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A Minute Amount of S-Puckered Sugars Is Sufficient for (6-4) Photoproduct Formation at the Dinucleotide Level

Céline Moriou,[†] Clément Denhez,[‡] Oleksandr Plashkevych,[§] Stéphanie Coantic-Castex,[‡] Jyoti Chattopadhyaya,[§] Dominique Guillaume,[‡] and Pascale Clivio*[,‡]

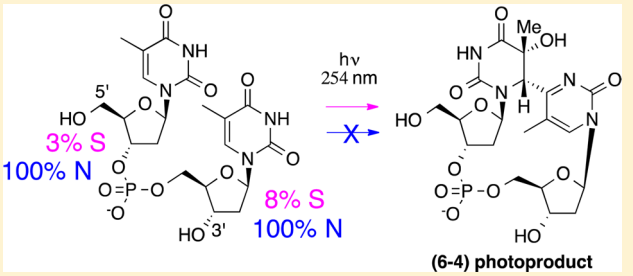
[†]Institut de Chimie des Substances Naturelles, CNRS, 91198 Gif-sur-Yvette Cedex, France

[‡]Université de Reims Champagne Ardenne, Institut de Chimie Moléculaire de Reims, CNRS UMR 7312, UFR de Pharmacie, 51 rue Cognacq-Jay, 51096 Reims Cedex, France

[§]Institute of Cell & Molecular Biology, Program of Chemical Biology, Box 581, Biomedical Center, University of Uppsala, S-75123, Uppsala, Sweden

Supporting Information

ABSTRACT: The di-2′-α-fluoro analogue of thymidylyl(3′,5′)-thymidine, synthesized to probe the effect of a minimum amount of S conformer on the photoreactivity of dinucleotides, is endowed with only 3% and 8% of S sugar conformation at its 5′- and 3′-end, respectively. This analogue gives rise to the (6-4) photoproduct as efficiently as the dithymine dinucleotide (74% and 66% at the 5′- and 3′-end, respectively) under 254 nm. Our results suggest that the 5′-N, 3′-S conformer gives rise to the (6-4) photoproduct.



Exposure of DNA to UV light results mostly in chemical modifications at adjacent pyrimidine sites. In cells, such modifications can be repaired or lead to senescence, death, or mutation.[1] Therefore, DNA photoproducts play a considerable role in human health through their involvement in skin cancer.[2] Cyclobutane pyrimidine dimers (CPDs) and (6-4) photoproducts ((6-4) PPs) represent the two major classes of DNA photoproducts (Scheme 1).[3] Although CPDs have been found to be the most deleterious PPs in repair-proficient cells,[4] (6-4) PPs would be considerably more dangerous in repair-deficient or repair-capacity-saturated cells.[5] Therefore, identification of the processes leading to (6-4) PP, including DNA conformational parameters, is of utmost importance. The dithymine dinucleotide TpT (**1**) is a good model to study DNA photoreactivity. As such, we currently dissect its photoreactivity as well as that of conformationally restricted analogues.[6−10]

Sugar conformation is one of the key factors governing global conformation of nucleic acids even at the dinucleotide level. In aqueous solution, the sugar pucker of nucleos(t)ides exists as an equilibrium mixture whose extreme forms are the North (N) and South (S) conformers.[11] Substitution at the nucleos(t)ide 2′-position dramatically influences this equilibrium.[12,13] Photochemical investigation of T_LpT_L, the locked TpT analogue, has evidenced that exclusive N conformation at each dinucleotide end precludes (6-4) PP formation, CPD being the sole PP formed.[10] This strongly suggests the importance of S-type sugar conformation to promote relevant intramolecular thymine moieties overlap geometry for (6-4) PP to form. Therefore, we decided to determine the minimum amount of S conformer necessary to get (6-4) PPs. To study this hypothesis, we designed a modified dithymine dinucleotide not locked as T_LpT_L[10] but whose expected N conformer population would nevertheless approach 100% as closely as possible at each end. 2′-α-Fluorosubstitution of pyrimidine nucleosides is known to drive the N population of the sugar conformation over 85%.[12b,c] Therefore, we synthesized the dinucleotide analogue of TpT containing a fluorine atom at the 2′-α-position of each sugar residue (2′-α-fluorothymidylyl-(3′,5′)-2′-α-fluorothymidine, $T_{\alpha F}pT_{\alpha F}$, **2**) to study its conformation and photoreactivity. Dinucleotide **2** was prepared from 2′-α-fluorothymidine following the procedure of Ikeda et al.[14] and Williams et al.[15] (Scheme 2). In brief, dimethoxytritylation of 2′-α-fluorothymidine **7**[14] provided **8** in quantitative yield.[14] 3′-Acetyl-2′-α-fluorothymidine **9** was prepared through a one-pot acetylation/detritylation procedure from **8** in 60% yield. Modified nucleoside **8** also afforded known phosphoramidite **10**,[14,15] which was then condensed with alcohol **9**. Condensation was performed in acetonitrile and in the presence of 5-ethylthiotetrazole as activating reagent, instead of the more common 1*H*-tetrazole, due to the known lower reactivity of 2′-fluorophosphoramidites compared to their unsubstituted counterparts.[16] The corresponding phosphite triester intermediate was subsequently oxidized in the presence of I_2 to give intermediate **11** in 60% (2 steps). Deprotection of **11**, performed using concentrated aqueous NH_4OH and then 80% aqueous acetic acid, afforded **2** in 73% yield (2 steps).

