), however in very low yield (13%). Extension of the reaction time to 8 h, however, increased the yield of **22** to 30% (Scheme 4). These results indicate that **17** is much more reactive to nucleophilic addition than *α*-isomer **21** under the conditions employed. This decrease in reactivity is likely due to steric hindrance at the lower face of the nucleoside. Dess−Martin oxidation of **22** provided the desired ketone (**23**) in 100% yield.

The final step in the acquisition of radical precursors **7a** and **7b** involves the deprotection of the 5′-hydroxyl. In the case of **19**, this was attempted with TFA/H_2O at low temperature.[30] The result was not the expected precursor, but decomposition of the starting material. Attempts were made with other reagents classically used to deprotect silylethers; however, none of these led to the formation of **7a**. We are currently investigating other alternatives to facilitate the acquisition of this compound. The treatment of **23** with TBAF in THF resulted in removal of the *t*-butyldiphenylsilyl group to deliver precursor **7b** in 44% yield (Scheme 4).

Phenyl selenide radical precursors **8a** and **b** were synthesized in a straightforward manner using known literature procedures (Scheme 5).[34] These substrates were previously synthesized employing 5′-trityl protection instead of the dimethoxytrityl groups reported herein. *β*-3′-Selenophenyl-C3′-deoxythymidine (**8a**)[34] was obtained through nucleophilic substitution of the mesyl group by the phenyl selenide ion followed by detritylation to deliver the phenyl selenide radical precursor in 91% yield. Analogously, dimethoxytrityl protected **27** was obtained through nucleophilic attack of the phenyl selenide anion, at the *α*-face of anhydrothymidine[35] **26** in good yield (89%). Subsequent detritylation with 80% acetic acid at 95 °C

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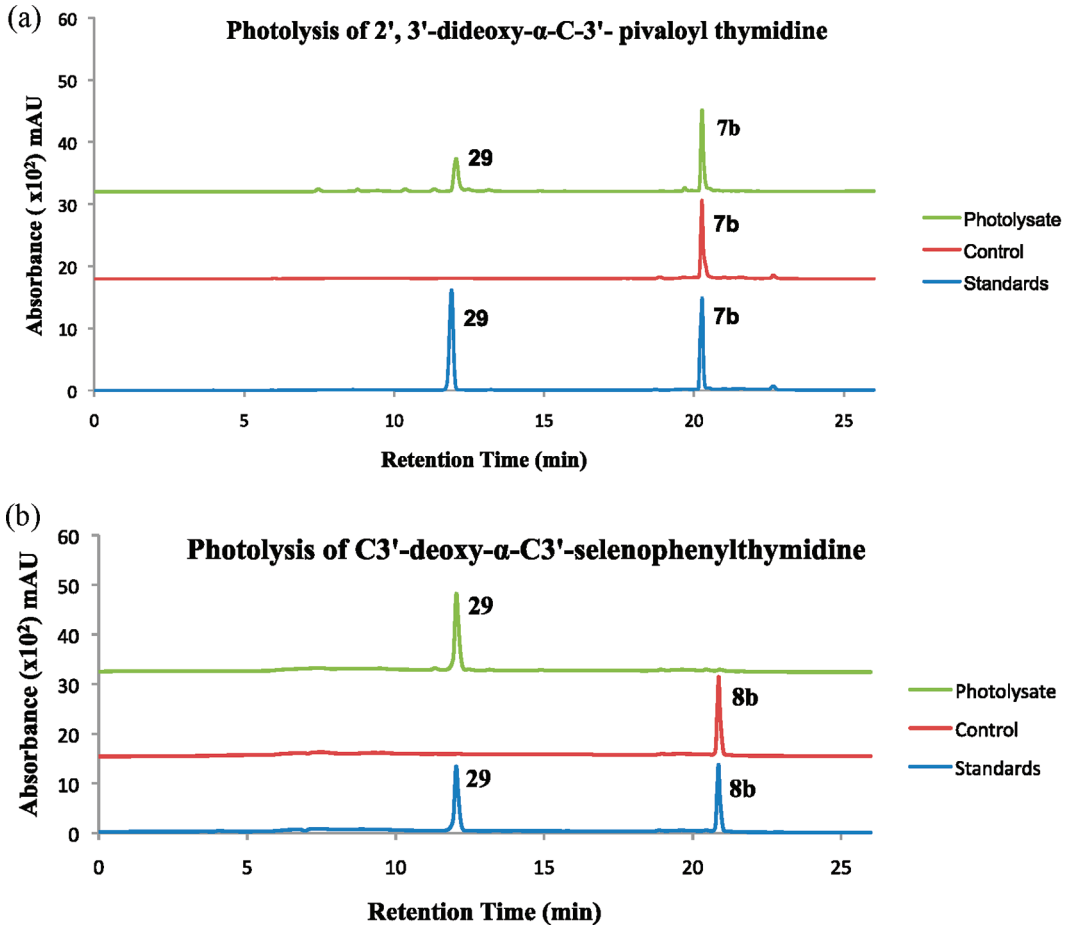

Figure 2. Reverse phase HPLC analyses of the photolyzates resulting from (a) **7b** and (b) **8b** (green). In each experiment, controls (red), standard **29** along with radical precursors **7b** or **8b** (blue) were injected separately.

afforded known **8b**[34] in 85% yield (Scheme 5). The related trityl protected derivative was obtained in slightly lower yield using the same procedures.

