JOC Article

Sugar Conformational Effects on the Photochemistry of Thymidylyl(3′-5′)thymidine

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The synthesis and conformational analysis of 2′-*O*,5-dimethyluridylyl(3′-5′)-2′-*O*,5-dimethyluridine (**1a**), the analogue of thymidylyl(3′-5′)thymidine (TpT; **1b**) in which a methoxy group replaces each 2′-α-hydrogen atom, are described. In comparison with TpT, such modification increases the population of the C3′-endo conformer of the sugar ring puckering at the 5′- and 3′-ends from 30 to 75% and from 37 to 66%, respectively. Photolyses of **1a** and TpT at 254 nm are qualitatively comparable (the cis-syn cyclobutane pyrimidine dimer and the (6-4) photoproduct are formed), although it is significantly faster in the case of **1a**. These results are explained by the increased propensity of the modified dinucleotide to adopt a base-stacked conformation geometry reminiscent of that for TpT.

Introduction

Exposure of DNA to solar ultraviolet light results in the formation of dipyrimidine photoproducts, the most common being cyclobutane pyrimidine dimers (CPD) and pyrimidine (6-4) pyrimidone photoproducts ((6-4)PP) with their Dewar valence isomers (Figure 1).[1] A good deal of evidence points to these photoproducts being at the origin of the molecular mechanism leading to skin carcinogenesis through their combined mutagenic and immunosuppressive properties.[2] However, the respective contribution of each class of photoproduct in skin cancer development is not yet fully known, nor are the factors that influence their respective formation. Indeed, understanding these factors is critical to further investigate photocarcinogenesis. At present, parameters such as photophysical properties of the targeted nucleobases, wavelength, flanking sequence context, and conformational features, including secondary structure and DNA−protein interaction, have been identified to play an important role in affecting photoproduct formation.[3] However, the rules that govern the relationships between DNA conformational parameters and preferential photoproduct formation are unclear.

We consider the elucidation of the factors which drive the photochemistry occurring at the dinucleotide level to be a prerequisite step in understanding the phenomena which take place within the DNA double helix. Consequently, we have recently started a program consisting of systematically comparing the photochemistry of thymine−thymine dinucleoside monophosphate analogues endowed with particular conformational properties with that of the parent deoxydinucleotide **1b** (TpT), a good B-DNA model. Such studies have already allowed us to show that, upon UV irradiation, peptide nucleic acids dimers *sensus stricto* do not give rise to (6-4) PP but lead exclusively to CPD.[4] Conversely, in the case of "amide-3" dimer, both (6-4) PP and CPD are formed.[5]

In continuing our work, we were interested in probing the effect of the major conformational parameter that distinguishes B-DNA from A-DNA, the sugar pucker, on the photochemistry of TpT. Interestingly, in the oligonucleotide series, the 2′-α-methoxy modification of 2′-deoxyribose has been shown to induce a significant shift of the conformational equilibrium of the sugar ring from the C2′-endo toward the C3′-endo conformation, resulting in a mimic A-conformation.[6] This trend is likely to be operative at the dinucleotide level for pyrimidines, since the ratio of C3′-endo conformers of ribodinucleotides[7] *vs*

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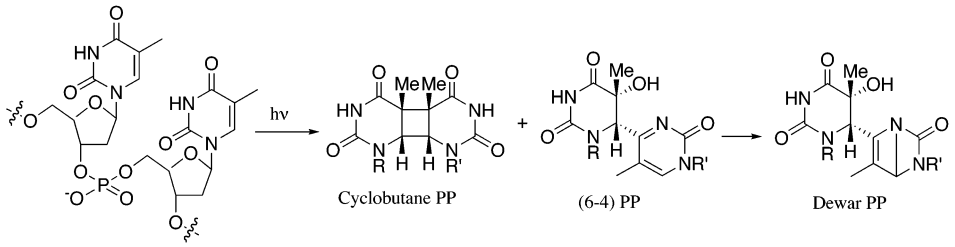


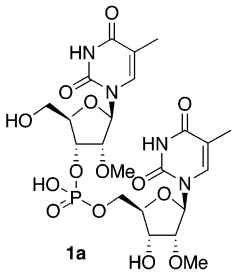
FIGURE 1. Major DNA photoproducts formed at adjacent thymine sites.