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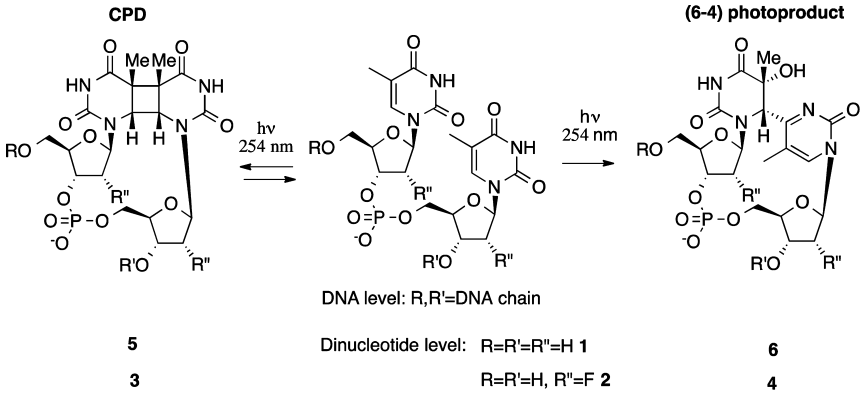
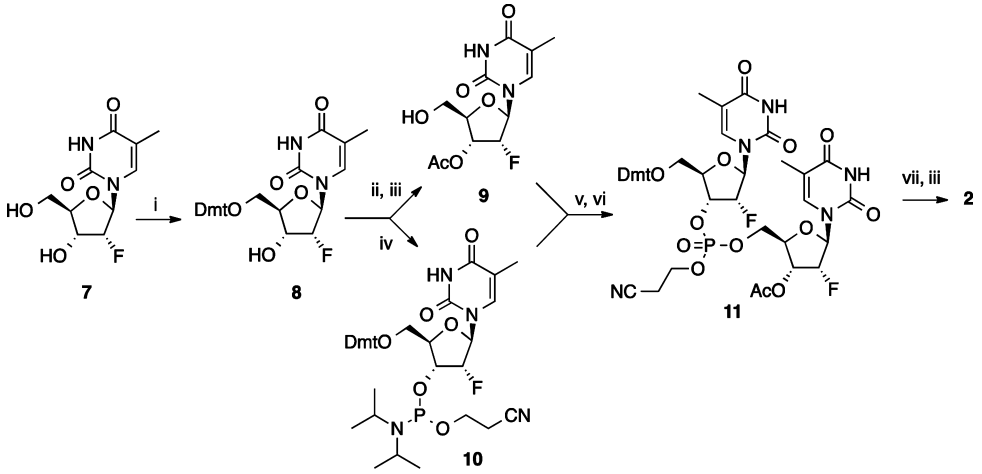
Scheme 1. Photochemical Behavior of a Dithymine Site in DNA and of TpT (1) and $T_{\alpha F}pT_{\alpha F}$ (2)



Scheme 2. Synthesis of 2[a]



[a]Conditions: (i) DmtCl, pyridine; (ii) Ac_2O, pyridine; (iii) 80% aqueous AcOH; (iv) $NC(CH_2)_2OP$-(Cl)$N(i\text{-}Pr)_2$, $(i\text{-}Pr)_2EtN$, CH_2Cl_2; (v) 5-ethylthiotetrazole, CH_3CN; (vi) I_2, THF/H_2O/2,6-lutidine; (vii) conc. aqueous NH_4OH.

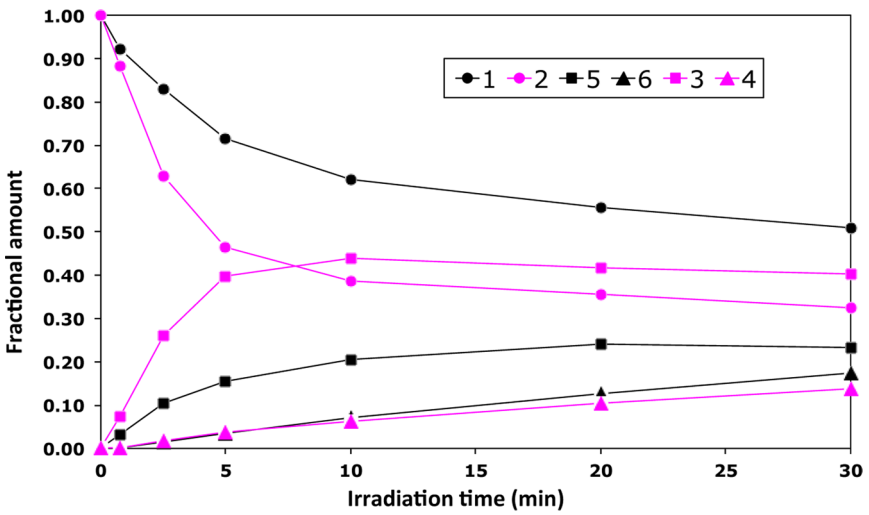


Figure 1. Fractional amount of the $T_{\alpha F}pT_{\alpha F}$ (2), TpT (1), and of their respective photoproducts as a function of irradiation time.