Photochemical Generation of the 2′,3′-Dideoxy-C3′-thymdinyl Radical from Radical Precursors. To determine the suitability of radical precursors **7b** and **8a**,**b** for generation of **28**, we probed their efficiencies through photochemical experiments in the presence of the hydrogen atom donor tributyltin hydride (Scheme 6). The reduction of **28** through rapid hydrogen atom donation should lead to the formation of 2,3-dideoxythymidine (**29**) as the sole product. Anaerobic photolysis ($\lambda \geq 320$ nm) of the three available precursors did indeed deliver modified nucleoside **29** as the sole product. Photochemical conversion of precursor **7b** to radical **28** was performed in a 1:1 mixture of acetonitrile/water containing an excess of tributyltin hydride. Photolysis of **8a** and **8b** was performed under similar conditions. However, in the case of **8a** the addition of THF (0.01%) to the solvent mixture was required because of solubility issues related to the precursor. Analysis of the photolyzates using reverse phase HPLC was easily accomplished using independently synthesized standard **29**.[36,37] The identity of the reduction product was additionally confirmed using ESI-MS. Figure 2 shows representative chromatograms obtained through analysis of the mixtures obtained from photolysis of α-3′-pivaloylthymidine **7b** (Figure 2a) and α-3′-phenylselenylthymidine **8b** (Figure 2b).

CONCLUSIONS

Collectively, the results described herein demonstrate that the $C3'_{dephos}$ thymdinyl radical (**28**) can be generated in nucleosides through exposure of precursors **7b**, **8a**, and **8b** to UV light of ≥320 nm. As these substrates will be further utilized in the synthesis of oliogonucleotide precursors of **2**, several factors are important in the determination of the most suitable precursor to pursue. The accessibility of the precursors is a significant consideration. It is clear that precursors **8a** and **8b** are much less synthetically challenging to obtain because of the high yielding transformations at each step and the overall length of the synthesis when compared to precursors **7a** and **7b**.

As mentioned earlier, pivaloyl-modified thymidine **7b** undergoes a Norrish type I photocleavage liberating two alkyl radicals upon loss of carbon monoxide, one of which is our radical of interest (**28**). The other radical partner, the *t*-butyl radical, is not believed to participate in the formation of secondary products in such processes in oligonucleotides.[28] Approximately 62% of **7b** is converted to **29** after 1 h of photolysis with no detection of side products. These results support the use of **7b** as a viable precursor of radical **2**.

In the case of the phenyl selenide precursors **8a**,**b**, the radical of interest is generated by photochemical scission of the PhSe–C bond to initiate a chain reaction that involves reaction with nBu$_3$SnH. This reaction is very efficient in the case of **8a**,**b** delivering reduction product **29** in high yield (59% for **8a** and 92% for **8b**) as the sole product after 15 min of photolysis. A

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difference in stereochemistry at the C3′-position in the phenyl selenides has an impact on photochemical conversion as indicated by the lower yield when **8a** is the precursor. This observation is likely due to the difference in accessibility of the phenyl selenide moiety to the tin radical needed to propagate this radical chain reaction. Studies in DNA oligomers will be performed under more physiological conditions employing thiol trapping agents as well as molecular oxygen to determine the ultimate fate of radical **28**. Even though the studies herein indicate that the radical of interest can be generated from precursors **8a** and **b**, their full utility will have to be determined in the absence of nBu$_3$SnH.

Of additional concern in the design of precursors **8a**,**b** is their ability to withstand the oxidative conditions required to obtain phosphate linkages from phosphites or H-phosphonates during automated DNA synthesis. RNA polymers containing alkyl selenide moieties were obtained through automated RNA synthesis using oxidative conditions identical to those found in DNA synthesis.[38] In these studies it was reported that exposure of the alkyl selenide containing oligomer to 20 mM I_2 for 20 s led to the formation of 2−5% selenoxide when the modification was near the 3′-terminus of the oligonucleotide. Our initial investigations into the synthesis of oligomers containing **8a**,**b** using the H-phosphonate method do not indicate the formation of the corresponding selenoxides using this methodology (data not shown). Collectively, these results indicate that both **7** and **8** have the potential to be efficient precursors of the radical of interest.

The identification of sugar damage fragments induced by the exposure of DNA to low energy electrons has been significantly hindered by a lack of knowledge of their mechanisms of formation. Radical precursors capable of generating one of the intermediates observed in this process, the 2′,3′-dideoxy-C3′-thymidinyl radical (**28**), will provide much needed information on the structure of these lesions and will facilitate the determination of the impact of these species on cellular processes.

