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3′-Modified oligodeoxyribonucleotides for the study of 2-deoxyribose damage in DNA

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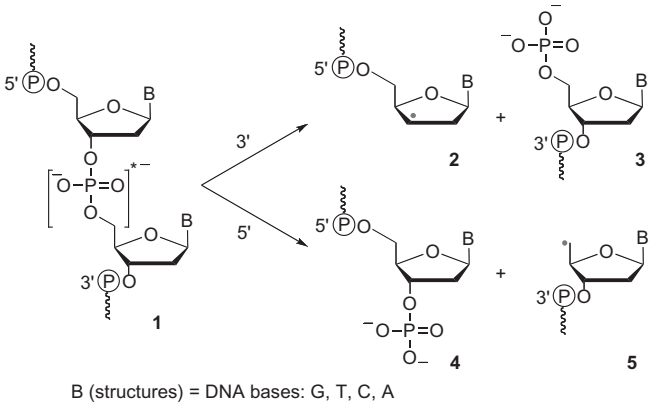
ABSTRACT

Well-defined substrates for the study of oxidative processes are important for the elucidation of the role of DNA damage in the etiology of diseases such as cancer. We have synthesized 3′-modified oligodeoxyribonucleotides (ODNs) using 5′ → 3′ 'reverse' DNA synthesis for the study of 2-deoxyribose oxidative damage to DNA. The modified monomers designed for these studies all share a common feature, they lack the naturally occurring 3′-hydroxyl group found in 2-deoxyribonucleosides. Modified H-phosphonates containing 3′-phenyl selenides as well as saturated and unsaturated sugars were obtained and incorporated in ODNs. These ODNs were used to investigate the fate of C3′-dideoxyribonucleotide radicals in DNA.

It is a long accepted fact that the primary cellular target of ionizing radiation is DNA.[1] The transfer of energy from ionizing radiation to cellular molecules such as proteins, water and DNA, results in the production of large numbers of electrons with energies of <30 eV.[2] To fully elucidate the effects of ionizing radiation on biological systems, it is important to determine the nature of the neutral products formed when these low energy electrons (LEEs) are captured by DNA. In recent years, it has been determined, through studies involving DNA in the condensed phase, that LEEs are easily transferred to the π^* orbitals of the nucleobases initiating DNA damage processes. This damage takes the form of bond dissociation either at the N-glycosidic bond of the nucleobase or cleavage of the C–O bond at the 3′- and/or 5′-phosphodiester moieties.[3,4]

When cleavage occurs at the carbon–phosphodiester bond C3′- and C5′-radical species **2** and **5** are formed along with phosphorylated DNA fragments **3** and **4** (Scheme 1). It was reported that in addition to stable fragments **3** and **4**, other fragments were also produced which have evaded identification.[3] To address this issue, the work described herein seeks to elucidate the structure of fragments originating from the degradation of **2**. Through the use of site-specifically modified oligodeoxyribonucleotides (ODNs) containing photolabile groups capable of generating the C3′-dideoxy-radical **2**, as well as expected degradation products, we will seek to determine the mechanism of degradation as well as the identity



Scheme 1. The proposed pathway of LEE induced formation of C3′ and C5′-deoxyribose radicals in DNA.[3]

of the end products obtained from this reactive intermediate. These investigations build on our long standing interest in the site-specific generation of sugar radicals in DNA oligomers.[5–7]

Modified ODNs suitable for the formation and study of **2** contain modifications at the C3′-position of the terminal 2-deoxyribose moiety. Due to the nature of these substrates, nucleotide monomers lacking the 3′-hydroxyl normally found at the C3′-position are required. The synthesis of ODNs containing modifications at their 3′-ends is conventionally accomplished through the use of specially prepared solid supports.[8,9] While this approach has made