Delightfully, the NMR-based PSEUROT+J_{HF}[12c] analysis of **2** indicated that, in solution, both sugar residues are highly predominantly, but not exclusively, in the N conformational domain. North conformer population was calculated to be 97% and 92% at the 5′- (P_N = 22.8°) and 3′- (P_N= 22.5°) end, respectively (Supporting Information). Exposure of an aqueous solution of **2** to 254 nm light afforded the c,s CPD (**3**) and the (6-4) PP (**4**) (Supporting Information). The mass spectra of **3** and **4** displayed a quasi-molecular ion peak at m/z 581 ($(M - H)^-$). The presence of two proton singlets at δ 7.88 and 5.12 and two methyl protons at δ 2.30 and 1.79 in the ^1H NMR spectrum of **4** was the signature of a (6-4) adduct while the presence in the ^1H

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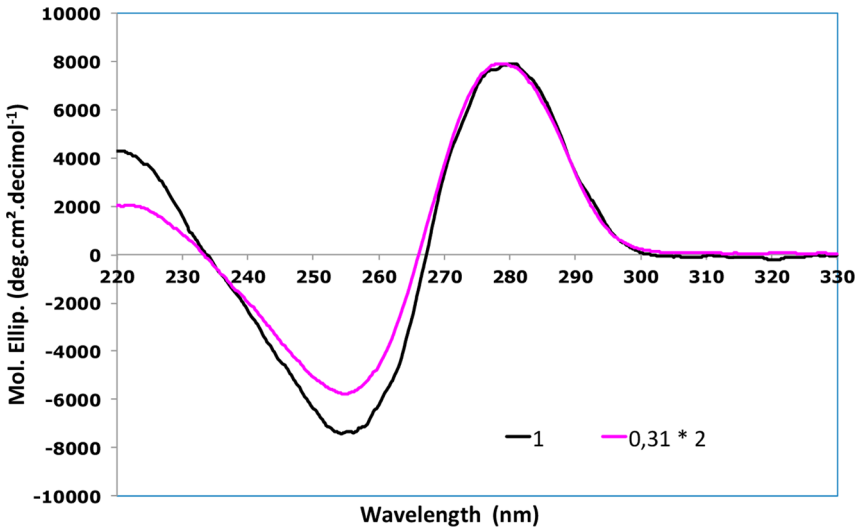

Figure 2. CD difference spectra at 20 °C of **1** and of **2** normalized with respect to the positive band amplitude of **1**.

NMR spectrum of **3** of two shielded methyl signals (δ 1.52 and 1.43) attested the presence of a CPD.[17,18] UV absorption of **4** at λ_{max} = 328 nm and lack of a UV absorption maximum above 240 nm for **3** further confirmed the assigned structures. The cis−syn (c,s) stereochemistry of the cyclobutane **3** was deduced from nOes between H6 $T_{\alpha F}$p- and H3′ $T_{\alpha F}$p-; H6 $T_{\alpha F}$p- and CH_3 $T_{\alpha F}$p-; H6 $T_{\alpha F}$p- and CH_3 -p$T_{\alpha F}$; CH_3 $T_{\alpha F}$p- and H6 -p$T_{\alpha F}$; H6 -p$T_{\alpha F}$ and H2′ -p$T_{\alpha F}$; H6 -p$T_{\alpha F}$ and CH_3 -p$T_{\alpha F}$. For the (6-4) PP **4**, observed nOes established the C5 (*R*) and C6 (*S*) stereochemistry (H6 $T_{\alpha F}$p- and H3′ $T_{\alpha F}$p-; H6 $T_{\alpha F}$p- and H2′ $T_{\alpha F}$p-; H6 $T_{\alpha F}$p- and CH_3 $T_{\alpha F}$p-; H6 $T_{\alpha F}$p- and CH_3 -p$T_{\alpha F}$; H6 -p$T_{\alpha F}$ and CH_3 -p$T_{\alpha F}$; H6 -p$T_{\alpha F}$ and H2′ -p$T_{\alpha F}$; H6 -p$T_{\alpha F}$ and H3′ -p$T_{\alpha F}$). Thus, **3** and **4** arise from cycloaddition reactions between the two thymine bases of **2** in an *anti* glycosidic bond conformation as in the case of **1**.

Photochemical reaction kinetics and quantum yield (Φ) of PP formation, from **2** with respect to **1**, were determined (Supporting Information) as previously reported.[9] Kinetic studies revealed a similar initial rate of formation of (6-4) PPs **4** and **6** obtained from UV exposure of **2** and **1**, respectively (Figure 1). The higher rate of disappearance of **2**, with respect to **1**, was nicely correlated with a higher initial rate of formation of CPD **3** compared to that of TpT-derived CPD **5** (Figure 1). While Φ of CPD formation from **2** ($(2.7 \pm 0.3) \times 10^{-2}$) was estimated to be *ca.* twice higher than that from **1** ($(1.1 \pm 0.05) \times 10^{-2}$),[19] Φ of (6-4) PP formation from **2** ($(0.13 \pm 0.02) \times 10^{-2}$) was surprisingly found to be quite similar to that from TpT **1** ($(0.10 \pm 0.05) \times 10^{-2}$),[19] whose S conformer population is 74% and 66% at its 5′- and 3′-end, respectively.[20]

Conformers in solution are in an equilibrium mixture of intramolecular stacked and unstacked species, among which only intramolecular stacked species can yield photoreaction products. Thus, we investigated the stacking properties of **2** by circular dichroism (Supporting Information). The CD difference spectrum of **2** and **1** is presented Figure 2.

