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Formation of a Methide Derivative upon Photolysis of Thymidine Bromohydrins

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Reaction of bromine with thymidine in aqueous solution produces, in high yield, the corresponding 5-bromo-6-hydroxy-5,6-dihydroderivative (thymidine bromohydrins). UVC photolysis of thymidine bromohydrins gives rise to a reactive intermediate that is converted into 5-(hydroxymethyl)-2′-deoxyuridine upon incubation in water. When the former compound is left in methanol, ethanol, or propanol, the corresponding 5-alkoxymethyl derivatives are produced. The proposed structure for the primary photolysis product of thymidine bromohydrins is a methide derivative of the thymine ring. This compound could be an interesting intermediate in the synthesis of methyl-substituted thymidine.

Introduction

Modification of the chemical structure of DNA bases is associated with deleterious cellular processes including lethality and mutagenicity. In that respect, radical-induced degradation of nucleobases has been extensively investigated on model systems such as nucleosides and short oligonucleotides.[1] Photochemical generation of nucleobase radicals is a powerful tool for the understanding of the degradation pathways.[2] Recently, we used thymine bromohydrins, previously used as synthetic precursors of pyrimidine hydroperoxides,[3] to photochemically trigger the formation of tandem lesions in a dinucleoside monophosphate carrying a vicinal guanine moiety.[4] In the latter experiment, products only modified on their thymine moiety were also obtained in high yield.

We presently report a more thorough study of the photolysis of thymine bromohydrins at the nucleoside level. The final stable products were characterized, on the basis of ^{1}H NMR and mass spectrometry analyses, as 5-(hydroxymethyl)-uracil nucleosides. Their formation involved an intermediate that was partially identified by ^{1}H NMR and mass spectrometry. A methide structure, in agreement with the reactivity in water and alcohols, was proposed for this transient bromohydrins photolysis product.

Results

Identification of the Final Stable Products of Photolysis of Thymidine Bromohydrins. (5*R*,6*S*)- and (5*S*,6*R*)-5-Bromo-6-hydroxy-5,6-dihydrothymidine (trans bromohydrins of thymidine, **2**) were synthesized in high yield as previously reported by addition of bromine to an aqueous solution of thymidine (**1**).[5] The reaction mixture was injected on a reverse-phase HPLC column, and a fraction containing the two bromohydrins was collected. The resulting solution was then exposed to the UVC light emitted by a germicidal lamp. Thymidine bromohydrins are very reactive under these conditions, since, after 30 min of irradiation, HPLC analysis shows that **2** is completely converted. Instead, the HPLC−UV chromatogram exhibits two main peaks corresponding to closely eluting photoproducts **3** and **3′** (yield ca. 95%) at shorter retention times than those obtained for **2** (Figure 1). Thymidine glycols that may arise from either the hydrolytic conversion of bromohydrins or the fate of the 6-hydroxy-5-yl radical are also obtained in low yield (<5%). When irradiated bromohydrins mixtures are left in aqueous solution at room temperature, two new compounds, **4** and **4′**, are produced at the expense of **3** and **3′**, as shown by HPLC−MS/MS analyses (vide infra). The same observations are made when the crude bromination mixture was irradiated instead of HPLC-purified bromohydrins.

4 and **4′** were isolated separately by reverse phase HPLC. Compound **4** was identified as 5-(hydroxymethyl)-2′-deoxyuridine (5-HMdUrd) on the basis of its chromatography, mass spectrometry (Figure 2),[6] and ^{1}H NMR

(1) (a) Breen, A. P.; Murphy, J. A. *Free Radical Biol. Med.* **1995**, *18*, 1033−1077. (b) Cadet, J.; Berger, M.; Douki, T.; Ravanat, J.-L. *Rev. Physiol. Biochem. Pharmacol.* **1997**, *131*, 1−87.