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3′-modified oligomers such as 3′-phosphoglycolate and 3′-phosphoglycolaldehyde easily obtainable,[10] it is limited in scope due to the need for highly functionalized substrates attached to solid supports. Dervan and co-workers reported the use of a 'reverse' synthesis approach for nucleic acids to facilitate the formation of ODNs containing 3′–3′ linkages.[11] The same concept has also been applied to the synthesis of ODNs containing 5′–5′,[12,13] linkages as well as oligonucleotide substrates containing segments in the opposite sense of the rest of the strand.[14] Additionally, almost a decade ago, Hecht and co-workers[15] reported 'reverse' (i.e., 5′ → 3′) automated synthesis of modified ODNs with a tyrosine residue at the 3′-end. The authors pointed out the generality of this approach for the introduction of modifications at the 3′-end of ODNs through the incorporation of modified phosphoramidites into a protected ODN during the final synthetic step. This strategy is highly appropriate for our purposes. The commercial availability of 5′-phosphoramidite monomers, appropriately functionalized for automated nucleic acid synthesis as well as solid supports with a 5′-linkage, makes this approach even more suitable. Due to our long-standing interest in the synthesis of modified ODNs using the H-phosphonate method,[7,16] we chose to develop methods for the 'reverse' synthesis of the modified ODNs of interest using 5′-*H*-phosphonates **6**–**9**.

Phenylselenide containing nucleosides and ODNs have been shown to efficiently generate radicals at both the 2-deoxyribose as well as the nucleobases of DNA under photochemical conditions facilitating the homolytic cleavage of the C–Se bond.[17–21] Using these substrates, radicals have been generated at the C4′-position of the sugar as well as the C5- and methyl substituent of thymine bases in ODNs. Our initial investigations indicate that phenylselenide containing nucleosides are efficient precursors of the radical of interest in their monomeric form.[22]

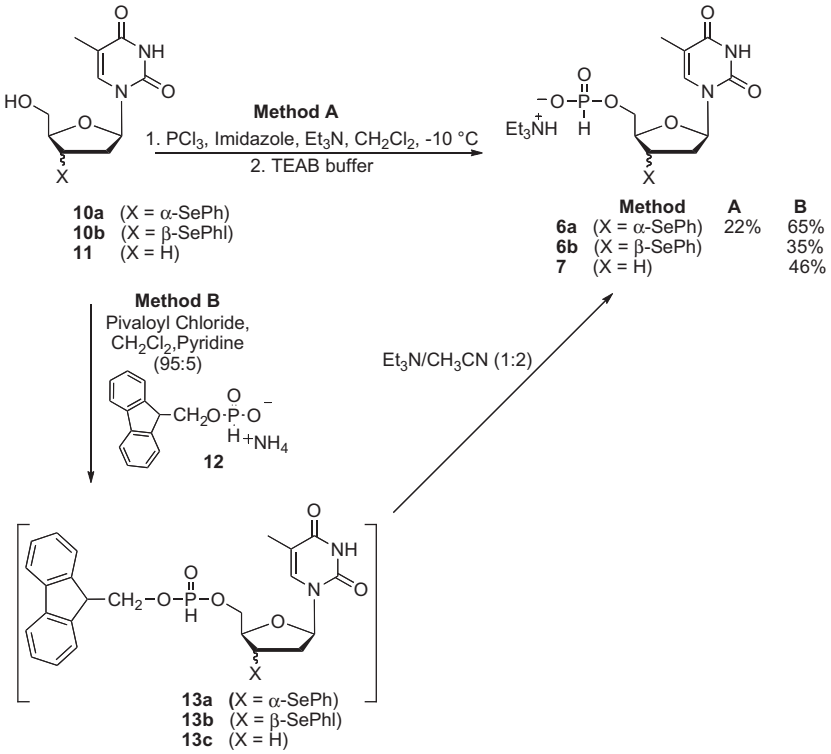
To determine their ability to perform the same task in ODNs, we have synthesized ODNs containing C3′-phenylselenide monomers **6** as well as unsaturated substrates **8** and **9**, representing radical disproportionation products that can arise from **2**, and compound **7**, the reduction product formed upon trapping of radical **2** by a hydrogen atom donor.