EXPERIMENTAL SECTION

General Methods. All reactions were carried out in oven-dried glassware under an atmosphere of argon unless otherwise stated. Anhydrous THF was dried over activated alumina. Column chromatography was performed on an automated chromatography system equipped with an in-line variable wavelength detector. TLC was performed on precoated TLC plates of silica gel 60 F-254. All nuclear magnetic resonance spectra were recorded on either a 400 or 600 MHz NMR in $CDCl_3$ or CD_3OD as solvent. All chemical shifts are reported in parts per million downfield from TMS. Coupling constants are given in Hertz. HPLC analysis was performed on a liquid chromatography system using a C-18 5 μm column (4.6 × 250 cm). Gradients: A stepwise gradient was applied. A: 50 mM TEAA buffer pH = 7.0. B: acetonitrile; 0−20% B over 15 min, 20−75% B over 3 min, 75−90% B linearly over 4 min. Flow rate, 1.0 mL/min.

5-*O*-(*tert*-Butyldimethylsilyloxymethyl)-4-*C*-(2-methyl-1,3-dioxolane)-tetrahydrofuran-2-one (12). A pyrex test tube (16 × 150 mm) was charged with protected butenolide **9** (0.40 g, 1.75 mmol), benzophenone (**11**) (0.32 g, 1.76 mmol), and 2-methyl-1,3-dioxolane **10** (14.0 mL). After degassing for 1 h under a constant stream of nitrogen, the stirred solution was irradiated for 2 h with a 500 W Hg arc source. The solution was then evaporated to dryness in vacuo to afford a clear, pale yellow oil. Purification by flash column chromatography (9:1 pentane/ethyl acetate) afforded the title compound as a clear colorless oil. Yield: 0.48 g (87%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.07 (3H, s), 0.08 (3H, s), 0.89 (9H, s), 1.30 (3H, s), 2.48 (1H, dd, J = 17.0, 3.4), 2.71 (2H, m), 3.67 (1H, dd, J = 11.2, 2.4), 3.91 (1H, dd, J = 11.6, 2.0), 3.99 (4H, m), 4.52 (1H, m); ^{13}C ($CDCl_3$, 400 MHz) δ −5.6, 18.2, 21.5, 25.8, 31.2, 44.1, 64.8, 65.0, 81.0, 109.5, 176.7. HRMS [M + H]$^+$ calc. for $C_{15}H_{29}O_5Si$ 317.1784, found 317.1785.

5-*O*-(*tert*-Butyldimethylsilyl)-3-*C*-(2-methyl-1,3-dioxolane)-2,3-dideoxy-α- and β-D-pentofuranose(13). A solution of lactone **12** (0.31 g, 0.98 mmol) in anhydrous dichloromethane (3.0 mL) was cooled to −78 °C, and diisobutylaluminum hydride (DIBAL-H, 0.93 mL) was added dropwise. After 5 h, methanol (0.16 mL) was added, and stirring was continued at −78 °C for another 1.5 h. Ethyl acetate (0.73 mL) and saturated $NaHCO_3$ (0.16 mL) were added, and the reaction was warmed to room temperature over a one-hour period. The solution was dried over MgSO4, filtered through Celite, and evaporated to dryness in vacuo to afford a clear oily residue. Yield: 0.30 g (97%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.06 (6H, s), 0.13 (6H, s), 0.89 (9H, s), 0.94 (9H, s), 1.30 (3H, s), 1.36 (3H, s), 1.90 (1H, dd, J = 13.8, 1.8), 2.01 (2H, m), 2.21 (1H, m), 2.53 (1H, m), 2.72 (1H, m), 3.62 (2H, m), 3.71 (1H, dd, J = 10.8, 3.6), 3.84 (1H, dd, J = 10.6, 2.2), 3.99 (8H, m), 4.18 (2H, m), 4.24 (1H, d, J = 9.2), 4.85 (1H, d, J = 11.2), 5.36 (2H, m); ^{13}C ($CDCl_3$, 400 MHz) δ −5.4, −5.2, 18.49, 18.5, 22.6, 22.9, 25.9, 26.0, 36.1, 38.4, 44.7, 46.9, 64.8, 64.91, 64.94, 65.6, 65.7, 79.6, 81.1, 81.2, 98.79, 98.8, 110.3, 110.5. HRMS [M − H]$^-$ calc. for $C_{15}H_{29}O_5Si$ 317.1784, found 317.1785.

3-*C*-Acetyl-2,3-dideoxy-α- and β-D-pentofuranose (14). A solution of lactone **13** (0.08 g, 0.25 mmol) in THF (4.29 mL) was cooled to 0 °C, and 1 M HCl (0.69 mL) was added dropwise. The reaction was warmed to room temperature, and stirring was continued for 48 h. The reaction mixture was neutralized with Amberlyst A-21 ion-exchange resin, filtered, and the solution was evaporated to dryness in vacuo to afford a grainy yellow oil. Purification by flash column chromatography (9:1 dicholoromethane/methanol) afforded the desired ketone as a yellow oil. Yield: 0.03 g (75%); ^{1}H NMR (CH_3OD, 400 MHz) δ 4 isomers 1.79−2.20 (2H, m), 2.21−2.25 (3H, m), 2.32−3.13 (1H, m), 3.63−4.15 (2H, m), 4.18−4.44, 4.64−4.68 (1H, m), 5.38−5.50 (1H, m); ^{13}C (CH_3OD, 600 MHz) δ 4 isomers 25.9, 27.4, 27.6, 27.7, 27.9, 29.4, 29.8, 30.1, 38.0, 38.3, 38.8, 38.9, 52.9, 53.4, 55.0, 56.7, 64.5, 65.1, 66.1, 66.6, 83.2, 92.0, 96.9, 99.6, 210.1, 210.4, 210.9, 211.6. HRMS [M + H]$^+$ calc. for $C_7H_{13}O_4$ 161.0814, found 161.0814.