The similar shape of the CD difference spectrum of **2** and **1** indicated that the average intramolecular base stacked population adopted by **2** and **1** is similar in terms of geometry (Figure 2).[21] However, the *ca.* 3-fold increase in molecular ellipticity at λ_{max} 278 nm supported the idea that $T_{\alpha F}pT_{\alpha F}$ (**2**) is 3 times more stacked than TpT.[21] Therefore, the increased formation efficiency of CPD **3**, compared to **5**, may be correlated with the elevated degree of stacking, associated with a favorable geometry, of its precursor (**2**), compared to TpT. This hypothesis is in line with the involvement of the initial ground-state geometry of the two reacting thymine residues in CPD formation.[22] In contrast, since Φ of (6-4) PP from **2** is similar to that from **1** and since **2** is 3 times more stacked than **1**, formation of (6-4) PP **4** is not linearly correlated with the stacking level of **2**. If the population of the S conformer is the rate-limiting step in (6-4) PP formation, this could indicate that (6-4) PP arises either from non-CD detectable ground state conformations of minor favorably stacked species[9,10] or from species whose geometry is different from the one of the ground state. If the population of the S conformer is not the rate-limiting step in (6-4) PP formation, this study supports the idea that other factors such as excited state geometries/properties govern the formation of (6-4) PP.[23−25]

In conclusion, whereas T_LpT_L is incompetent to afford (6-4) PP, $T_{\alpha F}pT_{\alpha F}$ **2**, whose S sugar conformation is 3% and 8% at the 5′- and 3′-end, respectively, can provide (6-4) PP as efficiently as TpT whose S conformer is 74% and 66% at the 5′- and 3′-end, respectively.[20] For **2**, such sugar conformational population represents dinucleotide-population conformer probabilities of NN (89.9%) population in equilibrium with 7.6, 2.3, and 0.2% of NS, SN, and SS species, respectively. Considered alone, the amount of S conformers cannot be used to anticipate the capacity of a dinucleotide to form (6-4) PP. However, our results clearly indicate that, at the dinucleotide level, conformational changes induced by the presence of S-puckered sugars in amounts as minute as 3% and 8% at the 5′- and 3′-end, respectively, are sufficient to allow (6-4) PP formation. Since an S sugar conformation at the 5′-end of a dinucleotide is known to be detrimental to intramolecular stacking[8a,9] and that only stacked species can afford PPs, this strongly supports the idea that an S conformer at the 3′-end is essential for (6-4) PP formation. The (6-4) photoproduct would, therefore, result from NS dinucleotide conformers.

EXPERIMENTAL SECTION

General Remarks. Solvents and chemicals used for the reactions were purchased from commercial suppliers. Dichloromethane, acetonitrile, *N*,*N*-diisopropylethylamine (DIPEA), ethyl acetate (AcOEt), and heptane were dried by distillation from calcium hydride. Pyridine and triethylamine (TEA) were dried by distillation from KOH and kept over KOH. TLC was performed on silica gel plates (Kieselgel 60, F_{254}) with

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detection by UV and visualization by spraying with a methanolic solution of sulfuric acid. Chromatography was performed on silica gel 60, particle size 35−70 μm, unless otherwise stated. Medium pressure reverse-phase chromatography was performed on a LiChroprep RP 18. ^{1}H NMR and ^{13}C NMR spectra were recorded on 300 or 500 or 600 MHz spectrometers. Observed chemical shift (δ) values are given in ppm and coupling constants (J) in Hz. The following abbreviations are used: s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublet), T$_{\alpha F}$p- and -pT$_{\alpha F}$ represent the 5′-end and the 3′-end nucleoside residues, respectively, of **2**−**4**. ^{1}H NMR and ^{13}C NMR chemical shifts were calibrated using residual solvent signals at the following values: CD_3OD δ_H 3.31 and δ_C 49.15, D_2O δ_H 4.80. For $CDCl_3$, δ_H and δ_C are reported from internal TMS (δ 0.00). ^{13}C NMR spectra recorded in D_2O were calibrated from dioxane (δ_C 67.8 ppm). ^{31}P NMR and ^{19}F NMR chemical shifts were reported from an external capillary standard of 85% phosphoric acid (δ_P 0.00 ppm) of TFA (δ_F −77.00 ppm), respectively. Superscript a and b labels denote interchangeable assignments within a compound. High-resolution mass spectra (HRMS) were determined on a liquid chromatography time-of-flight (LCT) spectrometer. Electron spray ionization mass spectrometry (ESI-MS) was measured on an LC/MS mass spectrometer.