Due to the absence of the 3′-hydroxyl, these 3′-modified nucleotides do not contain the dimethoxytrityl protected hydroxyl substituent at the 3′-position of the nucleoside usually found in 5′-phosphoramidites. This change makes these H-phosphonates significantly more polar than their unmodified counterparts. As reported by Kraszewski et al,[23] we found the isolation and purification of such H-phosphonate monomers to be highly inefficient when the synthesis was performed using traditional phosphonylation conditions.[24,25] This became evident in our synthesis of H-phosphonate derivative **6a** using standard phosphonylation conditions employing PCl_3 and imidazole in the presence of triethylamine (Method A, Scheme 2). This approach delivered **6a** as its triethylammonium salt in only 22% yield (Scheme 2). The solubility of **10a**[26] in the reaction solvent CH_2Cl_2 was limited and reaction workup was very tedious.

To overcome this problem, an H-phosphonylation reagent developed by Fang and co-workers was used facilitating a more efficient aqueous work-up and purification than Method A (Method B, Scheme 2).[27]

Method B is based on the use of the H-phosphonylation reagent, ammonium (9*H*-fluoren-9-yl)methyl H-phosphonate (**12**), to introduce a lipophilic handle into these polar nucleosides to increase solubility and to simplify purification. The inclusion of pyridine in the reaction mixture also increases the solubility of the starting material. Compound **12** is easily obtained through treatment of commercially available phosphonic acid with pivaloyl chloride followed by the addition of (9*H*-fluoren-9-yl)methanol.[27] The use of this reagent permitted simple aqueous work-up of the reaction mixture and at the same time easy removal of the reaction by-products at the end of the synthesis.

Treatment of nucleoside **10a** with **12** in the presence of pivaloyl chloride, which functions as an activator, allowed introduction of the lipophilic (9H-fluoren-9-yl)methyl handle to deliver **13a** as an oily residue. Without further workup, treatment of intermediate



Scheme 2. Synthesis of H-phosphonate monomers.

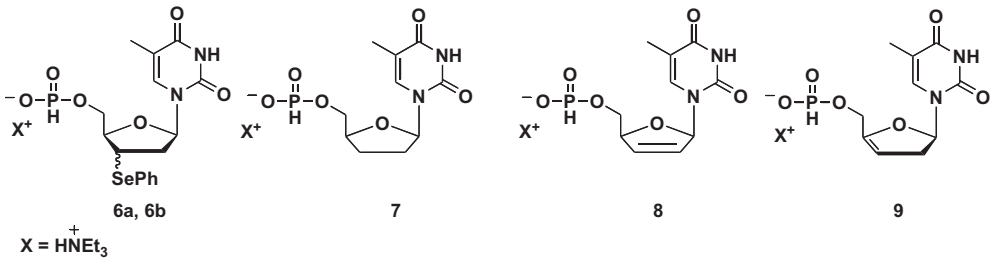


Figure 1. 2′,3′-dideoxyribonucleotide H-phosphonates.

13a with CH_3CN/triethylamine (2:1, v/v) at room temperature resulted in quantitative elimination of the (9*H*-fluoren-9-yl)methyl group (Scheme 2). Silica gel purification of the crude products afforded phenyl selenide derivatives **6a** and **6b** in 65 and 35% yield, respectively, while the 5′-*H*-phosphonate of 3′-deoxythymidine (**7**) was obtained in 46% yield. This approach was also extended to the synthesis of proposed disproportionation products (**8** and **9**). Using the same procedure described for the substrates above (**6**–**7**, Scheme 2) unsaturated H-phosphonates **8** and **9** (Fig. 1) were obtained in 50% and 27% yield, respectively.

While not impressive these yields are a substantial improvement over those obtained using traditional methods (data not shown). A possible explanation for the low yields obtained lies in the fact that the conversion of the nucleosides to intermediates **13** was found to be incomplete in all cases (based on TLC). Extending the reaction time as well as using more equivalents of phosphonylating agent **12** failed to lead to improvement of the yields. The utilization of 1-adamantanecarbonyl chloride as activator also did not result in improvement in yield (data not shown). Moreover, the synthesis of **9** was even more challenging due to the instability of the starting material (3′,4′-didehydro-2′,3′-dideoxythymidine).[28] Efforts are underway to improve the synthetic yields of H-phosphonates **6**–**9**.