(2) (a) Greenberg, M. M. *Chem. Res. Toxicol.* **1998**, *11*, 1235−1248. (b) Romieu, A.; Bellon, S.; Gasparutto, D.; Cadet, J. *Org. Lett.* **2000**, *2*, 1085−1088.

(3) (a) Cadet, J.; Téoule, R. *Biochim. Biophys. Acta* **1971**, *238*, 8−26. (b) Cadet, J.; Téoule, R. *C. R. Acad. Sci. Paris* **1973**, *276*, 1743−1746. (c) Wagner, R. J.; van Lier, J. E.; Berger, M.; Cadet, J. *J. Am. Chem. Soc.* **1994**, *116*, 2235−2242. (d) Tremblay, S.; Douki, T.; Cadet, J.; Wagner, R. *J. Biol. Chem.* **1999**, *274*, 20833−20838.

(4) Douki, T.; Rivière, J.; Cadet, J. *Chem. Res. Toxicol.* **2002**, *15*, 445−454.

(5) Ulrich, J.; Cadet, J.; Téoule, R. *Org. Mass Spectrom.* **1973**, *7*, 543−554.

(6) Frelon, S.; Douki, T.; Ravanat, J.-L.; Tornabene, C.; Cadet, J. *Chem. Res. Toxicol.* **2000**, *13*, 1002−1010.

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TABLE 1. Chemical Shifts (in ppm) and Coupling Constants (in Hz) of the Nonexchangeable Protons of Products 4 and 4′ [a]

proton	product **4**	product **4′**
H1′	6.31 ($J_{1'-2'} = 5.6$; $J_{1'-2''} = 6.5$)	6.11 ($J_{1'-2'} = 7.2$; $J_{1'-2''} = 2.7$)
H2′	2.25 ($J_{2'-2''} = -13.5$; $J_{2'-3'} = 6.1$)	2.05 ($J_{2'-2''} = -14.1$; $J_{2'-3'}$: 7.2)
H2″	2.27 ($J_{2''-3'} = 3.9$)	2.60 ($J_{2''-3'} = 2.6$)
H3′	4.51 ($J_{3'-4'} = 2.8$)	4.65 ($J_{3'-4'} = 2.4$)
H4′	4.10 ($J_{4'-5'} = 3.2$; $J_{4'-5''} = 4.7$)	4.35 ($J_{4'-5'} = 3.8$; $J_{4'-5''} = 5.4$)
H5′	3.80 ($J_{5'-5''} = -12.2$)	3.45 ($J_{5'-5''} = -12.6$)
H5″	3.90	3.55
H6	7.75	7.82
CH_2	4.23	4.22

[a] ^{1}H NMR (400 MHz) spectra were recorded in D_2O at 25 °C.

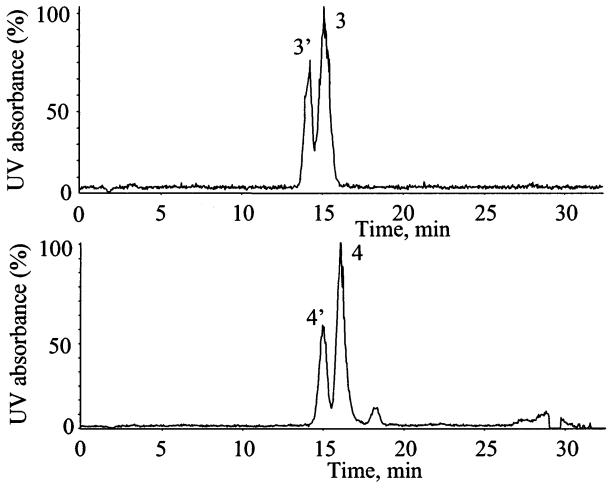


FIGURE 1. Reverse phase HPLC chromatograms of UVC-irradiated solution of thymidine bromohydrins **2** immediately after irradiation (upper panel) or following overnight incubation at 37 °C (lower panel).