3′-*O*-Acetyl-2′-α-fluorothymidine (9). To a solution of 5′-*O*-dimethoxytrityl-2′-α-fluorothymidine **8**[14] (0.216 g, 0.38 mmol) in dry pyridine (5.3 mL) was added Ac_2O (143 μL, 1.52 mmol). The mixture was stirred for 24 h at room temperature, poured onto ice, and extracted with AcOEt (2 × 20 mL). The organic layer was dried over anhydrous Na_2SO_4, and evaporated to give crude 3′-*O*-acetyl-5′-*O*-dimethoxytrityl-2′-α-fluorothymidine. The crude product was taken in 80% aqueous AcOH (19 mL) and stirred for 4 h at room temperature. The mixture was evaporated and the residual oil was purified by two successive flash silica gel chromatographies (30−100% AcOEt in heptane and 0−10% CH_3OH in CH_2Cl_2) to give **9** (0.070 g, 60%) as a white foam. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.53 (1H, s), 5.93 (1H, dd, J = 3.4, 16.8 Hz), 5.25 (1H, ddd, J = 3.4, 5.0, 52.8 Hz), 5.25 (1H, ddd, J = 5.0, 6.2, 12.2 Hz), 4.18 (1H, m), 3.89 (1H, J = 2.0, 12.5 Hz), 3.70 (1H, dd, J = 2.4, 12.5 Hz), 2.11 (3H, s), 1.85 (3H, s). ^{13}C NMR (62.5 MHz, $CDCl_3$): 170.4, 164.1, 150.5, 137.5, 111.7, 90.8 (d, J = 193.1 Hz), 90.7 (d, J = 33.7 Hz), 82.0, 69.7 (d, J = 14.8 Hz), 60.9, 20.7, 12.5. ^{19}F NMR (282 MHz, $CDCl_3$): δ −201.86. HRMS (ESI$^+$ mode) (M + Na)$^+$: calcd. for $C_{12}H_{15}O_6N_2FNa$ 325.0812, found 325.0813.

***P*-Cyanoethyl-5′-*O*-dimethoxytrityl-2′-α-fluorothymidylyl-(3′,5′)-3′-*O*-acetyl-2′-α-fluorothymidine (11).** Compounds **9**, **10**,[14,15] and 5-ethylthiotetrazole were dried over P_2O_5 under vacuum overnight. To a solution of **10** (0.293 g, 0.38 mmol) in anhydrous CH_3CN under argon at room temperature, **9** (0.097 g, 0.32 mmol) and 5-ethylthiotetrazole (0.138 g, 1.06 mmol) were added. The reaction was stirred for 30 min. A 0.2 M iodine solution (0.145 g, 0.57 mmol) in THF/H_2O/2,6-lutidine (50:25:25, v/v/v) (2.9 mL) was added to the reaction, and the mixture was stirred for 25 min. A saturated sodium thiosulfate solution was added until the solution became colorless. The mixture was diluted with CH_2Cl_2 and washed with water. The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated. The residue was purified by flash silica gel chromatography (50−100% AcOEt in heptane) to give **11** (0.185 g, 60%) as a white foam. ^{19}F NMR (282 MHz, $CDCl_3$): δ −195.98, −197.48, −201.54, −202.61. ^{31}P (121 MHz, $CDCl_3$): δ −2.35, −2.68. HRMS (ESI$^+$ mode) (M + Na)$^+$: calcd. for $C_{46}H_{48}O_{15}N_5F_2PNa$ 1002.2750, found 1002.2703.