2′-deoxyribodinucleotides[8] is increased and since 2′-*O*-methylation of ribodinucleotides induces an additional stabilization of the C3′-endo form.[9] Therefore, we propose to probe, at the single-stranded dinucleotide level, the effect of the C3′-endo conformational preference induced by 2′-methoxylation on the photochemistry of thymine.

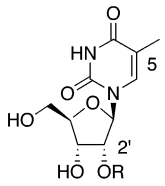
We herein report the synthesis of 2′-*O*,5-dimethyluridylyl(3′-5′)-2′-*O*,5-dimethyluridine (or 2′-α-methoxythymidylyl(3′-5′)-2′-α-methoxythymidine (TmopTmo)) **1a**, together with its sugar conformational analysis and photochemical behavior. In addition, to rationalize the observed photochemical results, the stacking properties of **1a** and **1b** are compared.

Results

Synthesis of 2′-*O*,5-Dimethyluridyl (3′-5′)-2′-*O*,5-dimethyluridine (1a). Whereas the synthesis of oligonucleotides containing 2′-*O*,5-dimethyluridine (**2a**) has been reported,[10] as far as we know, dinucleotide **1a** has never been prepared. We have obtained this compound as outlined in Scheme 1.



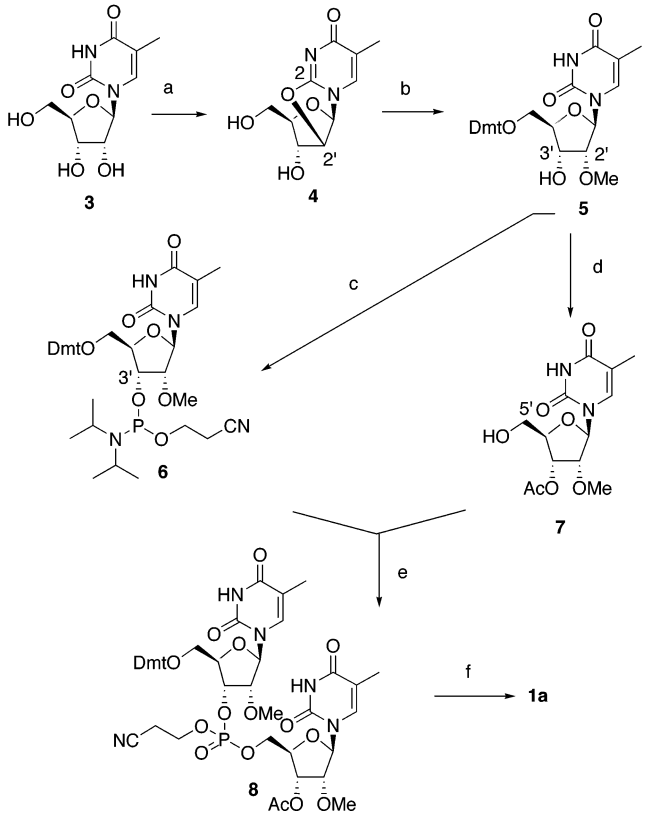
1a



2a R=OCH$_3$
2b R=H

Two general strategies have been developed to synthesize 2′-*O*,5-dimethyluridine nucleosides. One route uses a 2-*O*-methyl ribosylation procedure of thymine,[11] whereas the other one makes use of 5-methyluridine derivatives. In the latter case, the 2′-*O*-methyl group is obtained either by methylation of the 2′-hydroxyl group[10,12] or, to circumvent multiple blocking/deblocking steps, by a 3′-assisted regiospecific opening of a 2,2′-anhydro intermediate.[13] We decided to follow such an approach,

