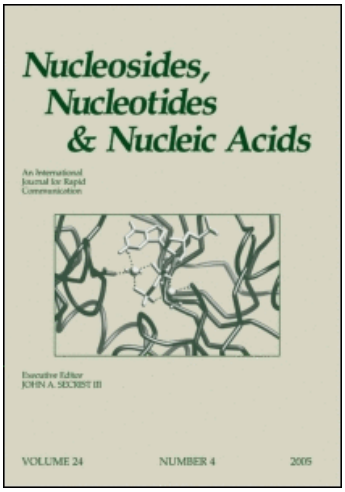

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5-(PHENYLTHIOMETHYL)-2′-DEOXYURIDINE AS AN EFFICIENT PHOTOREACTIVE PRECURSOR TO GENERATE SINGLE AND MULTIPLE LESIONS WITHIN DNA FRAGMENTS

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ABSTRACT

5-(Phenylthiomethyl)-2′-deoxyuridine was successfully incorporated into DNA oligomers by automated DNA synthesis using phosphoramidite chemistry. UV exposure of the latter thionucleoside containing oligonucleotides under anaerobic and aerobic conditions gives rise to specific base lesions. The photoproducts have been isolated and further characterized on the basis of NMR and mass spectrometric analyses.

During the last few 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. Indeed, the UV-mediated independant generation of a specific radical at a defined site in an

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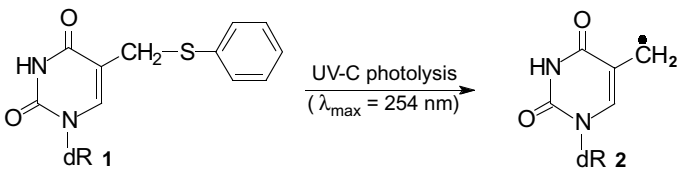
ODN enables one to investigate the chemistry of this radical under conditions in which other radicals derived from nucleosides are not formed. This approach offers a distinct advantage over the use of ionizing radiation that gives rise to multiple reactive intermediates.

In order to investigate the reactivity of the 5-(2′-deoxyuridilyl)methyl radical **2** in DNA oligomers, we have developed a similar methodology by synthesizing 5-(phenylthiomethyl)-2′-deoxyuridine **1**, a specific photoprecursor of radical **2** derived from thymidine. Radical **2** is one of the main reactive radiation-induced and type-I photosensitized decomposition intermediates of thymidine (2,3). 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 type I mechanism. Subsequent reaction with oxygen leads to the related hydroperoxide which decomposes into 5-(hydroxymethyl)-2′-deoxyuridine ($d^{HM}U$) and 5-formyl-2′-deoxyuridine ($d^{F}U$). Furthermore, the exocyclic radical is suspected to interact with neighboring guanines, resulting in the formation of DNA lesions as two adjacent modified bases ("tandem DNA lesions") (4,5).

With the aim to further substantiate the latter mechanistic pathways, thionucleoside **1** was site-specifically incorporated into several DNA oligomers using both phosphotriester and phosphoramidite chemistry (6). Then, radical **2** was generated by UV-C irradiation (λ_{max} = 254 nm) under both aerobic and anaerobic conditions (Scheme 1). The isolation and the characterization of single and multiple DNA damage induced via 5-(2′-deoxyuridilyl)methyl radical have been performed.

When 2-mer [5′-d(^{PhS}UG)-3′] **3** is UV-irradiated under aerobic conditions, high yields of [5′-d(^{HM}UG)-3′] **4** and [5′-d(^{F}UG)-3′] **5** are obtained. Structural insights into the modified dinucleoside monophosphates **4** and **5** were gained from ESI-MS measurements and enzymatic digestion after reversed-phase HPLC purification. Similar results were obtained with other $d^{PhS}U$ containing DNA sequences. Altogether, these results suggest that 5-(2′-deoxyuridilyl)methyl radical **2** is formed in a high yield and trapped efficiently by oxygen when **1** is UV-irradiated within these biopolymers.

Thereafter, photolysis of **3** in deaerated aqueous solution resulted in the formation of [5′-d(TG)-3′] as the major compound. This photoproduct is likely to arise from the trapping of **2** by thiophenol, an hydrogen atom donor, produced during photoirradiation. Furthermore, other products which illustrate the reactivity of



Scheme 1. Generation of **2** by UV-C photolysis of the thionucleoside **1**.