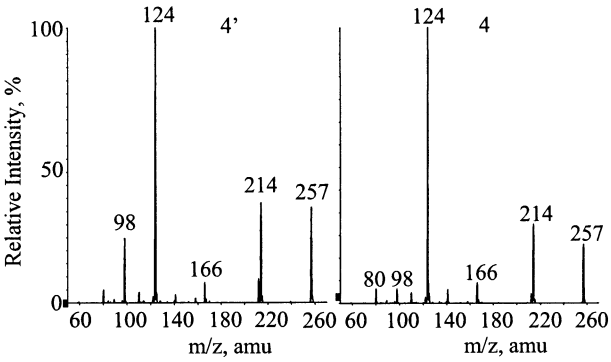


FIGURE 2. Fragmentation mass spectra of **4** and **4′** recorded by HPLC−MS/MS analysis in the negative mode. The daughter ion ($[M - H]^-$) was set at $m/z = 257$.

features (Table 1).[7] The fragmentation mass spectrum of **4′** is very similar to that of **4**, with major daughter ions at $m/z = 214$ and 124. In addition, the ^{1}H NMR chemical shifts of the H6 and methyl group of **4′** (7.82 and 4.22 ppm, respectively) are very similar to those of **4** (7.75 and 4.23 ppm, respectively). These spectroscopic similarities strongly suggest the presence of a 5-(hydroxymethyl)-uracil residue in **4′**, like in **4**.

The main difference in the ^{1}H NMR features of **4** and **4′** was found for their 2-deoxyribose moiety (Table 1). A downfield shift is observed for H3′ and H4′ of compound **4′**, with respect to those of **4**. In addition, the signals of H2′ and H2″ are observed at 2.05 and 2.60 ppm for **4′** in contrast to 2.25 and 2.27 ppm for **4**. These observations suggest that the 2-deoxyribose unit of compound **4′** is not in the usual β-furanosyl configuration. A pyranosyl structure for the 2-deoxyribose moiety of **4′** is ruled out, because these isomers exhibit chemical shifts around 5.5 and below 4 ppm for H1′ and H4′, respectively.[8] Characterization of the osidic moiety of **4′** was carried out by analysis of the coupling constants of the proton of the 2-deoxyribose unit. In particular, the $J_{1'-2''}$, $J_{2''-3'}$, and $J_{3'-4'}$ coupling constants are almost equal, which has previously been shown to be accounted for by the trans position of H2″ with respect to H1′ and H3′ in the α anomer of nucleosides.[8a,9] Moreover, the low value of the latter coupling constants strongly suggests that the 2-deoxyribose ring is in a $C2'_{endo}$ puckered conformation, with the base in an anti position along the *N*-glycosidic bond.[9] The latter feature is in agreement with the presence of a bulky substituent on the methyl group rather than at the C5 or C6 positions. These observations, together with the relatively large value of the $J_{1'-2'}$ coupling constant (7.2 Hz), provide definitive evidence that **4′** is the α-anomer of 5-HMdUrd.

Characterization and Decomposition of the Primary Photolysis Products. As mentioned above, photolysis of **2** yields primary photoproducts **3** and **3′** that are then converted into **4** and **4′** in aqueous solution. Interestingly, the four latter compounds exhibit the same molecular weight of 258, as determined by HPLC−MS. However, major differences were observed on the fragmentation mass spectra recorded in the negative mode. Indeed, the same major daughter ion is observed at $m/z = 98$ for **3** and **3′** (Figure 3), while signals at $m/z = 214$ and 124 are observed for **4** and **4′**. Further characterization of **3** and **3′** was first achieved by HPLC separation associated with a UV diode array detection. A single maximum at 210 nm is observed in the UV absorption spectra of both compounds. This strongly suggests a loss of aromaticity of the thymine moiety, likely due to the saturation of C5−C6 double bond.