Oligonucleotides containing 3′-modified nucleotides at their 3′-termini (**14**) were synthesized using the above-described H-phosphonates (**6**–**9**) through combined semi-automated and manual synthesis techniques. Beginning with commercially available supports and 5′-phosphoramidites, the unmodified portion of the sequence was formed on an Applied Biosystems 391 PCR-MATE DNA synthesizer. The final dimethoxytrityl protecting group of the unmodified oligomer was removed in the last automated step. The incorporation of the modified nucleotide of interest was then achieved utilizing the corresponding H-phosphonate derivatives via manual coupling as previously described.[16]

The resulting protected ODNs were cleaved from the solid support and fully deprotected via treatment with ammonium hydroxide for 15–18 h at 55 °C. The resulting crude oligonucleotides were purified using reverse phase HPLC (Fig. 2), **14a**. Oligomers **14a**–**f** were obtained in high yield, and purity as indicated by HPLC and MALDI-ToF MS analysis (See Table 1).

Table 1
MALDI-ToF MS analysis of ODNs **14a**–**f**

Sequence#	Sequences	Theoretical $[M+H]^+$	Experimental $[M+H]^+$
14a	TTATTp (**10a**)	1912.3	1912.2
14b	TTATTp (**10b**)	1912.3	1912.3
14c	TTATTp (**11**)	1756.3	1756.3
14d	TTATT (X)[a]	1754.3	1754.4
14e	TTATT (Y)[b]	1754.3	1754.2
14f	TTATC (Y) [b]	1739.3	1739.3

[a] X = 2′,3′-didehydro-2′,3′-dideoxythymidine.
[b] Y = 3′,4′-didehydro-2′,3′-dideoxythymidine.

The propensity of phenylselenides to undergo oxidation in the presence of reagents similar to those utilized for the conversion of the H-phosphonate moiety to the conrresponding phosphate prompted us to look at the extent of this conversion in the synthesis of ODNs **14a**–**b**. RNA polymers containing alkyl selenide moieties obtained through automated RNA synthesis[29] were reported to deliver 2–5% selenoxide upon exposure of the alkyl selenide containing oligomer to 20 mM I_2 for 20 s. This conversion was observed when the modification was near the 3′-terminus of the ODN.

In the synthesis of ODNs **14a**–**f**, the oxidation of the H-phosphonate linkage is performed using 4% I_2 in pyridine/H_2O/THF (1:1:8) with THF/H_2O/triethylamine (8:1:1). Under these conditions oxidation of the selenide moiety in ODNs **14a** and **b** could not be detected either by HPLC or by MALDI-ToF analysis (data not shown). These oligomers were also found to be very stable with no oxidation or degradation in the presence of air over several months.

Oligonucleotides **14c** and **d** were also shown to be highly stable over long periods of time. However, analysis of oligomers **14e** and **f** after storage at -20 °C in aqueous solution for several months indicated decomposition (Fig. 3). MALDI-ToF MS analysis detected the presence of a fragment corresponding to the product of base hydrolysis (**15**) as well as an oligomer containing a 3′-phosphate (**17**) resulting from the complete loss of the modified nucleoside (Scheme 3). This observation prompted us to further investigate the stability of the 3′,4′-unsaturated nucleotide containing ODN.

After incubation of **14f** for 45 min in 100 mM phosphate buffer, pH = 2.0, HPLC analysis showed the presence of remaining **14f**, along with a considerable amount of another fragment with a retention time of 12.47 min (see Supplementary data). ESI-MS analysis of the isolated peaks confirmed the presence of the starting material and fragment **15** (see Supplementary data). ODN **17** was not detected by HPLC analysis. The facile conversion of enol ethers similar to **14e**/**f** to the corresponding ketoaldehyde has been reported.[28]

With the required precursors in hand as well as a set of plausible degradation products, we turned to the identification of the lesions resulting from the photochemical generation of C3′-dideoxyradical **2** from C3′-phenylselenides. ODNs **14a** and **b** were photochemically activated under anaerobic conditions in 100 mM phosphate buffer. After photolysis, the crude photolysate was injected directly onto a reverse-phase column with detection at 260 nm. Elution was achieved by applying a linear gradient of 50 mM TEAA buffer (pH = 7.0) in acetonitrile. The fractions were collected and analyzed by MALDI-ToF MS, and the products were compared to independently synthesized standards. Standard curves of all oligonucleotides were constructed by injection of independently synthesized substrates with UV dection in order to quantify the products from the photolysis experiments (see Supplementary data).