1-[(5-*O*-*tert*-Butyldimethylsilyl)-3-*C*-(hydroxymethyl)-2,3-dideoxy-β-D-*threo*-pentofuranosyl)]thymine (16). To a solution of **15** (0.94 g, 2.66 mmol) in anhydrous THF (5.4 mL) under nitrogen was added BH_3/1,4-oxathiane (0.33 mL of a 7.8 M solution in oxathiane, 2.87 mmol) at room temperature. After cooling of this mixture to 0 °C, a 2 M solution of NaOH (1.5 mL) was slowly added, followed by the dropwise addition of 30% aqueous H_2O_2 (0.38 mL). Stirring was continued for 1 h at room temperature. The reaction mixture was poured into ice-water (68 mL) and extracted with diethylether (80 mL). The combined organic phase was washed with water (68 mL) and saturated aqueous $NaHCO_3$ (2 × 68 mL), dried (Na_2SO_4), and evaporated to dryness in vacuo. The product was purified on a silica gel column (50:50 ethylacetate/pentane) to give **16** as a clear colorless oil. Yield: 0.88 g (89%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.15 (6H, s, Si(CH_3)$_2$), 0.94 (9H, s, SiC(CH_3)$_3$), 1.81 (1H, m, 2′-H$_\beta$), 1.93 (3H, s, CH_3), 2.44 (1H, m, 2′-H$_\alpha$), 2.72 (1H, m, 3′-H), 3.34 (1H, t, J = 6.4 Hz, OH), 3.73−3.89 (3H, m, C$\underline{H}_2$OH, 5′-H), 4.00 (1H, m, 5′-H), 4.16 (1H, m, 4′-H), 6.06 (1H, dd, J = 8.2, 6.2 Hz, 1′-H), 7.48 (1H, s, 6-H), 9.44 (1H, br s, NH); ^{13}C ($CDCl_3$, 400 MHz) δ −5.3, 12.8, 18.4, 26.0, 34.0, 42.6, 61.8, 62.6, 79.8, 84.3, 111.0, 135.6, 150.8, 164.2. HRMS [M + H]$^+$ calc. for $C_{17}H_{31}O_5N_2Si$ 371.2002, found 371.2001.

1-[(5-*O*-*tert*-Butyldimethylsilyl)-3-*C*-(formyl)-2,3-dideoxy-β-D-*threo*-pentofuranosyl)]thymine (17). A solution of **16** (0.83 g, 2.24 mmol) in CH_2Cl_2 (8 mL) was cannulated into a solution of Dess−Martin periodinane (1.43 g, 3.37 mmol) in anhydrous CH_2Cl_2 (16 mL) at 0 °C. After the mixture was stirred overnight at room temperature, diethyl ether (70 mL) was added, and the solution was poured slowly into a solution of saturated $NaHCO_3$ (45 mL) containing $Na_2S_2O_3$·5H_2O (5.56 g). The organic layer was removed, and the aqueous layer was extracted with diethyl ether (95 mL). The

combined organic phase was washed with saturated $NaHCO_3$ (95 mL) followed by H_2O (95 mL), dried (Na_2SO_4), and evaporated to dryness in vacuo. The product was isolated as a white amorphous solid. Yield: 0.83 g (100%); ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.10 (6H, s, $Si(CH_3)_2$), 0.91 (9H, s, $SiC(CH_3)_3$), 1.94 (3H, d, J = 1.2 Hz, CH_3), 2.43 (2H, m, 2′-H), 3.27 (1H, m, 3′-H), 3.95 (2H, m, 5′-H), 4.40 (1H, m, 4′-H), 6.14 (1H, dd, J = 7.2, 6.60 Hz, 1′-H), 7.44 (1H, d, J = 1.2 Hz, 6-H), 8.27 (1H, br s, NH), 9.85 (1H, s, CHO); ^{13}C ($CDCl_3$, 600 MHz) δ −5.5, 12.7, 18.3, 26.0, 31.4, 51.0, 61.8, 80.3, 83.8, 111.4, 135.4, 150.9, 164.2, 199.5. HRMS $[M + H]^+$ calc. for $C_{17}H_{29}O_5N_2Si$ calc. 369.1846, found 369.1846.