(7) Decarroz, C.; Wagner, J. R.; van Lier, J. E.; Murali, K. C.; Riesz, P.; Cadet, J. *Int. J. Radiat. Biol.* **1986**, *50*, 491−505.

(8) (a) Berger, M.; Cadet, J. *Z. Naturforsch.* **1985**, *40b*, 1519−1531. (b) Douki, T.; Voituriez, L.; Cadet, J. *Chem. Res. Toxicol.* **1995**, *8*, 244−253. (c) Greenberg, M. M.; Hantosi, Z.; Wieder, C. J.; Rithner, C. D. *Biochemistry* **2001**, *40*, 15856−15861

(9) Cadet, J.; Taïeb, C.; Remin, M.; Niemczura, W. P.; Hruska, F. E. *Biochim. Biophys. Acta* **1980**, *608*, 435−445.

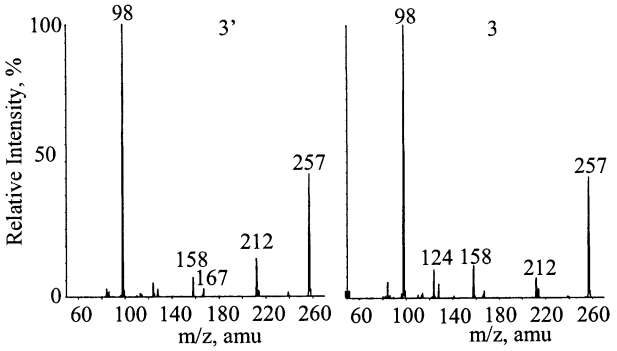
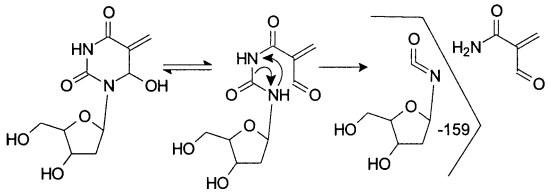


FIGURE 3. Fragmentation mass spectra of **4** and **4′** recorded by HPLC-MS/MS analysis in the negative mode. The daughter ion ($[M - H]^-$) was set at $m/z = 257$.

Attempts were made to isolate **3** and **3′** by HPLC and characterize them by ^{1}H NMR. Unfortunately, the compounds are not stable during the lyophilization step aimed at removing the HPLC buffer. The same observation was made following rotary evaporation under vacuum. Interestingly, **3** and **3′** were found to yield **4** and **4′**, respectively. Similar results were obtained when HPLC fractions containing either **3** or **3′** were left overnight at room temperature. A more quantitative study of the hydrolysis of **3** and **3′** into **4** and **4′** was carried out on the basis of HPLC−MS/MS analyses. Indeed, **3**, **3′**, **4**, and **4′** were closely eluted from the reverse phase HPLC column. This would have hampered the quantification of the products by using UV detection. Therefore, the differences in fragmentation pattern of the compounds were used to set up a multiple reaction monitoring method. **3** and **3′** were quantified by detecting the 257 → 98 transition. The rate constant for the disappearance of **3** and **3′** was thus determined to be 5×10^{-4} min^{-1} for both compounds. The response of the MS/MS detector, set on the 257 → 124 transition, for **4** and **4′** was calibrated with authentic 5-HMdUrd. It was thus possible to calculate the formation rate constant of the two latter compounds in the same samples rather than the decomposition of **3** and **3′**. Both **4** and **4′** were produced with the same rate that was found to be 6×10^{-4} min^{-1}. The good agreement between these rate constants was additional evidence for the conversion of **3** and **3′** into **4** and **4′**.

