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# Synthesis and UV Photolysis of Oligodeoxynucleotides That Contain 5-(Phenylthiomethyl)-2′-deoxyuridine: A Specific Photolabile Precursor of 5-(2′-Deoxyuridilyl)methyl Radical

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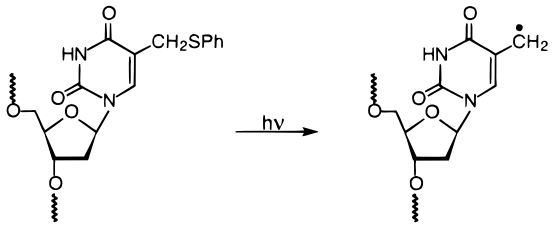
## ABSTRACT




**The title exocyclic radical (2) is generated via photochemical cleavage of 5-(phenylthiomethyl)-2′-deoxyuridine (8). The latter thionucleoside (8) was successfully incorporated into DNA oligomers by automated DNA synthesis using phosphoramidite chemistry. UV exposure of 8 containing oligonucleotides under (an)aerobic conditions gives rise to specific base lesions. The photoproducts have been isolated and further characterized on the basis of detailed NMR and mass spectrometric analyses.**

During the past five years, many efforts have been devoted to the synthesis of oligodeoxynucleotides (ODNs) that contained photoreactive precursors of radical intermediates involved in the radiation-induced decomposition of purine and pyrimidine nucleic acid components.[1] These modified DNA fragments are powerful tools for mechanistic studies aimed at elucidating the role of individual nucleobase and osidic reactive intermediates in the formation of nucleic acid lesions. These include oxidized bases, abasic sites, and DNA strand breaks. Indeed, the UV-mediated independent generation of a specific radical at a defined site in an ODN enables one to investigate the chemistry of this radical under conditions in which other radicals derived from nucleosides are not formed. This approach offers a distinct advantage over the use of ionizing radiation that gives rise to multiple reactive intermediates. Interestingly, the chemistry of the osidic radicals resulting from C-1′- and C-4′-hydrogen atom abstraction has been studied in detail by using such modified ODNs. The latter mechanistic investigations have involved extensive product analysis, together with kinetics and EPR measurements.[1,2]

To investigate the reactivity of the 5-(2′-deoxyuridilyl)-methyl radical **2** in DNA oligomers, we have developed a similar methodolgy by synthesizing **8**, a specific photopre-

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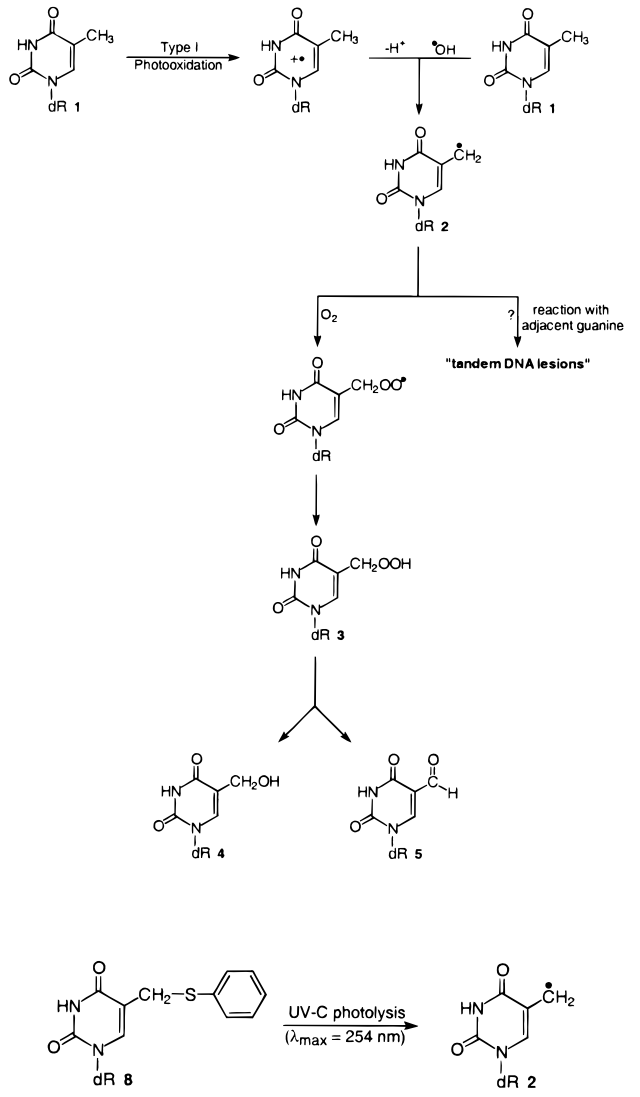
cursor to radical **2** derived from thymidine. Radical **2** is one of the main reactive radiation-induced and type I photosensitized decomposition products of thymidine **1**.[3,4] It results from either •OH-mediated hydrogen atom abstraction from the 5-methyl group or deprotonation of the radical cation intermediate produced by photosensitizers operating through a type I mechanism such as benzophenone or menadione (Scheme 1). Subsequent reaction with oxygen leads to