1-[(5-*O*-*tert*-Butyldimethylsilyl)-3-*C*-(2,2-dimethyl-1-hydroxypropyl)-2,3-dideoxy-β-D-*threo*-pentofuranosyl)]thymine (18). Cerium chloride was dried as described by Kamiya.[39] To a suspension of dry $CeCl_3$ (16.7 g, 44.8 mmol) in THF (105 mL) at −78 °C was added a 1.6 M solution of *t*-butyllithium (28.0 mL, 44.8 mmol) in pentane. This was allowed to stir for 1.5 h before a solution of **17** (0.83 g, 2.24 mmol) in THF (41 mL) was added dropwise at the same temperature. The reaction stirred at this temperature for 3.5 h, after which it was quenched by the addition of aqueous NH_4Cl (200 mL) and allowed to warm to room temperature. The product was then extracted with CH_2Cl_2 (6 × 250 mL), the organic layer was dried over Na_2SO_4, and the solvent was removed in vacuo. The crude product was purified on a silica gel column (40% ethyl acetate in hexane) to give **18** as a diastereomeric mixture of a clear colorless oil. Only one isomer was successfully purified and characterized. Yield: 0.42 g (44%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.16 (6H, s), 0.94 (18H, 4s), 1.93 (3H, d, J = 1.2 Hz), 2.15 (1H, m), 2.27 (1H, m), 2.69 (1H, m), 3.47 (1H, d, J = 3.2), 3.60 (1H, d, J = 2.8 Hz), 3.90 (1H, m), 4.03 (2H, m), 6.08 (1H, dd, J = 9.0, 5.8 Hz), 7.50 (1H, d, J = 1.2 Hz), 8.8 (1H, br s); ^{13}C ($CDCl_3$, 400 MHz) δ −5.2, 12.8, 18.5, 26.0, 27.0, 31.5, 35.5, 40.8, 63.0, 75.9, 80.5, 83.9, 111.1, 135.9, 150.8, 163.9. HRMS $[M + H]^+$ calc. for $C_{21}H_{39}O_5N_2Si$ 427.2628, found 427.2629.

1-[(5-*O*-*tert*-Butyldimethylsilyl)-3-*C*-(2,2-dimethyl-1-oxopropyl)-2,3-dideoxy-β-D-*threo*-pentofuranosyl)]thymine (19). To a solution of Dess−Martin periodinane (0.30 g, 0.71 mmol) in anhydrous CH_2Cl_2 (3 mL) was cannulated a solution of **18** (0.20 g, 0.47 mmol) in CH_2Cl_2 (2 mL) at 0 °C. Stirring was continued at 0 °C for 15 min and then at room temperature for 4 h. Diethylether (14 mL) was added, and the solution was poured slowly into a solution of (1.16 g, 4.69 mmol) $Na_2S_2O_3{\cdot}5H_2O$ in saturated $NaHCO_3$ (9 mL). The organic phase was washed with saturated $NaHCO_3$ (20 mL), followed by H_2O (20 mL) and saturated NaCl (20 mL), dried with $MgSO_4$, and evaporated to dryness in vacuo. The product was isolated as a white solid. Yield: 0.19 g (96%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.06 (6H, s, $Si(CH_3)_2$), 0.88 (9H, s, $SiC(CH_3)_3$), 1.16 (9H, s, $COC(CH_3)_3$), 1.96 (3H, d, J = 0.8 Hz, CH_3), 2.06 (1H, m, 2′-H_β), 2.49 (1H, m, 2′-H_α), 3.68 (2H, m, 5′-H), 3.81 (1H, m, 3′-H), 4.20 (1H, m, 4′-H), 6.14 (1H, dd, J = 8.0, 6.0 Hz, 1′-H), 7.51 (1H, d, J = 0.8 Hz, 6-H), 9.23 (1H, br s, NH); ^{13}C ($CDCl_3$, 400 MHz) δ −5.1, 12.9, 18.7, 26.0, 26.2, 36.7, 44.2, 45.0, 62.6, 80.8, 84.3, 111.4, 135.6, 150.8, 164.0, 214.1. HRMS $[M + H]^+$ calc. for $C_{21}H_{37}O_5N_2Si$ 425.2472, found 425.2473.

1-[5-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-3-*C*-(2,2-dimethyl-1-hydroxypropyl)-β-D-*erythro*-pentofuranosyl]thymine (22). Cerium chloride was dried as described by Kamiya.[39] To a suspension of dry $CeCl_3$ (8.32 g, 22.34 mmol) in THF (52 mL) was added dropwise 1.7 M *t*-butyllithium in pentane (13.14 mL, 22.34 mmol) at −78 °C. The solution was allowed to stir at this temperature for 1.5 h. Aldehyde **21** (0.55 g, 1.117 mmol) in THF (20 mL), which was coevaporated with dry THF, was added over 25 min and allowed to stir at the same temperature for 8 h, before being quenched by the addition of of saturated NH_4Cl (101 mL) at −78 °C. The reaction mixture was extracted with dichloromethane (× 6), the organic layer was dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure to afford a brownish foam. The crude mixture was purified by column chromatography with 1:1 ethyl acetate/hexane to afford **22** as a diastereomeric mixture in the form of a colorless foam. Yield: 0.17 g (30%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88−0.92 (18H, 2s), 1.1 (18H, s), 1.61 (6H, s), 1.85 (1H, m), 2.02 (1H, d, J = 5.6), 2.24 (1H, m), 2.45 (1H, d, J = 5.6 Hz), 2.71 (2H, m), 3.19 (1H, d, J = 5.2 Hz), 3.33 (1H, t, J = 4.99 Hz), 3.81, 3.88 (2H, 2 dd, J = 11.4, 2.8 Hz), 3.98 (1H, m), 4.07 (2H, m), 4.33 (1H, m), 6.13 (1H, t, J = 6.8 Hz), 6.23 (1H, t, J = 6.4 Hz), 7.36−7.69 (22H, m), 9.08 (1H, br s), 9.17 (1H, br s); ^{13}C ($CDCl_3$, 400 MHz) δ 12.3, 19.6, 26.6, 26.7, 27.2, 32.8, 35.8, 36.1, 38.5, 40.1, 40.6, 63.3, 66.0, 77.4, 82.2, 82.9, 84.3, 85.4, 85.6, 111.0, 128.03, 128.07, 128.12, 130.11, 130.15, 130.19, 130.24, 132.74, 132.96, 133.27, 133.34, 135.57, 135.69, 135.75, 135.81, 150.6, 150.7, 164.2. HRMS $[M + Na]^+$ calc. for $C_{31}H_{42}O_5N_2SiNa$ 573.2761, found 573.2760.