The impossibility of recording ^{1}H NMR spectra of HPLC-purified **3** and **3′** was partly overcome by carrying out synthesis and photolysis of bromohydrins in deuterated water. The high thymidine concentration used (41 mM) made difficult the precise control of the pH. As a result, **2** was partly converted into thymidine glycols (≈50%). However, 200 MHz ^{1}H NMR analysis of the crude UVC-irradiated reaction mixture unambiguously showed the presence of a product (yield ≈40%), exhibiting signals corresponding to two conjugated protons ($J = 8.5$ Hz) at chemical shifts of 5.87 and 5.63 ppm. The latter values are distinct from those reported for the H6 of bromohydrins and glycols[10] and correspond to ethylenic protons exhibiting a geminate coupling. Keeping in mind that the C5−C6 double of **3** and **3′** is saturated, a structure exhibiting an exocyclic double bond at the C5 position may be proposed for their base moiety. The molecular mass of 258 Da also indicates the presence of a hydroxyl group. A likely position is at C6, like in the starting bromohydrins. The resulting structure, characterized by the presence of a methide function, would also account for the observed fragmentation mass spectrum, which exhibits a daughter ion arising from the loss of a 159 Da fragment (Scheme 1). A similar rearrangement has been previously reported for the diastereoisomers of 5,6-dihydroxy-5,6-dihydro-thymidine[6] that also exhibit a saturated C5−C6 bond together with a hydroxyl group at the 6 position. The great similarities of their spectroscopic properties indicate that the difference in the chemical structure of **3** and **3′** involves their 2-deoxyribose ring rather than their base moiety. The respective conversion of the two latter products into **4** and **4′** strongly suggests that **3** and **3′** are the β and α anomers, respectively, of the thymidine methide.

SCHEME 1: Proposed Pathway for the Collision-Induced Fragmentation of Compounds 3 and 3′



Decomposition of the Thymidine Methide in Alcohols. The ability of the primary photolysis products of **2** to undergo nucleophilic addition was then investigated. Indeed, the latter feature is a characteristic of the reactivity methide derivatives and may thus be used as an additional evidence for the proposed structure. For this purpose, compounds **3** and **3′** were prepared as mentioned above and diluted 10 times in either methanol, ethanol, 1-propanol, or 2-propanol. The samples were left at room temperature for 24 h and then analyzed by HPLC−MS and HPLC−MS/MS. In the four cases, major products correspond to either methyl-, ethyl-, or propyl-substituted 5-HMdUrd, as inferred from their molecular weight found by HPLC−MS to be 272, 286, 300, and 300, respectively. The presence of alkyl substituents of increasing hydrophobicity on a hydroxymethyl group is also shown by the variation in the retention time on the reverse phase HPLC system (11.0, 13.4, 15.6, 18.1, and 18.9 min for the decomposition product of **3** in water, methanol, ethanol, 2-propanol, and 1-propanol, respectively). Further structural evidence was inferred from the analysis of the fragmentation mass spectra (Figure 4 and Table 2). A major ion for 5-HMdUrd as well as for the alcohol degradation products of **3** and **3′** is observed at $[M - H - 43]^-$ as the result of ring opening and fragmentation. An additional major pathway for the alkylated derivatives is the loss of the 2-deoxyribose unit ($[M - H - 116]^-$). Interestingly, the major loss of a neutral fragment of 133 Da observed upon fragmentation of 5-HMdUrd is not observed for the decomposition products of thymidine methide in alcohols. This fragmentation pathway corresponds to the loss, from the ring opened form of 5-HMdUrd, of the 2-deoxyribose ring

(10) Cadet, J.; Ducolomb, R.; Hruska, F. E. *Biochim. Biophys. Acta* **1979**, *563*, 206−215.