SCHEME 1. Synthesis of 1a[a]



[a] Legend: (a) ref 17; (b) (i) DmtCl, pyridine, (ii) Mg(OCH$_3$)$_2$, DMF, 68% yield from **4**; (c) refs 11 and 12; (d) (i) Ac$_2$O, pyridine, (ii) TFA/CH$_2$Cl$_2$ 3%, 71% yield from **5**; (e) (i) tetrazole, CH$_3$CN, (ii) I$_2$, THF/H$_2$O/2,6-lutidine; (f) (i) NH$_4$OH/MeOH, (ii) 80% aqueous AcOH, 26% yield from **7**.

starting from 5-methyluridine and employing Mg(OMe)$_2$ as a source of methoxide for the 3′-assisted regiospecific opening of its corresponding 2,2′-anhydro intermediate as described for the synthesis of 2′-*O*-methyluridine (Scheme 1).[14] Thus, 5-methyluridine (**3**)[15] was treated with diphenyl carbonate under basic catalysis conditions to give the anhydro derivative **4**[16] according to the procedure described for the synthesis of 2,2′-cyclouridine.[17] Then, compound **4** was dimethoxytritylated and the 2,2′-anhydro linkage was regio- and stereoselectively

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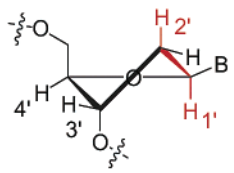
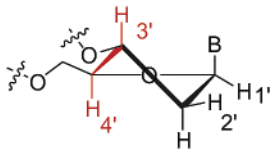
TABLE 1. $J_{1'2'}$ and $J_{3'4'}$ Coupling Constants (Hz)[a] and Population Distribution (%) of Conformers of 1a





	Tmop-	-pTmo
$J_{1'2'}$	2.4	3.2
$J_{3'4'}$	7.4	6.2
C2′-endo[b]	25	34
C3′-endo	78,[c] 75[b]	65,[c] 66[b]

[a] Coupling constants are accurate to ±0.1 Hz. [b] Calculated from $J_{1'2'}/(J_{1'2'} + J_{3'4'})$. [c] Calculated from $J_{3'4'}/(J_{1'2'} + J_{3'4'})$.

opened at the sugar 2′-position by magnesium methoxide, affording **5**[12] in 68% yield (from **4**, two steps). Phosphitylation of **5** with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite in the presence of Hünig's base led to phosphoramidite **6**[11,12] in 80% yield. Acetylation of **5** (Ac_2O, pyridine) followed by detritylation (TFA/CH_2Cl_2 3%) gave 3′-*O*-acetyl-2′-*O*,5-dimethyluridine **7** in 71% yield. Finally, phosphoramidite **6** was condensed with **7** in the presence of tetrazole in acetonitrile, and subsequent oxidation with iodine in THF/H_2O/2,6-lutidine afforded the corresponding phosphotriester **8**. Dimer **1a** was obtained after treatment of **8** with aqueous ammonia−methanol and then 80% aqueous acetic acid. The overall yield of the condensation/oxidation/deprotection steps was 26% (based on **7**, four steps).

NMR-Based Conformation of 2′-*O*,5-Dimethyluridyl(3′-5′)-2′-*O*,5-dimethyluridine (1a). The 2′-*O*-methylribose protons of **1a** were assigned using the 2D COSY technique. Signals of the 3′- and 5′-end sugar protons were differentiated from the multiplicity of the H3′ signal of the 5′-end sugar and confirmed by a ^{31}P decoupling experiment.