1-[5-(*tert*-Butyldiphenylsilyl)-2,3-dideoxy-3-*C*-pivaloyl-β-D-*erythro*-pentofuranosyl]thymine (23). A solution of **22** (0.17 g, 0.309 mmol) in anhydrous CH_2Cl_2 (1 mL) was added to a solution of the Dess−Martin periodinane (0.2 g, 0.472 mmol) in anhydrous CH_2Cl_2 (2.2 mL) at 0 °C. Stirring was continued at 0 °C for 15 min and then at room temperature overnight. The reaction mixture was diluted with diethyl ether (10 mL), poured into ice-cold aqueous saturated $NaHCO_3$ (6.2 mL) containing $Na_2S_2O_3{\cdot}5H_2O$ (0.77 g, 3.09 mmol), and stirred for 10 min. The organic layer was washed with saturated $NaHCO_3$, H_2O, and saturated NaCl, dried over Na_2SO_4, and the solvent was removed under reduced pressure to afford **23** as colorless foam. Yield: 0.17 g (100%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.11 (18H, s, $SiC(C\underline{H}_3)_3$ and $COC(C\underline{H}_3)_3$), 1.62 (3H, d, J = 0.8, CH_3), 2.24 (1H, m, 2′-H_β), 2.43 (1H, m, 2′-Hα), 3.66 (1H, dd, J = 11.8, J = 2.6, 5′-H), 3.84 (1H, m, 3′-H), 4.09 (1H, dd, J = 12, J = 2, 5′-H), 4.25 (1H, dt, J = 6.8, J = 2.4, 4′-H), 6.22 (1H, dd, J = 6.8 Hz, J = 5.2 Hz, 1′-H), 7.37−7.47 (6H, m, Ar), 7.53 (1H, d, J = 0.8, 6-H), 7.64−7.68 (4H, m, Ar), 8.88 (1H, br s, NH); ^{13}C ($CDCl_3$, 400 MHz) δ 12.3, 19.6, 26.0, 27.3, 30.5, 39.0, 43.7, 44.9, 63.5, 83.8, 85.6, 111.1, 128.2, 130.3, 132.8, 135.6, 150.3, 164.1, 215.3. HRMS $[M + Na]^+$ calc. for $C_{31}H_{40}O_5N_2SiNa$ 571.2604, found 571.2617.

1-(2,3-Dideoxy-3-*C*-pivaloyl-β-D-*erythro*-pentofuranosyl)-thymine (7b). To a solution of **23** (0.15 g, 0.27 mmol) in THF (2.3 mL) was added a 1 M solution of TBAF in THF (0.41 mL) at room temperature. Stirring was continued for 1 h. The solvent was removed in vacuo, and the crude mixture was purified by column chromatography with ethyl acetate to afford **7b** as a colorless foam. Yield: 0.04 g (44%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.18 (9H, s, $COC(C\underline{H}_3)_3$), 1.93 (3H, s, CH_3), 2.44 (2H, m, 2′-H), 2.65 (1H, dd, J = 6.2 Hz, J = 3.4, 5′-OH), 3.62 (1H, m, 3′-H), 3.97 (2H, m, 5′-H), 4.29 (1H, dt, J = 8 Hz, J = 2.4 Hz, 4′-H), 6.0 (1H, dd, J = 7.2 Hz, J = 4.4 Hz, 1′-H), 7.48 (1H, d, J = 0.8 Hz, 6-H), 8.67 (1H, br s, NH); ^{13}C ($CDCl_3$, 400 MHz) δ 12.8, 25.8, 38.7, 43.3, 45.2, 61.9, 84.7, 88.2, 110.9, 137.6, 150.4, 164.1, 215.8. HRMS $[M + Na]^+$ calc. for $C_{15}H_{22}O_5N_2Na$ 333.1426, found 333.1419.