TABLE 2. HPLC−MS/MS Features of the Products Obtained upon Decomposition of 3 and 3′ in Alcohols[a]

alcohol	R_t (min)	$[M - H]^-$	daughter 1	daughter 2	daughter 3	daughter 4
methanol	13.4	271 (100%)	228 (9%)	209 (5%)	196 (5%)	155 (14%)
ethanol	15.6	284 (100%)	241 (5%)	196 (5%)	169 (28%)	
1-propanol	18.9	299 (100%)	256 (5%)	209 (5%)	196 (7%)	183 (17%)
2-propanol	18.1	299 (100%)	256 (5%)	209 (8%)	196 (5%)	183 (12%)

[a] The retention time together with the mass-to-charge ratio (*m*/*z*) of the main daughter ions is listed. The values in parentheses represent the relative intensity of the signals in the mass spectra.

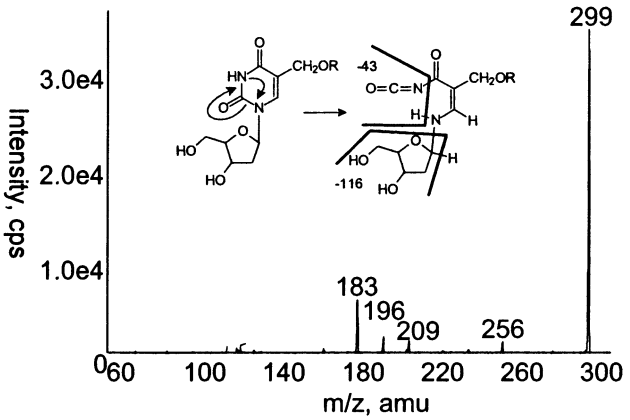


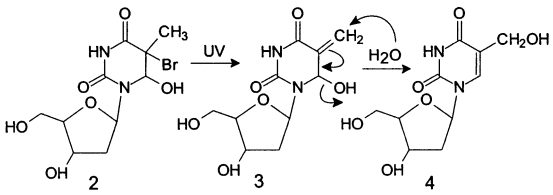
FIGURE 4. Fragmentation mass spectrum of the decomposition product of the thymidine methide in 1-propanol.

carrying the N1 nitrogen as an amino group. This rearrangement is only possible when labile hydrogen atoms, like the allylic hydroxyl group of **4** and **4′**, are available. The observation that this fragmentation does not occur in the alcohol decomposition products of **3** and **3′** provides further evidence that they exhibit an 5-(alkoxymethyl)-uracil moiety.

Discussion

The present results show that photolysis of thymidine bromohydrins, a thymidine derivative modified on the C5−C6 double bond, leads to the formation of 5-(hydroxymethyl)-2′-deoxyuridine, a methyl oxidation product. This is rather unexpected, since the best documented mechanism of formation of 5-HMdUrd involves the initial generation of the 5-peroxymethyl radical of thymidine.[1b] However, the latter intermediate was not detected by HPLC analysis of the bromohydrins photolysis mixture. In addition, 5-formyl-2′-deoxyuridine, the main decomposition product of the latter peroxide,[7] was not detected in significant amount (data not shown). The identification of a methide derivative as primary product of the photolysis of thymidine bromohydrins shed some light on these observations. Indeed, methide substituents are highly electrophilic groups described as intermediates in a series of enzymatic metabolic processes.[11] Interestingly, quinone methide derivatives have been found to be DNA alkylating agents.[12]

SCHEME 2: Formation of 5-HMdUrd upon Photolysis of Thymidine Bromohydrins



Therefore, it might be proposed that 5-HMdUrd arises from the nucleophilic addition of water to the exocyclic double bond of the thymidine methide (Scheme 2). The reaction of **3** and **3′** with alcohols, which yields alkoxymethyl derivatives of 5-HMdUrd, provides further support for this proposal. Interestingly, formation of a methide derivative of thymidine has previously been proposed, even though the latter transient was not characterized, to account for the hydrolytic conversion of addition products of 2,2,6,6-tetramethyl-1,4-piperidone-*N*-oxyl (TAN) to the 6-hydroxy-5,6-dihydrothymidine-5-yl radical.[13] In the trans diastereoisomers of the latter adduct, a Cope rearrangement was proposed to yield the methide derivative presently identified. Water and hydrogen peroxide were found to add to the latter transient yielding 5-HMdUrd and 5-(hydroperoxymethyl)-2′-deoxyuridine, respectively.