In aqueous solution, the sugar pucker of nucleosides and nucleotides exists as an equilibrium mixture of C2′-endo and C3′-endo conformers, whose respective populations can be determined by ^1H NMR using vicinal spin-coupling constants of the sugar protons.[18] The population of the C3′-endo conformers can be estimated using the sum of $J_{1'2'} + J_{3'4'}$ and the observed $J_{1'2'}$ and $J_{3'4'}$ values (Table 1).[18,19] We used a value of $J_{1'2'} + J_{3'4'} = 9.5$ Hz corresponding to the ribodinucleotide series to compute the population of the C3′-endo conformer of **1a**.[7]

The maximum observed deviation in the values computed separately from $J_{1'2'}$ and $J_{3'4'}$ was 3%. We considered only the $J_{1'2'}$-derived value, since its determination was more accurate than that of $J_{3'4'}$ due to complex multiplicity. Calculations revealed that both the 5′- and 3′-terminal 2′-*O*-methylribose units of **1a** preferentially adopt a C3′-endo conformation (75% and 66%, respectively). Thus, in comparison to TpT (**1b**) (30% of C3′-endo for Tp- and 37% of C3′-endo for -pT),[8] 2′-α-methoxylation increases, as expected, significantly the population of the C3′-endo pucker of each sugar, from 30 to 75% and from 37 to 66% for the Tmop- and -pTmo residues, respectively. The influence of bis-methoxylation on the sugar pucker of a dinucleoside monophosphate was unknown. In fact, only the effect of 2′-*O*-methylation of the 5′-unit of pyrimidine ribodinucleotides on puckering had been studied.[9] Considering that the conformation of a sugar is not critically influenced by the nature of the adjacent sugar,[20] the effect of the 2′-methoxylation of TpT at the 5′-end (Δ% = 45%) is in accordance with the observed stabilization of the C3′-endo form when comparing the 5′-sugar puckers of dCpdC[8] and CmopC[9a] (Δ% = 47%). In contrast, the effect of C2′-methoxylation on the C3′-endo conformational equilibrium is less pronounced at the 3′-end sugar of TpT (Δ% = 29%). This trend clearly demonstrates the effect of the phosphate group in stabilizing the C3′-endo conformation of the 5′-end sugar. Previously observed at the monomer level, this behavior is due to the repulsive steric effect of the phosphate group in the C2′-endo form and the methoxy group.[9b] Otherwise, the C3′-endo conformational preference is due to the electronegativity of the 2′-substituent[21] (*gauche* stereoelectronic effect of the O4′−C1′−C2′−O2 fragment)[22] and to repulsive steric effects between the 2′-methoxy group and the 2-carbonyl group of the pyrimidine base.[9b] The combination of these factors is undoubtedly responsible for the observed predominance of the C3′-endo sugar puckering of **1a**.

Photochemical Behavior of 2′-*O*,5-Dimethyluridyl(3′-5′)-2′-*O*,5-dimethyluridine (1a). The photochemical behavior of **1a** was studied in aqueous solution at 254 nm. Examination of the ^1H NMR spectrum of the crude irradiation mixture of **1a** revealed the formation of two photoproducts (**9a** and **10a**) whose respective yield varied with the photolysis time (Scheme 2).

Signals of the base portion of the photoproducts could be easily observed. Photoproduct **9a** displayed two singlets (one proton each) at δ 7.92 and 5.04 and two singlets (three protons each) at δ 2.27 and 1.75. Comparison of these signal chemical shifts with those of key protons of **9b**, the (6-4) PP of TpT (H6 -pT, δ 8.00; H6 Tp-, δ 5.10; CH_3 -pT, δ 2.32; CH_3 Tp-, δ 1.76)[23] suggested that **9a** was the 2′-dimethoxy (6-4) analogue of TpT. In addition, HPLC analysis using a photodiode detector showed, for **9a**, a UV chromophore of the (6-4) type (λ_{max} 329 nm). Compound **10a** displayed a UV spectrum reminiscent of the cyclobutane class (low absorption above 254 nm). This was confirmed by the presence of two shielded methyl signals at δ 1.49 and 1.44 and the lack of two vinylic H6 protons compared with **1a**. Unfortunately, comparison of the methyl or the H1′ signal chemical shifts of **10a** with those of the cis-syn (**10b**) and trans-syn I CPD of TpT[24]