In the presence of 6 mM glutathione (GSH), photochemical cleavage of the C3′-selenium bond upon exposure to UV light, $\geqslant$320 nm, resulted in the formation of the same products from

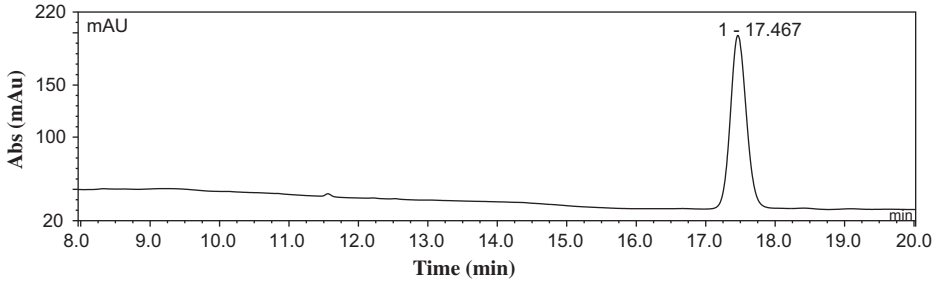


Figure 2. HPLC chromatogram of oligonucleotide **14a**.

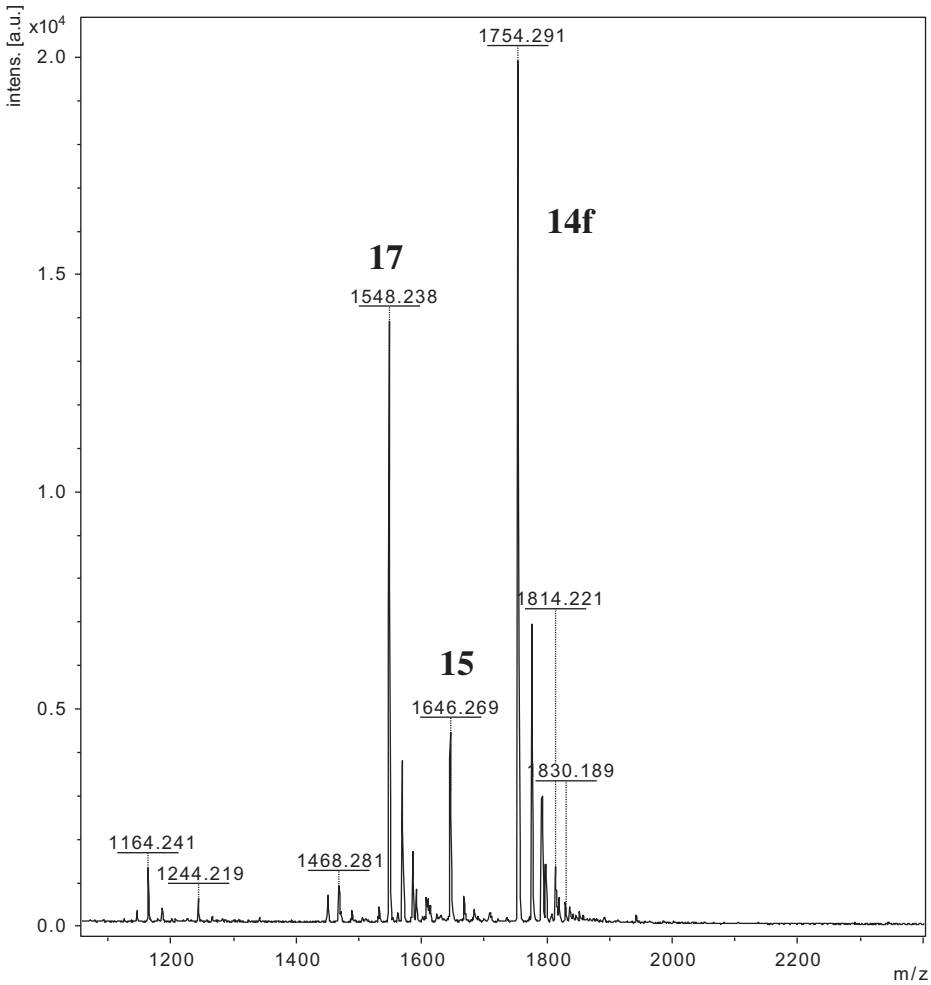
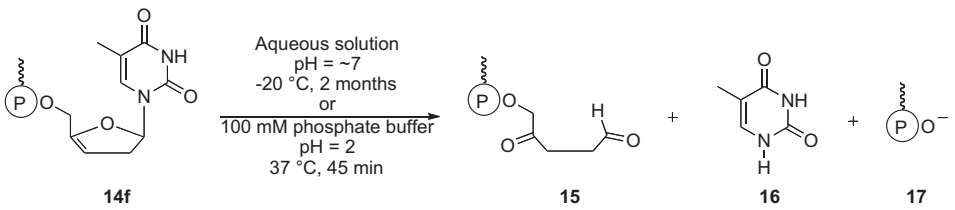
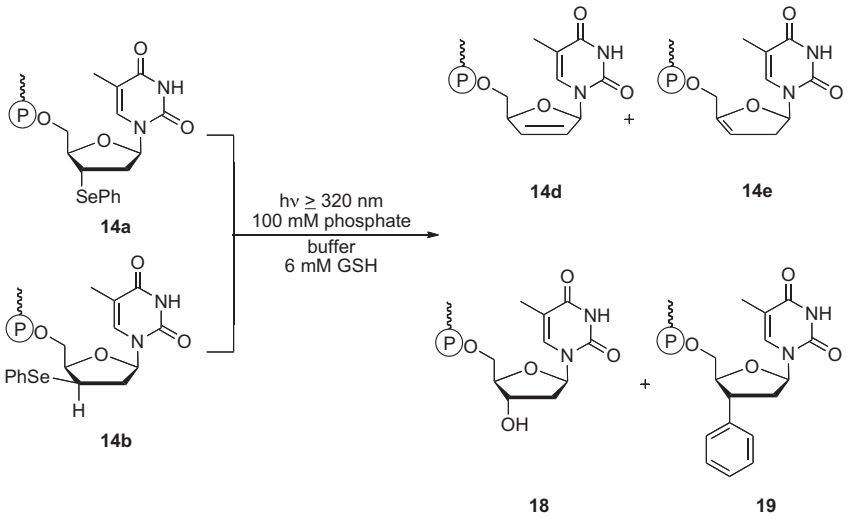


Figure 3. MALDI-ToF MS analysis of an aqueous solution of **14e** stored at -20 °C for 2 months (compound **14e**: expected 1754.3 *m*/*z* found 1754.3; compound **15**: expected 1646.3 found 1646.3; compound **17**: expected 1548.3 [M+H] found 1548.2 [M+H]).



Scheme 3. Decompostion products of **14f**.

both precursors. Utilizing the independently synthesized oligomers reported herein, we were able to easily identify the unsaturated sugar containing oligomers **14d** and **14e** as the major degradation products observed in this system with **14d** formed in an ~3:1 ratio relative to **14e** in the case of alpha phenylselenide **14a** and 6:1 in the case of beta isomer **14b**. In addition,



Scheme 4. Products identified in the photolysate derived from radical precursors **14a** and **14b** in the presence of glutathione.