The exact photochemical process leading to the formation of the thymidine methide remains to be elucidated. However, a radical reaction triggered by homolytic cleavage of the C5−Br bond can be ruled out. Indeed, the resulting carbon-centered radical rapidly reacts with oxygen to yield a 6-hydroxy-5-peroxy-5,6-dihydro pyrimidine radical. The latter transient has been extensively studied following exposure of thymidine to ionizing radiation and was found to mostly lead to the formation of thymidine glycols together with ring rearrangement and fragmentation products.[1b] It is therefore likely that the initial step of the presently reported photochemical reaction involves formation of a thymine cation and a bromide ion. The formation of the methide cannot then be simply explained by the deprotonation of the methyl group. Indeed, anomerization was also found to take place during the photochemical step. This is shown by the quantitative conversion of **3** into **4** and **3′** into **4′**, without conversion of **3** into **3′**. This shows that the thymidine methide itself does not undergo anomerization and that the isomerization of the osidic moiety occurs during the formation of the latter compound.

Altogether, it may be concluded that the photolysis of thymidine bromohydrins produces, in high yield, a methide intermediate. The latter transient might be attacked by nucleophilic reagents, as shown by the present

(11) (a) Iverson, S. L.; Shen, L.; Anlar, N.; Bolton, J. L. *Chem. Res. Toxicol.* **1996**, *9*, 492−499. (b) Bolton, J. L.; Sevestre, H.; Ibe, B. O.; Thompson, J. A. *Chem. Res. Toxicol.* **1990**, *3*, 65−70. (c) Guyton, K. Z.; Thompson, J. A.; Kensler, T. W. *Chem. Res. Toxicol.* **1993**, *6*, 731−738.

(12) (a) Chatterjee, M.; Rokita, S. E. *J. Am. Chem. Soc.* **1994**, *116*, 1690−1697. (b) Lewis, M. A.; Graff Yoerg, D.; Bolton, J. L.; Thompson, J. A. *Chem. Res. Toxicol.* **1996**, *9*, 1368−1374. (c) Veldhuyzen, W. F.; Shallop, A. J.; Jones, R. A.; Rokita, S. E. *J. Am. Chem. Soc.* **2001**, *123*, 11126−11132.

(13) Berger, M.; Cadet, J.; Ulrich, J. *Can. J. Chem.* **1984**, *62*, 6−14.

results with a series of alcohols. Even though applications to other nucleophiles might be hampered by the required aqueous conditions and the anomerization, this photoreaction represents a new synthetic route toward methyl-substituted derivatives of thymine.

Experimental Section

HPLC Analyses. HPLC−UV analyses were performed on an Uptisphere ODB column (4 × 250 mm, 5 μm particle size) connected to a Waters 990 diode array detector. UV spectra were recorded at wavelengths ranging between 190 and 300 nm. A gradient of acetonitrile in a 10 mM aqueous solution of ammonium formate, provided by a pump, was used at a flow rate of 1 mL/min. The proportion of organic phase increased from 0 to 20% within 15 min. The latter composition was then maintained for 45 min. For purification purposes, the HPLC system was used with a UV spectrophotometer set at 230 nm. Samples were also analyzed by use of an HPLC system coupled to mass spectrometry. An Uptisphere ODB column (2 × 150 mm, particle size 5 μm) was used with a gradient of acetonitrile in a 2 mM aqueous solution of ammonium formate (flow rate 200 μL/min). The amount of acetonitrile was raised from 0 to 20% within 15 min, and the latter proportion was kept constant for another 15 min. The output of the column was connected to a UV detector. The eluent was then mixed with methanol (200 μL/min) and directed toward a triple quadrupole mass spectrometer used with a turbo-ionspray source. Samples were analyzed either by single mass spectrometry (mass range 50−350) or in the product ion scan mode. In the latter experiments, specific pseudomolecular ions were selected and then fragmented. The resulting ions were analyzed (mass range 50−350). Analyses were performed in the negative mode.