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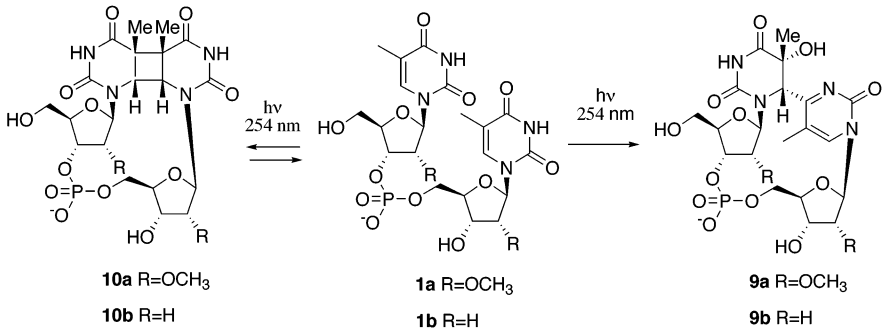
SCHEME 2. Photoproducts Formed upon 254 nm Photolysis of 1a and 1b



10a R=OCH_3 **1a** R=OCH_3 **9a** R=OCH_3

10b R=H **1b** R=H **9b** R=H

could not ascertain **10a** stereochemistry at the C5 and C6 positions. This ambiguity was solved by considering the stereochemical pathway leading to photoproducts. Indeed, photolysis of **1b** has been shown to lead to two major adducts, the (6-4) adduct **9b** and the cis-syn cyclobutane isomer **10b**,[23,25] both resulting from a photochemical process between two bases in an anti glycosidic bond conformation. Since the (6-4) adduct **9a** results from a photochemical process between two bases in an anti glycosidic bond conformation, it is reasonable to propose that **10a** also results from a similar stereochemical pathway and consequently has the same stereochemistry as the cis-syn cyclobutane photoproduct **10b**. Prolonged irradiation of **1a** led exclusively to **9a**, an observation consistent with the known photoreversal of the cis-syn CPD upon UV C exposure that led to accumulation of the (6-4) photoproduct.[25] Hence, this photochemical study demonstrated that increasing the ratio of the C3′-endo pucker from 30 to 75% and from 37 to 66% for the 5′- and 3′-residues, respectively, does not qualitatively modify the photochemical behavior of the dimer, since (6-4) and cyclobutane photoproducts **9a** and **10a** were formed.

To compare more closely the photochemical behavior of **1a** and **1b**, we photolyzed a solution prepared by mixing volume/volume solutions of these compounds having the same optical density and monitored the reaction by HPLC.[26] After 1 h of irradiation, *ca.* 58% of **1b** was consumed and the peak area ratio of **1b**/**1a** was 1.5. Considering that both CPDs have a similar photoreversal rate, which is quite reasonable for the photosplitting reaction involving the same cyclobutane, this means that the photolysis of **1a** is 33% more rapid than that of **1b** after 1 h. Assuming similar molar extinction coefficients for the corresponding adducts in the 2′-deoxyribose and 2′-*O*-methylribose series, UV detection could be used for determining the formation efficiency of photoproducts in each series. After 1 h of UV exposure, the yield of CPD in the 2′-*O*-methylribose series was 1.5 times higher than that in the TpT series and the (6-4) PP formation was found to be 1.3 times more efficient in the 2′-*O*-methylribose series. To determine if the formation of one class of adduct was favored within a series, we then compared the (6-4)/CPD ratio in each class after 1 h. The small difference obtained (8%) indicated that none of the (6-4) or CPD photochemical pathways were significantly favored.