2′-α-Fluorothymidylyl-(3′,5′)-2′-α-fluorothymidine (2). A solution of **11** (0.170 g, 0.17 mmol) in conc. aqueous NH_4OH (2.3 mL) was stirred overnight at room temperature. The solution was concentrated under reduced pressure. The residue was taken in 80% aq. acetic acid (2.3 mL). After 5 h, the mixture was concentrated in vacuo and the resulting residue was dissolved in water (10 mL). The aqueous solution was washed with CH_2Cl_2 (2 × 10 mL), and concentrated. The crude product was purified on a medium pressure reverse-phase chromatography (0−15% CH_3CN in water). Fractions were analyzed by UV, and those containing **2** were pooled, concentrated, and lyophilized to give 0.073 g of a white solid (73% yield). ^{1}H NMR (600 MHz, D_2O, 27 °C): δ 7.83 (1H, s, H6 T$_{\alpha F}$p-), 7.73 (1H, s, H6 -pT$_{\alpha F}$), 6.16 (1H, d, $J_{1',F}$ = 18.0 Hz, H1′ -pT$_{\alpha F}$), 5.95 (1H, d, $J_{1',F}$ = 17.7 Hz, H1′ T$_{\alpha F}$p-), 5.35 (1H, dd, $J_{2',F}$ = 51.5 Hz, $J_{2',3'}$ = 4.2 Hz, H2′ T$_{\alpha F}$p-), 5.14 (1H, dd, $J_{2',F}$ = 52.2 Hz, $J_{2',3'}$ = 4.2 Hz, H2′ -pT$_{\alpha F}$), 4.66 (1H, dtd, $J_{3',F}$ = 21.2 Hz, $J_{3',P}$ = 8.7 Hz, $J_{3',4'}$ = 8.7 Hz, $J_{3',2'}$ = 4.2 Hz, H3′ T$_{\alpha F}$p-), 4.53 (1H, ddd, $J_{3',F}$ = 23.2 Hz, $J_{3',4'}$ = 8.8 Hz, $J_{3',2'}$ = 4.2 Hz, H3′ -pT$_{\alpha F}$), 4.40 (1H, br d, $J_{5',5''}$ = 12.0 Hz, H5′ -pT$_{\alpha F}$), 4.34 (1H, br d, $J_{4',3'}$ = 8.7 Hz, H4′ T$_{\alpha F}$p-), 4.31 (1H, br d, $J_{4',3'}$ = 8.8 Hz, H4′ -pT$_{\alpha F}$), 4.19 (1H, br d, $J_{5',5''}$ = 12.0 Hz, H5′ -pT$_{\alpha F}$), 4.09 (1H, dd, $J_{5',5''}$ = 13.3 Hz, $J_{5',4'}$ = 1.5 Hz, H5′ T$_{\alpha F}$p-), 3.91 (1H, dd, $J_{5',5''}$ = 13.3 Hz, $J_{5',4'}$ = 3.1 Hz, H5′ T$_{\alpha F}$p-), 1.88 (3H, s, CH_3 T$_{\alpha F}$p-), 1.87 (3H, s, CH_3 -pT$_{\alpha F}$). ^{13}C NMR (75 MHz, D_2O): δ 167.2 (C4 T$_{\alpha F}$p-), 167.0 (C4 -pT$_{\alpha F}$), 152.2 and 152.1 (C2 T$_{\alpha F}$p- and C2 -pT$_{\alpha F}$), 138.0 (C6 T$_{\alpha F}$p-), 137.3 (C6 -pT$_{\alpha F}$), 112.6 (C5 -pT$_{\alpha F}$), 112.1 (C5 T$_{\alpha F}$p-), 94.8 (d, $J_{2',F}$ = 186 Hz, C2′ -pT$_{\alpha F}$), 93.1 (d, $J_{2',F}$ = 188 Hz, C2′ T$_{\alpha F}$p-), 90.0 (d, $J_{1',F}$ = 34 Hz, C1′ T$_{\alpha F}$p-), 89.1 (d, $J_{1',F}$ = 35 Hz, C1′ -pT$_{\alpha F}$), 82.9 (d, $J_{4',P}$ = 8.5 Hz, C4′ T$_{\alpha F}$p-), 81.9 (d, $J_{4',P}$ = 9.8 Hz, C4′ -pT$_{\alpha F}$), 71.2 (dd, $J_{3',F}$ = 16 Hz, $J_{3',P}$ = 5 Hz, C3′ T$_{\alpha F}$p-), 68.3 (d, $J_{3',F}$ = 16 Hz, C3′ -pT$_{\alpha F}$), 63.8 (d, $J_{5',P}$ = 4 Hz, C5′ -pT$_{\alpha F}$), 59.7 (C5′ T$_{\alpha F}$p-), 12.8 and 12.7 (CH_3 T$_{\alpha F}$p- and CH_3 -pT$_{\alpha F}$). ^{19}F NMR (282 MHz, D_2O): δ −200.24 (ddd, $J_{F,H1'}$ = 17.4 Hz, $J_{F,H3'}$ = 21.2 Hz, $J_{F,H2'}$ = 51.5 Hz, T$_{\alpha F}$p-), −201,79 (ddd, $J_{F,H1'}$ = 18.0 Hz, $J_{F,H3'}$ = 23.2 Hz, $J_{F,H2'}$ = 52.2 Hz, -pT$_{\alpha F}$). ^{31}P NMR (121 MHz, D_2O): δ −0.94. HRMS (ESI$^-$ mode) (M − H)$^-$: calcd. for $C_{20}H_{24}N_4O_{12}F_2P$ 581.1096, found 581.1074.

UV Irradiation Conditions. Analytical studies were conducted as previously described[9] except that the OD of the 1/1 mixture of compound **1** and **2** was 5.3. For preparative purposes, aqueous solutions of **2** (10 mg in 20 mL) was degassed under argon for 30 min and then exposed for 30 min to the 254 nm light (12 × 8 W; T8C UVC 7H 8W lamps) and then concentrated to dryness. The experiments were repeated several times.

HPLC Analyses and Purifications. **Analytic HPLC**: A 25 μL aliquot of the irradiation mixture was injected on a Symmetry C18 (5 μm, 4.6 × 250 mm) column using a 50 min, 1 mL·min^{-1} gradient of 0−15% CH_3CN in 0.05 M aqueous ammonium acetate. A photodiode array detector was used. Peak area was measured at 230 nm. *Retention time* (min): **4**, 17.1; **3**, 19.1; **2**, 37.2. **Preparative HPLC**: A prepurification of the crude irradiation mixture (108 mg) was performed by medium pressure reverse-phase chromatography using a gradient of CH_3CN in H_2O (0−10%). Fractions containing recovered starting material were pooled and concentrated to dryness to yield 28 mg of **2** (26%). Fractions containing the photoproducts were combined and repurified by HPLC on a SymmetryPrep C18 column (7 μm; 7.8 × 300 mm) using a 30 min, 2.8 mL·min^{-1} linear gradient of 0−9% CH_3CN in 0.05 M aqueous ammonium acetate. The detection was set at 230 nm. The (6-4) PP **4** (rt 19 min, 10 mg, 9%) and the CPD **3** (rt 21 min, 18 mg, 17%) were obtained after evaporation of the appropriate fractions.