**Scheme 1**[a]

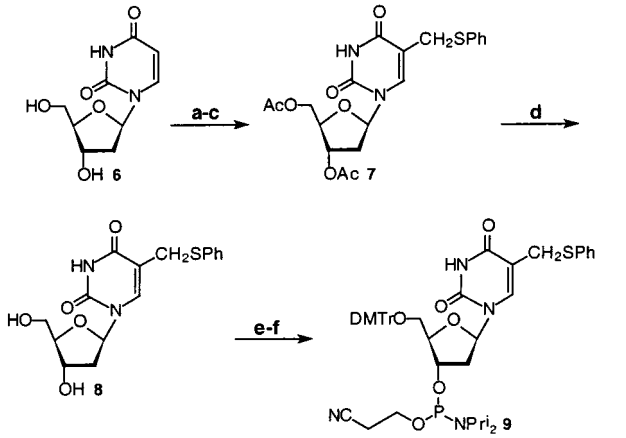



[a] (a) The 5-(2′-deoxyuridilyl)methyl radical **2** reaction manifold. (b) Generation of **2** by UV−C photolysis of the thionucleoside **8**.

hydroperoxide **3** which decomposes into 5-(hydroxymethyl)-2′-deoxyuridine ($d^{HM}U$) **4** and 5-formyl-2′-deoxyuridine ($d^{F}U$) **5**. Furthermore, the exocyclic radical is suspected to interact with neighboring guanines, resulting in the formation of DNA lesions with two adjacent modified bases ("tandem DNA lesions").[5,6] With the aim to further substantiate the latter mechanistic pathways, thionucleoside **8** was site-specifically incorporated into synthesized ODNs via phosphoramidite **9**. Then, radical **2** was independently generated by UV-C irradiation ($\lambda_{max}$ = 254 nm) under both aerobic and anaerobic conditions.

We report the synthesis of these modified ODNs together with the isolation and the characterization of DNA damage induced via the 5-(2′-deoxyuridilyl)methyl radical. It is assumed that **2** is generated via photochemical homolytic cleavage of the C−SPh bond of 5-(phenylthiomethyl)-2′-deoxyuridine ($d^{PhS}U$) **8**. This is supported by the formation of cyclonucleosides from several thionucleosides possessing this photoreactive group via the generation of similar alkyl radicals.[7−9] The synthesis of **8** and **9** is outlined in Scheme 2. Hydroxymethylation of 2′-deoxyuridine **6** with paraform-