These photochemical data indicate that 2′-α-methoxylation of **1b** results in an enhancement of photoproduct formation rate. Most probably, the C3′-endo conformational preference derived from 2′-α-methoxylation induces other conformational changes to be responsible for the observed photolysis difference. This may be an increased population of stacked species, since 2′-*O*-methylation of ribodinucleotides can enhance intramolecular base stacking.[27] Such stacked species should have a higher propensity to undergo photoproduct formation. To investigate the hypothesis that **1a** exhibits an increased stacking ability, in comparison to **1b**, we performed circular dichroism (CD) studies.

Circular Dichroism Studies. CD spectra of **1b** and **1a** were obtained in the 225−330 nm region over the temperature range 20−80 °C (Figures 2 and 3, respectively). The CD spectrum of TpT (**1b**) was consistent with previously published results.[28] At 20 °C, the CD amplitude of **1a** was found to be much larger than that of **1b** (a factor of 3 at about 280 nm).

It is generally accepted that the CD spectra of dinucleotides result from two contributions: one from each mononucleoside and a second due to the asymmetric interaction between the two bases. To examine the possible monomer contribution in the observed magnitude enhancement of the CD of **1a** with respect to **1b**, we recorded the CD spectrum of the respective mononucleoside constituents (2′-*O*,5-dimethyluridine **2a** and thymidine **2b**) (Figures 2 and 3). The intensity of the positive band of **2a** was found to be larger than that of **2b**. However, if methoxy substitution *per se* contributes, in part, to the CD difference between **1a** and **1b**, it is not totally responsible for this difference and other factors, such as the geometry of interaction between the two chromophores and/or the fraction of stacked conformation, must be involved (see below).

For compounds **1a** and **1b**, comparison of the CD spectrum of the dimer with that of its corresponding mononucleoside (Figures 2 and 3) revealed large differences, as already reported for **1b** and **2b**.[28] The differences between monomer and dimer spectra are clear evidence of a nonrandom conformation of bases in the dimer and arise from the presence of base-stacked conformations.[28] Assuming a two-state model, where a stacked and an unstacked form of the dimer are in equilibrium, it has been proposed that the CD difference

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(26) The concentration of **1b** was 0.19 mM ($\epsilon_{267} = 1.85 \times 10^4$).[25] An independent analysis of the separate photolysis of **1a** and **1b** resolved without ambiguity the origin of each photoproduct.

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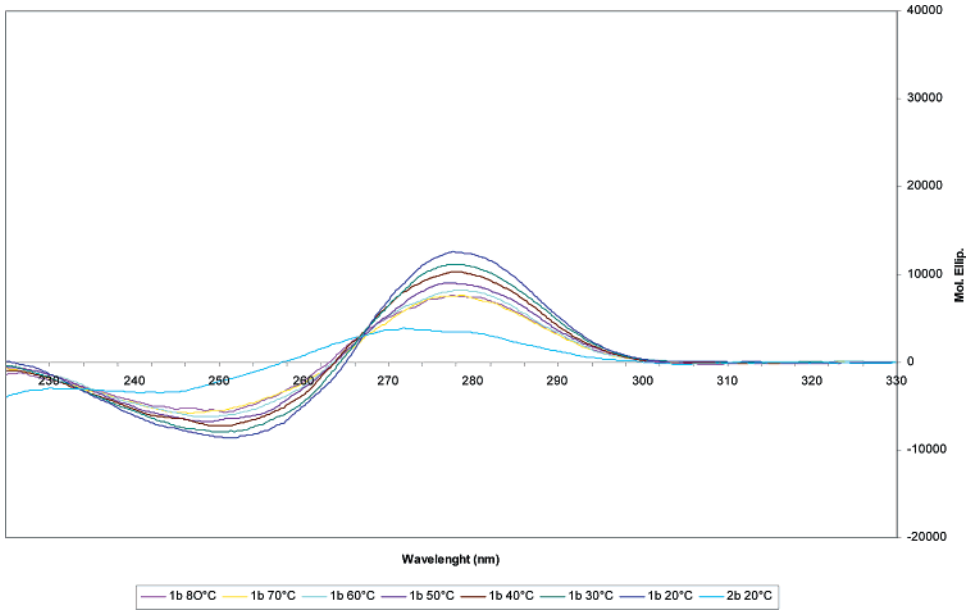

FIGURE 2. Influence of the temperature on the CD spectrum of TpT **1b** in a buffer containing 0.1 M Na phosphate, 1 M NaCl, pH 7.0. The CD intensity decreases as the temperature was increased from 20 to 80 °C by 10 °C increment. The light blue line corresponds to the monomer **2b**. CD data are expressed per residue.