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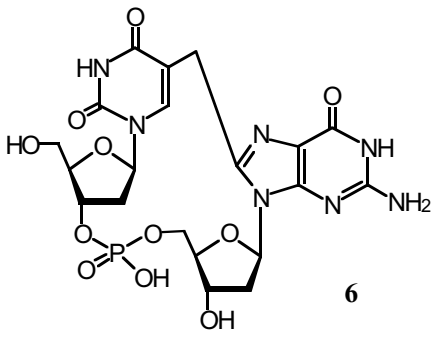

6

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 agreement with the calculated mass of 569.4 for the thymine-guanine tandem lesion [5′-d(T^{MeC8}G)-3′] **6** which has a covalent bond between the methyl carbon atom of thymine and the C-8 carbon atom of guanine.

The structure of the vicinal DNA lesion **6** was confirmed using tandem mass spectrometry (ESI-MS/MS) which allows the determination of the fragmentation pattern of the molecule (Fig. 1). Thus, a characteristic 568 ⇒ 470 transition which results from the loss of the sugar residue at the 5′-end of the dinucleoside monophosphate **6** was observed in the MS/MS spectra obtained in the negative mode. In the latter fragmentation, the thymine remains covalently attached through the methylene bridge. The ^{1}H NMR spectrum of **6** collected from HPLC runs unambiguously confirms the assigned structure of **6**. 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 (7–9). 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. Thus, under anoxic conditions, 5-(2′-deoxyuridilyl)methyl radical can react with adjacent guanine base to give [5′-d(T^{MeC8}G)-3′]. Moreover, it should be added that similar results have been obtained by UV-C irradiation of the opposite sequence [5′-d(GU^{SPh})-3′], which gives rise to the formation of the [5′-d(G^{C8Me}T)-3′] tandem lesion in a high yield.

In conclusion, the present studies demonstrated that 5-(phenylthiomethyl)-2′-deoxyuridine is an efficient photolabile precursor of 5-(2′-deoxyuridilyl)methyl radical within DNA oligomers. Work is in progress to generate **6** and other "tandem DNA lesions" in longer ODNs which will be suitable for studies aimed at determining both the biochemical (mutagenesis, repair) and conformational features of the latter damage. In addition, availability of such modified substrates