**Scheme 2.** Synthetic Reactions Used for the Preparation of the Phosphoramidite Synthon of $d^{PhS}U$[a]




[a] (a) $(CH_2O)_n$, TEA, $H_2O$, 60 °C, 66 h; (b) $(CH_3CO)_2O$, pyridine, 15 h; (c) PhSH, TEA, DMF, 70 °C, 24 h; (d) $NH_4OH$ 32% aqueous, $CH_3OH$, 15 h, 13% (a + b + c + d); (e) DMTrCl, pyridine, 23 h, 64%; (f) $NCCH_2CH_2OP(Cl)NPr^i_2$, DIEA, $CH_2Cl_2$, argon, 30 min, 96%.

aldehyde in alkaline medium followed by acetylation and selective substitution of the benzylic acetate group by

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(9) Recently, Mehl and Begley reported the synthesis of a bispyrimidine model system containing the PhS group as a precursor to the C-6 spore photoproduct radical: Mehl, R. A.; Begley, T. P. *Org. Lett.* **1999**, *1*, 1065−1066.

thiophenol produced the fully protected thionucleoside **7**.[10] Deprotection of **7** afforded d$^{PhS}$U **8**, which was further converted into phosphoramidite **9** via the protection of the primary alcohol with dimethoxytrityl chloride (DMTrCl)[11] followed by treatment with 2-cyanoethyl *N*,*N*-diisopropyl-lphosphoramidochloridite.[12] It was found that **8** is stable in the presence of the reagents (trichloroacetic acid, tetrazole, iodine/pyridine/THF/$H_2O$, and acetic anhydride/*N*-methylimidazole) used in automated chemical DNA synthesis. However, **8** was fully decomposed under standard base-deprotecting conditions (30% ammonium hydroxide, 55 °C, 12 h).[13] This was circumvented by using the highly alkali-labile amino-protecting groups developed by Schulhof et al.,[14] which allow for a complete deprotection of synthetic oligonucleotides in 30% aqueous ammonia at room temperature for 4 h. In the latter conditions, no degradation of the modified nucleoside **8** was detected.

The modified ODNs **10**−**12** (Table 1) were synthesized according to the solid-phase phosphoramidite method (1 and 10 $\mu$mol scales) on an automated DNA synthesizer using **9** and commercially available phenoxyacetyl-dA-, phenoxyacetyl-dG-, and isobutyryl-dC-cyanoethyl phosphoramidites. After cleavage of the support and removal of the alkali-labile groups with 30% ammonium hydroxide at room temperature for 4 h, the 5′-DMTr-oligomers were purified and deprotected on line by reversed-phase HPLC.[7a] Electrospray ionization mass spectrometry (ESI-MS) measurements of the modified ODNs (Table 1) confirmed the incorporation of d$^{PhS}$U (**8**) and the purity of oligomers **10**−**12**. Furthermore, HPLC analysis of the nucleoside mixture obtained by enzymatic digestion of 9-mer **12** by nuclease P1 followed by alkaline phosphatase provided dC, dG, dT, dA, and d$^{PhS}$U in a 2:1:3:2:1 ratio, confirming the structure (Figure 1).

**Table 1.** Sequences and Relative Molecular Masses (Da) of the Modified ODNs

| name | sequences[a] | length | mass (calcd) (Da) | mass[b] (found) (Da) |
|---|---|---|---|---|
| **10** | **X**G | 2 | 679.40 | 679.40 ± 0.10 |
| **11** | C**X**GA | 4 | 1281.80 | 1281.51 ± 0.31 |
| **12** | ATC**X**GTACT | 9 | 2796.80 | 2797.23 ± 1.74 |
| **13** | HMUG | 2 | 587.40 | 587.50 ± 0.10 |
| **14** | FUG | 2 | 585.40 | 585.40 ± 0.10 |
| **15** | C$^{HM}$UGA | 4 | 1189.80 | 1190.00 ± 0.10 |
| **16** | C$^{F}$UGA | 4 | 1187.80 | 1187.90 ± 0.10 |
| **17** | ATC$^{HM}$UGTACT | 9 | 2704.80 | 2704.50 ± 0.27 |
| **18** | ATC$^{F}$UGTACT | 9 | 2702.80 | 2701.80 ± 1.50 |
| **19** | TG | 2 | 571.40 | 571.40 ± 0.10 |
| **20** | T$^{MeC8}$G | 2 | 569.40 | 569.40 ± 0.10 |
| **21** | dimeric TG | 2 | 1140.90 | 1140.90 ± 0.10 |