1-[5-*O*-(4,4′-Dimethoxytrityl)-2,3-dideoxy-3-*C*-selenophenyl-β-D-*threo*-pentofuranosyl]thymine (25). To a cold solution (0 °C) of diphenylselenide (2.9 g, 9.3 mmol) in THF (116 mL) was added $LiAlH_4$ (0.26 g, 6.9 mmol) portion-wise under N_2 (g). Upon warming, the solution turned colorless. To this was added methanesulfonate **24** (3.6 g, 5.8 mmol) in anhydrous THF (58 mL). The mixture was heated at reflux for 2 h and then allowed to cool. The reaction mixture was quenched with methanol (58 mL), and the solvent was removed in vacuo. The residue was treated with saturated NH_4Cl solution (116 mL) and extracted with ethyl acetate (3 × 116 mL). The organic layer was washed with water (2 × 58 mL) and brine (58 mL), dried over $MgSO_4$, and concentrated. The crude product was purified by silica gel column chromatography with ethyl acetate/hexane (1:3) to afford a white foam. Yield: 2.4 g (78%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.46 (3H, s), 2.37 (1H, m), 2.82 (1H, m), 3.48 (1H, dd, J = 10.4 Hz, J = 4.4 Hz), 3.62 (1H, dd, J = 10.8 Hz, J = 2.8 Hz), 3.79 (6 H, s), 3.85 (1H, q, J = 16.0 Hz, J = 7.6 Hz), 4.4 (1H, m), 6.16 (1H, t, J = 6.6 Hz), 6.8−7.5 (18H, m), 7.72 (1 H, s), 9.47 (1H, bs); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 11.9, 40.2, 41.3, 55.4, 65.1, 81.0, 84.1, 87.4, 111.1, 113.2, 113.2, 127.3, 128.0, 128.7, 129.5, 130.6, 130.6, 134.0, 135.3, 136.2, 144.2, 150.8, 158.8, 164.2. HRMS $[M + Na]^+$ calc. for $C_{37}H_{36}O_6N_2SeNa$ 707.1636, found 707.1649.

1-(2,3-Dideoxy-3-*C*-selenophenyl-β-D-*threo*-pentofuranosyl]thymine (8a).[34] To compound **25** (1.19 g, 1.74 mmol) was added a solution of acetic acid/water (4:1 v/v), and the

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reaction mixture was heated to 85 °C for 1 h. After cooling to room temperature, the solvent was removed in vacuo. The residue was coevaporated with several portions of methanol. Precipitation of the pure product was achieved by addition of dichloromethane followed by ethyl acetate. The white solid was isolated by filtration. Yield: 0.65 g (91%); ^{1}H NMR (CD_3OD, 400 MHz) δ 1.92 (3H, d, J = 0.8 Hz), 2.3 (1H, m), 2.8 (1H, m), 3.92 (2H, m), 4.05 (1H, q, J = 7.6 Hz), 4.36 (1H, m), 6.06 (1H, t, J = 6.6 Hz), 7.3 (3H, m), 7.54 (2H, m), 8.03 (1H, d, J = 0.8 Hz); ^{13}C (CD_3OD, 400 MHz) δ 12.7, 41.0, 42.2, 64.7, 71.7, 83.8, 86.0, 111.2, 128.9, 130.6, 131.4, 134.76, 138.6. HRMS [M + Na]$^+$ calc. for $C_{16}H_{18}O_4N_2SeNa$ 405.0329, found 405.0305.

1-[5-*O*-(4,4′-Dimethoxytrityl)-2,3-dideoxy-3-*C*-selenophenyl-β-D-*erythro*-pentofuranosyl]thymine (27). Diphenylselenide (2.0 g, 5.00 mmol) was rendered anhydrous by repeated evaporation with pyridine. The residue was dissolved under argon in anhydrous THF (32 mL) and cooled to 0 °C. $LiAlH_4$ (142.5 mg, 3.75 mmol) was added portion-wise, and the solution was allowed to warm to room temperature, resulting in the formation of a clear solution. To this was added a THF (6.5 mL) solution of anhydrothymidine **26** (1.5 g, 3.2 mmol). The reaction mixture was heated under reflux for 2 h, allowed to cool, and quenched with methanol (6.5 mL). The solvent was removed under reduced pressure, and the residue was treated with saturated NH_4Cl solution (65 mL) and extracted with ethylacetate (3 × 65 mL). The organic layer was washed with water (2 × 33 mL) and brine (33 mL), dried over $MgSO_4$, and concentrated. The crude product was purified by silica gel column chromatography CH_2Cl_2/MeOH (98:2) to afford a white foam. Yield: 1.95 g (89%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.39 (3H, d, J = 0.8 Hz), 2.52 (2H, m), 3.39 (1H, dd, J = 10.8 Hz, J = 3.2 Hz), 3.57 (1H, dd, J = 11.0 Hz, J = 2.4 Hz), 3.79 (6H, s), 3.85 (1H, q, J = 8.4 Hz), 4.05 (1H, dt, J = 8.4 Hz, J = 2.4 Hz), 6.1 (1H, dd, J = 6.6 Hz, J = 3.6 Hz), 6.8–7.5 (18H, m), 7.72 (1H, d, J = 0.8 Hz), 9.35 (1H, bs); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 12.0, 36.9, 40.9, 55.4, 62.2, 85.0, 85.8, 86.9, 110.8, 113.3, 127., 127.3, 128.1, 128.3, 128.7, 129.5, 130.3, 135.6, 135.8, 144.5, 150.5, 158.8, 164.3. HRMS [M + Na]$^+$ calc. for $C_{37}H_{36}O_6N_2SeNa$ 707.1636, found 707.1663.