Table 2
Product yields from the photolysis of phenylselenides **14a** and **14b** in the presence of GSH

Phenyl selenide	**14d**	**14e**	**18**	**19**	Inversion products		Mass balance (%)
					14a	**14b**	
14a	42.8	15.7	4.8	3.2	—	29.2	95.7
14b	43.6	7.3	2.0	5.2	21.6	—	79.7

C3′-phenylselenide containing oligomers (**14a**–**14b)** with inverted configuration at the C-Se bond, relative to the starting material (**14a** → **14b**; **14b** → **14a**), as well as C3′-phenylthymidine containing oligomers (**19**) were observed. Finally, trace amounts of thymidine terminated oligomers (**18**) were also observed (Scheme 4). It should be noted that these precursors do not deliver either the expected reduction product **14c** or a 3′-phosphorylated ODN resulting from the loss of the modified nucleotide (see Table 2).

Hence, the majority of products formed upon photolysis of phenylselenides **14a** and **14b** are derived from the generation of the phenylselenyl radical in a solvent cage.[30] This phenomenon, which is the result of the slow diffusion of the selenyl radical from the site of formation due to its hydrophobicity, facilitates the intramolecular reaction of the resulting radicals to give unsaturated sugars **14d** and **14e**.

These modified oligomers are formed by hydrogen atom abstraction at the C2′- and C4′-positons of the 2′,3′-dideoxyradical, respectively. Additional evidence of this phenomenon is found in the formation of the phenylated oligomer **19**. Related 4′-phenylated oligomers were found to be a major reaction product in the generation of the C4′-radical from phenylselenide precursors. The formation of these oligomers was believed to result from the addition of the sugar radical to the *ipso* position of the phenylselenyl radical.[21] This delivered a biradical species which loss selenium to deliver the modified oligomer. Due to the hydrophobic character of the phenylselenide radical, access to the sugar radical is completely hindered and reduction by GSH prohibited. The rate of the hydrogen atom abstraction at the alpha sugar carbons successfully competes with all other radical reactions to deliver the unsaturated sugars as the major products. The formation of thymidine terminated oligomers can be explained by the oxidation of the initially formed radical followed by solvolysis.

These data indicate that the introduction of 3′-deoxynucleosides of varying structure can be easily accomplished through 'reverse' (5′ → 3′) oligonucleotide synthesis using 5′-*H*-phosphonates. The required H-phosphonates are easily obtained using an alternative synthesis strategy involving the introduction of a lipophilic moiety at the 5′-*H*-phosphonate. Even though this method facilitated the delivery of the desired H-phosphonates, efforts are underway to increase the conversion of the modified nucleosides to intermediate **13** and subsequently to increase the yields. Substrates containing phenyl selenide substituents at the 3′-position are obtained in high purifity with no indication of oxidation through exposure to the dilute iodide reagent required for oxidation of the H-phosphonate linkage. 2′,3′-Dideoxynucleosides can also be converted to their corresponding H-phosphonates and incorporated in ODNs using these methods.

These substrates have been successfully used to determine the products obtained from the generation of the 2′,3′-thymidinyl radical in DNA oligomers from phenylselenide precursors under anaerobic conditions. Due to the nature of these precursors several products were observed which are specifically related to reactions of the radical pair. Even though these selenide precursors do not appear to function well for our purposes under anaerobic conditions, we will utilize these substrates for future investigations to determine the products of degradation of the 3′-deoxy-*C*3′-thymidinyl radical under aerobic conditions. The high rate of trapping of the radical of interest by oxygen may suppress the side reactions we have seen in the presence of the weak hydrogen atom donor GSH. We will also utilize stronger hydrogen atom donors to potentially suppress the unwanted side reactions observed under anaerobic conditions.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.11.050.