[a] **X** is $^{PhS}$U. [b] All the ODN masses were obtained by ESI-MS in the negative mode.

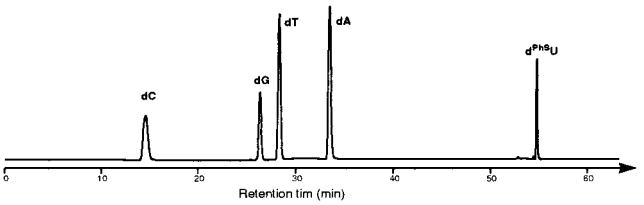


**Figure 1.** HPLC elution profile of the enzymatic digestion mixture of 9-mer d(ATC$^{PhS}$UGTACT) **12**.

When 2-mer [5′-d($^{PhS}$UG)-3′] **10** is UV-irradiated under aerobic conditions, high yields of [5′-d($^{HM}$UG)-3′] **13** and [5′-d($^{F}$UG)-3′] **14** are obtained (Figure 2a). Structural insights into the modified dinucleoside monophosphates **13** and **14** were gained from ESI-MS measurements (Table 1). In addition, both HPLC peaks were identified by comigration with authentic samples of **13** and **14** which were prepared by using synthetic methodologies described in the literature.[15,16] Similar results were obtained with the other d$^{PhS}$U-containing ODNs **11**−**12**. Altogether these results suggest that the 5-(2′-deoxyuridilyl)methyl radical **2** is formed in a high yield and trapped efficiently by oxygen when **8** is UV-irradiated within these biopolymers.

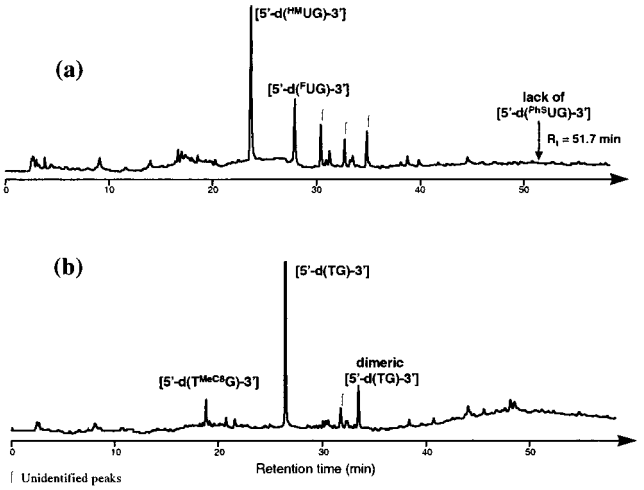


**Figure 2.** HPLC elution profiles of UV-irradiated d($^{PhS}$UG) **10** (a) under atmospheric $O_2$ and (b) $N_2$-purged solution.

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(16) For [5′-d($^{F}$UG)-3′], see: Berthod, T. Pétillot, Y.; Guy, A.; Cadet, J.; Forest, E.; Molko, D. *Nucleosides Nucleotides* **1996**, *15*, 1287−1305.