1-(2,3-Dideoxy-3-*C*-selenophenyl-β-D-*erythro*-pentofuranosyl)thymine (8b).[34] A solution of water/glacial acetic acid (1:4 v/v) was added to compound **27** (1.7 g, 2.5 mmol), and the mixture was heated to 85 °C for 2 h. The reaction mixture was cooled to room temperature, and the solvent was removed in vacuo. The residue was coevaporated with methanol and purified on silica gel with ethyl acetate–hexane (1:3) to afford a white foam. Yield: 0.92 g (85%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.9 (3H, d, J = 0.8 Hz), 2.27 (1H, t, J = 4.8 Hz), 2.45 and 2.56 (2H, each as m), 3.79 (2H, m), 4.02 (2H, m), 6.02 (1H, dd, J = 6.9 Hz, J = 3.8 Hz), 7.3–7.6 (6H, m), 8.5 (1H, bs); ^{13}C NMR ($CDCl_3$, 400 MHz) δ 12.8, 35.9, 40.0, 61.2, 85.7, 86.6, 110.9, 126.7, 128.9, 129.7, 135.9, 136.8, 151, 164. HRMS [M + Na]$^+$ calc. for $C_{16}H_{18}O_4N_2SeNa$ 405.0329, found 405.0322.

5′-*O*-(*tert*-Butyldimethylsilyl)-2′,3′-dideoxythymidine (30). A solution of 5′-*O*-(*tert*-butyldimethylsilyl)-3′-*O*-(phenoxythiocarbonyl)-thymidine.[40] (1.83 g, 3.72 mmol), *tri*-butyltin hydride (2.7 g, 9.28 mmol), and 2,2′-azobisisobutyronitrile (0.24 g, 1.5 mmol) in absolute toluene (50 mL) was heated at 80 °C for 2 h. Upon reaction completion, the solvent was evaporated to dryness in vacuo. The crude product was then subjected to silica gel flash chromatography. Elution was performed with ethylacetate/hexane (1:3) to give **30** as a white powder. Yield: 1.19 g (94%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.005 (3H, s), 0.82 (9H, s), 1.81 (3H, d, J = 0.8 Hz), 1.87 (2H, m), 2.25 (1H, m), 3.6 (1H, dd, J = 11.4 Hz, J = 2.8 Hz), 3.88 (1H, dd, J = 11.6 Hz, J = 2.8 Hz), 4.04 (1H, m), 5.98 (1H, dd, J = 6.2 Hz, J = 4.6 Hz), 7.47 (1H, d, J = 0.8 Hz), 9.57 (1H, s); ^{13}C ($CDCl_3$, 400 MHz) δ −5.17, 12.79, 18.65, 25.48, 26.1, 32.73, 64.67, 81.08, 85.94, 110.41, 135.86, 150.8, 164.45. HRMS [M + Na]$^+$ calc. for $C_{16}H_{28}O_4N_2SiNa$ 363.1716, found 363.1717.

2′,3′-Dideoxythymidine (29). To a solution of **30** (0.47 g, 1.38 mmol) in anhydrous THF (27 mL) was added dropwise tetra-*n*-butylammonium fluoride (1 M in THF, 5.5 mL, 5.5 mmol), and the mixture was allowed to stir at room temperature for 30 min. Then the reaction mixture was evaporated to dryness in vacuo. The crude product was chromatographed on silica gel with 6% methanol in dichloromethane to afford compound **29** as a white powder. Yield: 0.3 g (96%); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.92 (3H, d, J = 0.8 Hz), 2.06 (3H, m), 2.4 (1H, m), 3.74 (1H, d, J = 12.0 Hz), 4.01 (1H, d, J = 12.0 Hz), 4.19 (1H, m), 6.12 (1H, dd, J = 6.8 Hz, J = 4.0 Hz), 7.53 (1H, d, J = 0.8 Hz), 8.72 (1H, bs); ^{13}C ($CDCl_3$, 400 MHz) δ 12.83, 25.34, 32.33, 63.71, 81.39, 86.39, 110.79, 136.48, 150.52, 163.96. HRMS [M + Na]$^+$ calc. for $C_{10}H_{14}O_4N_2Na$ 249.0851, found 249.0846.

Standard Photolysis Conditions. A solution of either radical precursor **8b**/**7b** (1 mM in 1:1 CH_3CN/H_2O) or **8a** (1 mM in 1:1:0.01 CH_3CN/H_2O/THF) was transferred to a quartz cuvette and degassed by bubbling argon through the solution for 20 min. *tri*-Butyltin hydride (1000 equiv) was added under an argon atmosphere. The mixture was immediately photolyzed (**8** for 15 min, **7b** for 60 min) at 15 °C. The photolysis was performed using an 500 W high-pressure mercury arc lamp fitted with an IR filter, focusing lens, and 320 nm cutoff filter. The irradiation mixture was analyzed directly without workup by analytical HPLC with UV detection at 254 nm. Products were identified by ESI-MS and by comparison with authentic samples. Product yields were determined using standard curves.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

HPLC chromatograms. ^{1}H, ^{13}C, and ^{1}H-NOESY NMR spectra. Standard curves. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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