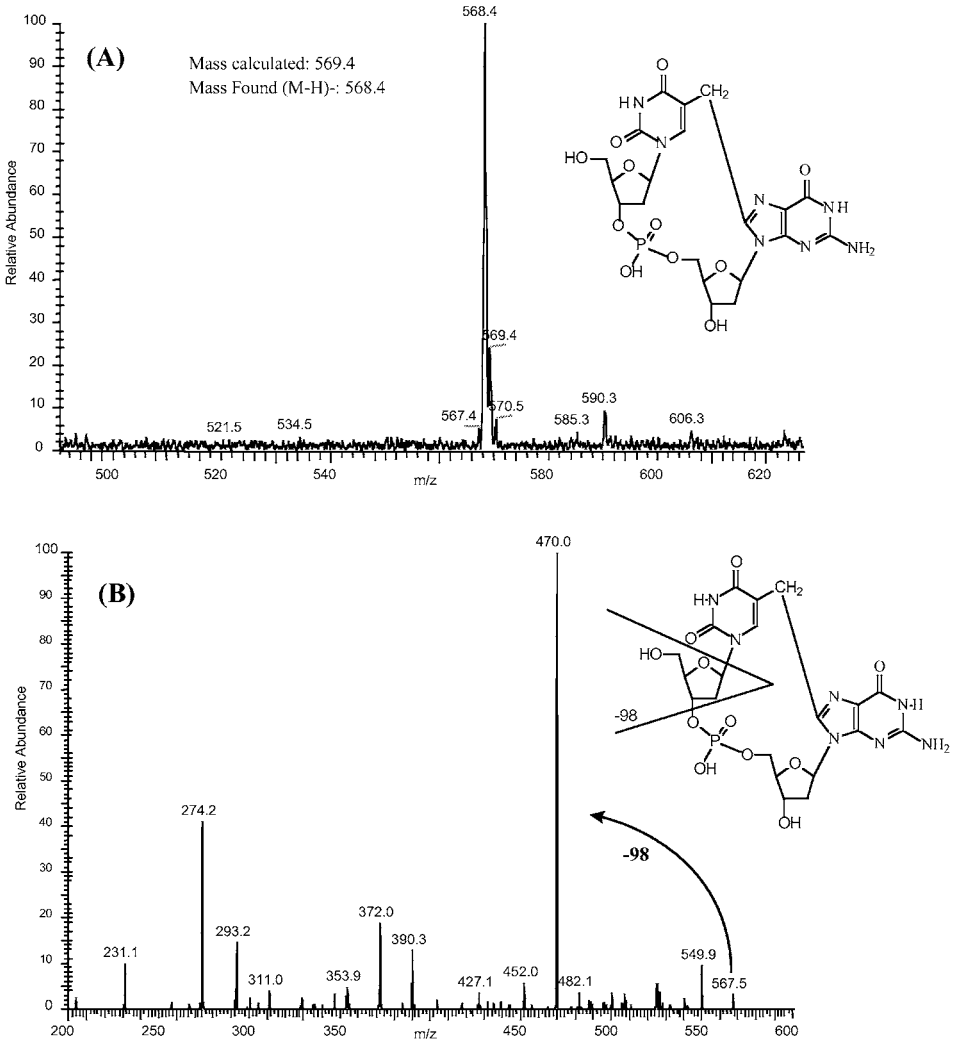

Figure 1. ESI-MS (A) and ESI-MS/MS (B) spectra (in the negative mode) of the modified dinucleoside monophosphate **6**.

will facilitate the development and the optimization of HPLC-MS/MS assay for monitoring the formation of these vicinal lesions within isolated and cellular oxidized DNA.

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REFERENCES

1. For reviews, see: Greenberg, M. M. *Chem. Res. Toxicol*., **1998**, *11*, 1235–1248. Beckwith, A. L. J.; Crich, D.; Duggan, P. J.; Yao, Q. *Chem. Rev.*, **1997**, *97*, 3273–3312.
2. Cadet, J.; Delatour, T.; Douki, T.; Gasparutto, D.; Pouget, J.-P.; Ravanat, J.-L.; Sauvaigo, S. *Mutat. Res*., **1999**, *424*, 9–21.
3. (a) Decarroz, C.; Wagner, J. R.; van Lier, J. E.; Muri-Krishna, C.; Riesz, P.; Cadet, J. *Int. J. Rad. Biol*., **1986**, *50*, 491–505. (b) Wagner, J. R.; van Lier, J. E.; Decarroz, C.; Berger, M.; Cadet, J. *Methods Enzymol*., **1990**, *186*, 502–511. (c) Delatour, T.; Douki, T.; D′Ham, C.; Cadet, J. *J. Photochem. Photobiol. B*., **1998**, *44*, 191–198.
4. Delatour, T.; Douki, T.; Gasparutto, D.; Brochier, M.-C.; Cadet, J. *Chem. Res. Toxicol*., **1998**, *11*, 1005–1013.
5. (a) Box, H. C.; Budzinski, E. E.; Dawidzik, J. D.; Wallace, J. C.; Evans, M. S.; Gobey, J. S. *Radiat. Res*., **1996**, *145*, 641–643. (b) Budzinski, E. E.; Dawidzik, J. D.; Rajecki, M. J.; Wallace, J. C.; Schroder, E. A.; Box, H. C. *Int. J. Rad. Biol*., **1997**, *71*, 327–336. (c) Box, H. C.; Budzinski, E. E.; Dawidzik, J. D.; Gobey, J. S.; Freund, H. G. *Free Radic. Biol. Med*., **1997**, *23*, 1021–1030. (d) Box, H. C.; Budzinski, E. E.; Dawidzik, J. D.; Wallace, J. C.; Iijima, H. *Radiat. Res*., **1998**, *149*, 433–439.
6. Romieu, A.; Bellon, S.; Gasparutto, D.; Cadet, J. *Org. Lett*., **2000**, *2*, 1085–1088.
7. Gasparutto, D.; Ravanat, J.-L.; Gérot, O.; Cadet, J. *J. Am. Chem. Soc*., **1998**, *120*, 10283–10286.
8. Romieu, A.; Gasparutto, D.; Molko, D.; Ravanat, J.-L.; Cadet, J. *Eur. J. Org. Chem*., **1999**, 49–56.
9. Romieu, A.; Gasparutto, D.; Cadet, J. *J. Chem. Soc, Perkin Trans*. **1999**, *1*, 1257–1263.

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