Synthesis and Photolysis of (5*R*,6*R*)- and (5*S*,6*S*)-5-Bromo-6-hydroxy-5,6-dihydrothymidine. Thymidine (10 mg) was solubilized in 15 mL of 10 mM aqueous phosphate buffer. Bromine (7 mg in solution in 1 mL of water) was added dropwise to the resulting mixture under magnetic stirring on an ice bath. The pH of the solution was controlled and kept above 5 by addition of 0.1 M sodium hydroxide when necessary. The reaction mixture was analyzed by HPLC−UV to check the completion of the reaction. When thymidine was totally consumed, air was bubbled for 15 min to remove traces of excess bromine. The crude reaction mixture was then exposed, on an ice bath and under magnetic stirring, to the UVC radiation (2 kJ m^{-2} min^{-1} mostly at 254 nm) emitted by a 2 × 15 W germicidal lamp. The pH was maintained above 5 by addition of sodium hydroxide. Completion of the reaction was controlled by HPLC−UV.

Characterization of the Bromohydrins Photolysis Products. The UV absorption spectra of the two bromohydrins photolysis products were recorded by using HPLC with diode array detection. The mass and fragmentation spectra were obtained by HPLC-mass spectrometry analysis. Partial ^{1}H NMR characterization of the thymidine methide was achieved by preparing a mixture of 10 mg of thymidine and 10 μL of 1 M pH 7 phosphate buffer in 1 mL of 99.9% D_2O. The resulting solution was freeze-dried and further solubilized in 1 mL of 99.9% D_2O. While the mixture was stirred on an ice bath, bromine (7 mg in suspension in 100 μL of 99.9% D_2O) was added. The pH of the solution was set at 5 by addition of 0.1 M NaOD in 99.9% D_2O. Excess bromine was removed by air bubbling. The resulting mixture was irradiated for 30 min in a watch-glass under stirring at 0 °C. The ^{1}H NMR spectrum of the obtained solution was then recorded on a spectrometer. The HDO signal was used as secondary reference and set at 4.9 ppm.

Characterization of the Thymidine Methide Decomposition Products. Products **4** and **4′**, corresponding to the two main peaks observed on the UV chromatogram of the irradiated bromohydrins solution left overnight at 37 °C, were collected separately. The samples were then freeze-dried, and the obtained residue was solubilized in 99.9% D_2O. The resulting solutions were evaporated under vacuum. The samples were then solubilized in 99.96% D_2O prior to 400 MHz ^{1}H NMR analysis carried out on a spectrometer. The HDO signal was used as secondary reference and set at 4.9 ppm. UV absorption, mass, and fragmentation spectra were obtained as described above. The decomposition rate constant of thymidine methide was determined by injecting the crude photolysis mixture at increasing periods of time after irradiation on the HPLC−MS/MS system used in the multiple reaction monitoring mode.

Decomposition of Thymidine Methide in Alcohols. Thymidine methide was prepared as described above from 25 mL of a 1 mM aqueous solution of thymidine. Following bromination and photolysis, aliquot fractions of 5 mL were mixed with 45 mL of either water, methanol, ethanol, 1-propanol, or 2-propanol. Samples were left overnight at room temperature and then evaporated to dryness. The resulting residue was solubilized in 5 mL of water. The obtained solutions were analyzed by HPLC−MS/MS in the product ion scan mode. Fragmentation of deprotonated pseudomolecular ions at m/z = 257, 271, 284, and 299 was monitored. The sample diluted in water yielded **4** and **4′** as the main products. For each of the samples diluted in alcohol, one major peak was observed on the chromatogram.

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