Thereafter, photolysis of **10** in deaerated aqueous solution resulted in the formation of [5′-d(TG)-3′] **19** as the major compound (Figure 2b). This product is likely to arise from trapping of **2** by thiophenol, a hydrogen atom donor, produced during photoirradiation. Furthermore, two other products which illustrate the reactivity of the 5-(2′-deoxyuridilyl)methyl radical under anaerobic conditions have been detected in the crude photolysate. The most polar compound was isolated in 12% yield and subjected to mass spectrometry measurements. The molecular weight of 569.4 is in good agreement with the calculated mass of 569.4 for the thymine-guanine tandem lesion [5′-d($T^{MeC8}$G)-3′] **20** which has a covalent bond between the methyl carbon atom of thymine and the C-8 carbon atom of guanine. The latter product was first identified by Box et al. in DNA oligomers, namely, [5′-d(CGTA)-3′] and [5′-d(ACGT)-3′], exposed to X irradiation in deoxygenated aqueous solution. This was achieved by $^1$H NMR spectroscopy.[6a,c] The structure of the vicinal DNA lesion **20** was confirmed by using tandem mass spectrometry (ESI-MS/MS) which allows the determination of the fragmentation pattern of the molecule. Thus, a characteristic 568−470 transition which results from a loss of the sugar residue at the 5′-end of the dinucleoside monophosphate **20** was observed in the MS/MS spectra in the negative mode. In the latter fragment, the thymine remains covalently attached through the methylene bridge. To generate enough product **20** for more extensive NMR studies, [5′-d($^{PhS}$UG)-3′] was synthesized in large amounts (1 mmol scale) by solution phosphotriester chemistry and subsequently irradiated. The $^1$H NMR spectrum of **20** collected from HPLC runs, exhibits the same features as those previously described by Box et al. for the analogous product in the [5′-d(CGTA)-3′] oligomer. As striking characteristics, the thymine methyl proton resonance and the guanine H-8 resonance are absent. Moreover, two doublet signals which correspond to the nonmagnetically equivalent methylenic protons are observed (chemical shifts at 3.65 and 3.95 ppm, respectively). This unambiguously confirms the assigned structure of **20**. The resistance of the latter adduct to enzymatic digestion by several endo- and exonucleases, namely, nuclease P1, calf spleen phosphodiesterase, and snake venom phosphodiesterase, was investigated as previously described.[7,17−19] No hydrolysis of the phosphodiester bond was observed under the three enzymatic degradation conditions used as inferred from RP-HPLC and mass spectrometry analyses of the reaction mixtures. The last compound isolated from the HPLC elution profile of the photolysis reaction has a molecular weight of 1140.9. This suggests a dimeric structure of [5′-d(TG)-3′] where the two methyl carbon atoms of thymines are connected by a covalent bond (calculated 1140.9). This structure was confirmed by submitting the compound to nuclease P1 and alkaline phosphatase digestion. RP-HPLC analysis of the hydrolysis mixture followed by tandem mass spectrometry measurements of the collected peaks revealed the presence of a resistant and characteristic d($T^{MeMe}$T) residue (mass calculated 482.4; mass found 482.2). Thus, under anaerobic conditions, the 5-(2′-deoxyuridilyl)-methyl radical can react with an adjacent guanine base to give [5′-d($T^{MeC8}$G)-3′] or undergo a radical pair combination to generate the dimeric adduct derived from [5′-d(TG)-3′].

In summary, the present studies demonstrated that 5-(phenylthiomethyl)-2′-deoxyuridine is an efficient photoprecursor of the 5-(2′-deoxyuridilyl)methyl radical within DNA oligomers. Work is in progress to generate **20** in longer ODNs which will be suitable for studies aimed at determining both the biochemical (mutagenesis, repair) and conformational features of the tandem lesion. In addition, availability of such modified ODNs will facilitate the development and the optimization of HPLC-MS/MS assay for monitoring the formation of the latter damage within isolated and cellular oxidized DNA.

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**Supporting Information Available:** Typical experimental procedures and characterization of compounds **8** and **9** and modified oligonucleotides. This material is available free of charge via the Internet at http://pubs.acs.org.

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