Cyclobutane Photoproduct 3. ^{1}H NMR (600 MHz, D_2O): δ 6.07 (1H, d, $J_{1',F}$ = 21.6 Hz, H1′ T$_{\alpha F}$p-), 5.73 (1H, dd, $J_{1',F}$ = 17.7 Hz, $J_{1',2'}$ = 2.0 Hz, H1′ -pT$_{\alpha F}$), 5.71 (1H, dd, $J_{2',F}$ = 52.1 Hz, $J_{2',3'}$ = 3.4 Hz, H2′ T$_{\alpha F}$p-), 5.05 (1H, ddd, $J_{2',F}$ = 51.8 Hz, $J_{2',3'}$ = 5.2 Hz, $J_{2',1'}$ = 2.0 Hz, H2′ -pT$_{\alpha F}$), 4.85 (1H, d, $J_{6,6}$ = 6.5 Hz, H6 T$_{\alpha F}$p-), 4.46 (1H, dddd, $J_{3',F}$ = 26.3 Hz , $J_{3',4'}$ = 10.9 Hz, $J_{3',2'}$ = 3.4 Hz, $J_{3',P}$ = 9.4 Hz, H3′ T$_{\alpha F}$p-), 4.32 (1H, dd, $J_{5',5''}$ = 10.6 Hz, $J_{5',4'}$ = 3.7 Hz, H5′ -pT$_{\alpha F}$), 4.30 (1H, m, H4′ -pT$_{\alpha F}$), 4.27 (1H, dd, $J_{5',5''}$ = 10.6 Hz, $J_{5',4'}$ = 4.5 Hz, H5′ -pT$_{\alpha F}$), 4.24 (1H, d, $J_{6,6}$ = 6.5 Hz, H6 -pT$_{\alpha F}$), 4.13 (1H, m, H4′ T$_{\alpha F}$p-), 4.07 (1H, m, H3′ -pT$_{\alpha F}$), 4.07 (1H, dd, $J_{5',5''}$ = 13.4 Hz, $J_{5',4'}$ = 1.6 Hz, H5′ T$_{\alpha F}$p-), 3.93 (1H, dd, $J_{5',5''}$ = 13.4, $J_{5',4'}$ = 2.8 Hz, H5′ T$_{\alpha F}$p-), 1.52 (3H, s, CH_3 -pT$_{\alpha F}$), 1.43 (3H, s, CH_3 T$_{\alpha F}$p-). ^{13}C NMR (150 MHz, D_2O): δ 175.3 (C4 T$_{\alpha F}$p-), 173.0 (C4 -pT$_{\alpha F}$), 155.5 (C2 T$_{\alpha F}$p-), 154.4 (C2 -pT$_{\alpha F}$), 94.2 (d, $J_{2',F}$ = 188 Hz, C2′ -pT$_{\alpha F}$), 93.9 (d, $J_{2',F}$ = 182 Hz, C2′ T$_{\alpha F}$p-), 91.2 (d, $J_{1',F}$ = 34 Hz, C1′ -pT$_{\alpha F}$), 88.6 (d, $J_{1',F}$ = 38 Hz, C1′ T$_{\alpha F}$p-), 81.5 (d, $J_{4',F}$ or $J_{4',P}$ = 9 Hz, C4′ -pT$_{\alpha F}$), 80.0 (d, $J_{4',F}$ or $J_{4',P}$ = 7 Hz, C4′ T$_{\alpha F}$p-), 71.3 (dd, $J_{3',F}$ = 17 Hz, $J_{3',P}$ = 5 Hz, C3′ T$_{\alpha F}$p-), 69.4 (d, $J_{3',F}$ = 16 Hz, C3′ -pT$_{\alpha F}$), 67.5 (d, $J_{5',P}$ = 5 Hz, C5′ -pT$_{\alpha F}$), 59.5 (C5′ T$_{\alpha F}$p-), 58.9 (C6 -pT$_{\alpha F}$), 55.0 (C6 T$_{\alpha F}$p-), 53.0 (C5 T$_{\alpha F}$p-), 48.4 (C5 -pT$_{\alpha F}$), 17.7 (CH_3 -pT$_{\alpha F}$), 17.4 (CH_3 T$_{\alpha F}$p-). ^{19}F NMR (282 MHz, D_2O): δ −200.21 (ddd, $J_{F,H2'}$ = 52.1 Hz, $J_{F,H3'}$ = 26.3 Hz, $J_{F,H1'}$ = 21.6 Hz, T$_{\alpha F}$p-) et −202.2 (ddd, $J_{F,H2'}$ = 51.8 Hz, $J_{F,H3'}$ = 19.7 Hz, $J_{F,H1'}$ = 17.7 Hz, -pT$_{\alpha F}$). ^{31}P NMR (121 MHz, D_2O): δ −0.23 (bd, $J_{H3',P}$ = 9.4 Hz). HRMS (ESI$^-$ mode) (M − H)$^-$: calcd. for $C_{20}H_{24}N_4O_{12}F_2P$ 581.1096, found 581.1091.