References and notes

1. Sonntag, C. v. *Free-Radical-Induced DNA Damage and its Repair: A Chemical Perspective*; Springer: Berlin, London, 2011.
2. Pimblott, S. M.; LaVerne, J. A. *Radiat. Phys. Chem.* **2007**, *76*, 1244.
3. Zheng, Y.; Cloutier, P.; Hunting, D. J.; Sanche, L. O.; Wagner, J. R. *J. Am. Chem. Soc.* **2005**, *127*, 16592.
4. Zheng, Y.; Cloutier, P.; Hunting, D. J.; Wagner, J. R.; Sanche, L. *J. Am. Chem. Soc.* **2004**, *126*, 1002.
5. Becker, D.; Bryant-Friedrich, A.; Trzasko, C.; Sevilla, M. D. *Radiat. Res.* **2003**, *160*, 174.
6. Bryant-Friedrich, A. C. *Org. Lett.* **2004**, *6*, 2329.
7. Lahoud, G. A.; Hitt, A. L.; Bryant-Friedrich, A. C. *Chem. Res. Toxicol.* **2006**, *19*, 1630.
8. Lyttle, M. H. *Curr. Pro. Nucl. Acid Chem.* **2001**, 4.6.1.
9. Greenberg, M. M. *Curr. Pro. Nucl. Acid Chem.* **2001**, 4.5.1.
10. Urata, H.; Akagi, M. *Tetrahedron Lett.* **1993**, *34*, 4015.
11. Horne, D. A.; Dervan, P. B. *J. Am. Chem. Soc.* **1990**, *112*, 2435.
12. Koga, M.; Moore, M. F.; Beaucage, S. L. *J. Org. Chem.* **1991**, *56*, 3757.
13. Masakazu Koga, O.; Wilk, A.; Moore, M. F.; Scremin, C. L.; Zhou, L.; Beaucage, S. L. *J. Org. Chem.* **1995**, *60*, 1520.
14. van de Sande, J. H.; Ramsing, N. B.; Germann, M. W.; Elhorst, W.; Kalisch, B. W.; von Kitzing, E.; Pon, R. T.; Clegg, R. C.; Jovin, T. M. *Science* **1988**, *241*, 551.
15. Claeboe, C. D.; Gao, R.; Hecht, S. M. *Nucleic Acids Res.* **2003**, *31*, 5685.
16. Lahoud, G.; Fancher, J.; Grosu, S.; Cavanaugh, B.; Bryant-Friedrich, A. *Bioorg. Med. Chem.* **2006**, *14*, 2581.
17. Hong, I. S.; Ding, H.; Greenberg, M. M. *J. Am. Chem. Soc.* **2006**, *128*, 485.
18. Hong, I. S.; Greenberg, M. M. *J. Am. Chem. Soc.* **2005**, *127*, 10510.
19. Hong, I. S.; Greenberg, M. M. *Org. Lett.* **2004**, *6*, 5011.
20. Tallman, K. A.; Greenberg, M. M. *J. Am. Chem. Soc.* **2001**, *123*, 5181.
21. Giese, B.; Beyrich-Graf, X.; Erdmann, P.; Petretta, M.; Schwitter, U. *Chem. Biol.* **1995**, *2*, 367.
22. Audat, S. A. S.; Trzasko Love, C.; Al-Oudat, B. A. S.; Bryant-Friedrich, A. C. *J. Org. Chem.* **2012**, *77*, 3829.
23. Romanowska, J.; Szymanska-Michalak, A.; Pietkiewicz, M.; Sobkowski, M.; Boryski, J.; Stawinski, J.; Kraszewski, A. *Lett. Org. Chem.* **2009**, *6*, 496.
24. Garegg, P. J.; Regberg, T.; Stawinski, J.; Stroemberg, R. *Chem. Scr.* **1986**, *26*, 59.
25. Gosselin, G.; Perigaud, C.; Lefebvre, I.; Pompon, A.; Aubertin, A.-M.; Kirn, A.; Szabo, T.; Stawinski, J.; Imbach, J.-L. *Antivir. Res.* **1993**, *22*, 143.
26. Cosford, N. D. P.; Schinazi, R. F. *J. Org. Chem.* **1991**, *56*, 2161.
27. Yang, Z.-W.; Xu, Z.-S.; Shen, N.-Z.; Fang, Z.-Q. *Nucleosides Nucleotides* **1995**, *14*, 167.
28. Imwinkelried, P., The University of Basel, 1995.
29. Carrasco, N.; Buzin, Y.; Tyson, E.; Halpert, E.; Huang, Z. *Nucleic Acids Res.* **2004**, *32*, 1638.
30. Ouchi, A.; Liu, S.; Li, Z.; Kumar, S. A.; Suzuki, T.; Hyugano, T.; Kitahara, H. *J. Org. Chem.* **2007**, *72*, 8700.