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(6-4) Photoproduct 4. ^{1}H NMR (600 MHz, D_2O): δ 7.88 (1H, s, H6 -pT$_{\alpha F}$), 6.43 (1H, d, $J_{1',F}$ = 19.0 Hz, H1′ -pT$_{\alpha F}$), 6.13 (1H, d, $J_{1',F}$ = 23.7 Hz, H1′ T$_{\alpha F}$p-), 5.85 (1H, dd, $J_{2',F}$ = 49.8 Hz, $J_{2',3'}$ = 4.5 Hz, H2′ -pT$_{\alpha F}$), 5.12 (1H, s, H6 T$_{\alpha F}$p-), 4.90 (1H, ddd, $J_{3',F}$ = 22.0 Hz, $J_{3',4'}$ = 6.5 Hz, $J_{3',2'}$ = 4.5 Hz, H3′ -pT$_{\alpha F}$), 4.36 (1H, m, H4′ -pT$_{\alpha F}$), 4.30 (1H, dd, $J_{2',F}$ = 52.9 Hz, $J_{2',3'}$ = 4.1 Hz, H2′ T$_{\alpha F}$p-), 4.12 (1H, ddd, $J_{5',5''}$ = 12.2 Hz, $J_{5',4'}$ = 5.7 Hz, $J_{5',P}$ = 1.5 Hz, H5′ -pT$_{\alpha F}$), 4.05 (1H, dd, $J_{5',5''}$ = 13.7 Hz, $J_{5',4'}$ = 2.0 Hz, H5′ T$_{\alpha F}$p-), 4.03 (1H, m, H4′ T$_{\alpha F}$p-), 3.95 (1H, dd, $J_{5',5''}$ = 12.2 Hz, $J_{5',4'}$ = 2.2 Hz, H5′ -pT$_{\alpha F}$), 3.86 (1H, dd, $J_{5',5''}$ = 13.7 Hz, $J_{5',4'}$ = 3.5 Hz, H5′ T$_{\alpha F}$p-), 3.66 (1H, dddd, $J_{3',F}$ = 25.7 Hz, $J_{3',P}$ = 10.6 Hz, $J_{3',4'}$ = 10.6 Hz, $J_{3',2'}$ = 4.1 Hz, H3′ T$_{\alpha F}$p-), 2.30 (3H, s, CH_3 -pT$_{\alpha F}$), 1.79 (3H, s, CH_3 T$_{\alpha F}$p-). ^{13}C NMR (150 MHz, D_2O): δ 175.5 (C4 -pT$_{\alpha F}$), 175.0 (C4 T$_{\alpha F}$p-), 157.6 (C2 -pT$_{\alpha F}$), 154.9 (C2 T$_{\alpha F}$p-), 145.8 (C6 -pT$_{\alpha F}$), 117.9 (C5 -pT$_{\alpha F}$), 93.3 (d, $J_{2',F}$ = 184 Hz, C2′ -pT$_{\alpha F}$), 92.3 (dd, $J_{2',F}$ = 186 Hz, $J_{2',P}$ = 6 Hz, C2′ T$_{\alpha F}$p-), 90.9 (d, $J_{1',F}$ = 36 Hz, C1′ -pT$_{\alpha F}$), 88.5 (d, $J_{1',F}$ = 35 Hz, C1′ T$_{\alpha F}$p-), 84.6 (d, $J_{4',F}$ or $J_{4',P}$ = 8 Hz, C4′ -pT$_{\alpha F}$), 79.8 (d, $J_{4',F}$ or $J_{4',P}$ = 4 Hz, C4′ T$_{\alpha F}$p-), 73.7 (C5 T$_{\alpha F}$p-), 71.2 (dd, $J_{3',F}$ = 17 Hz, $J_{3',P}$ = 5 Hz, C3′ T$_{\alpha F}$p-), 70.3 (d, $J_{3',F}$ = 16 Hz, C3′ -pT$_{\alpha F}$), 65.4 (d, $J_{5',P}$ = 5 Hz, C5′ -pT$_{\alpha F}$), 59.5 (C5′ T$_{\alpha F}$p-), 59.2 (C6 T$_{\alpha F}$p-), 26.3 (CH_3 T$_{\alpha F}$p-), 14.7 (CH_3 -pT$_{\alpha F}$). ^{19}F NMR (282 MHz, D_2O): δ −196.4 (ddd, $J_{F,H2'}$ = 52.9 Hz, $J_{F,H3'}$ = 25.7 Hz, $J_{F,H1'}$ = 23.7 Hz, T$_{\alpha F}$p-) et −208.0 (ddd, $J_{F,H2'}$ = 49.8 Hz, $J_{F,H3'}$ = 22.0 Hz, $J_{F,H1'}$ = 19.0 Hz, -pT$_{\alpha F}$). ^{31}P NMR (121 MHz, D_2O): δ −0.41 (m). HRMS (ESI$^-$ mode) (M − H)$^-$: calcd. for $C_{20}H_{24}N_4O_{12}F_2P$ 581.1096, found 581.1099.

Circular Dichroism Conditions. CD spectra were recorded on a spectropolarimeter equipped with a Peltier temperature controller. Conditions were those reported in ref 8a except that the buffer was 0.01 M Na phosphate, 0.1 M NaCl. The molar extinction coefficient at 267 nm of **1** and of its nucleoside constituent thymidine (T)[26] ($2 \times 9.65 \times 10^3$ and 9.65×10^3 M^{-1} cm^{-1}, respectively) were used for **2**, and for its nucleoside constituent 2′-α-fluorothymidine 7.

■ ASSOCIATED CONTENT

Supporting Information

NMR spectra of all new compounds, variable temperature CD spectra of **2**, and CD spectrum of **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: pascale.clivio@univ-reims.fr (P.C.).

Notes

The authors declare no competing financial interest.

For convenience, the name 2′-α-fluorothymidine and those of its derivatives are preferred instead of 2′-deoxy-2′-fluoro-5-methyluridine.

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