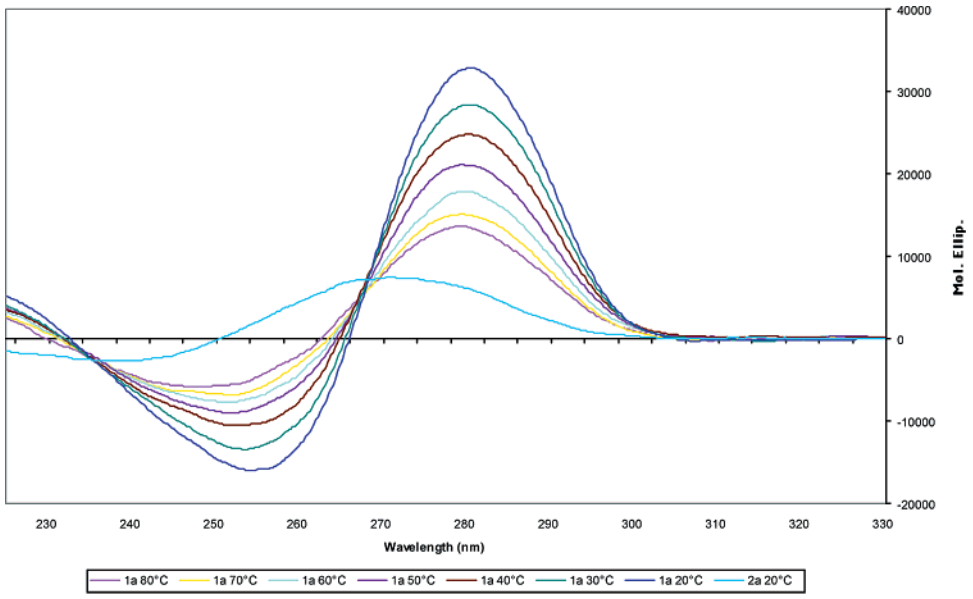

FIGURE 3. Influence of the temperature on the CD spectrum of TmopTmo (**1a**) in the same buffer as in Figure 2. The CD intensity decreases as the temperature was increased from 20 to 80 °C by 10 °C increments. The light blue line corresponds to the monomer **2a**. CD data are expressed per residue.

spectra between the dimer and its constituent monomers depends only on the geometry of interaction of the two base chromophores and on the fraction of the stacked form.[29] If two dimers with the same chromophore have the same stacked geometry, the two experimental difference curves should have the same shape and differ only by a constant positive multiplier. The difference curve of **1a** can be roughly considered as a simple multiple of that of **1b** (*ca.* 3) (Figure 4).

Then, if the two-state model of stacking applies to **1a** and **1b**, the low-temperature stacked geometries of **1a** and **1b** must be very similar, if not identical. In addition, at 20 °C, the fraction of the stacked form for **1a** is approximately 3 times higher than that of **1b**.

Furthermore, both dimers unstack with increasing temperature (Figures 2 and 3). The effects of temperature upon the amplitude of the CD difference spectra of **1a** and **1b** were recorded in the melting curves (Figure 5). At 80 °C, the melting curves converged toward low CD amplitude limits corresponding to the unstacked conformation of the dimers without attaining them. These limits are known to be difficult to reach.[27] The low-temperature upper CD amplitude limit representing the fully stacked conformation of the dimer was not observed

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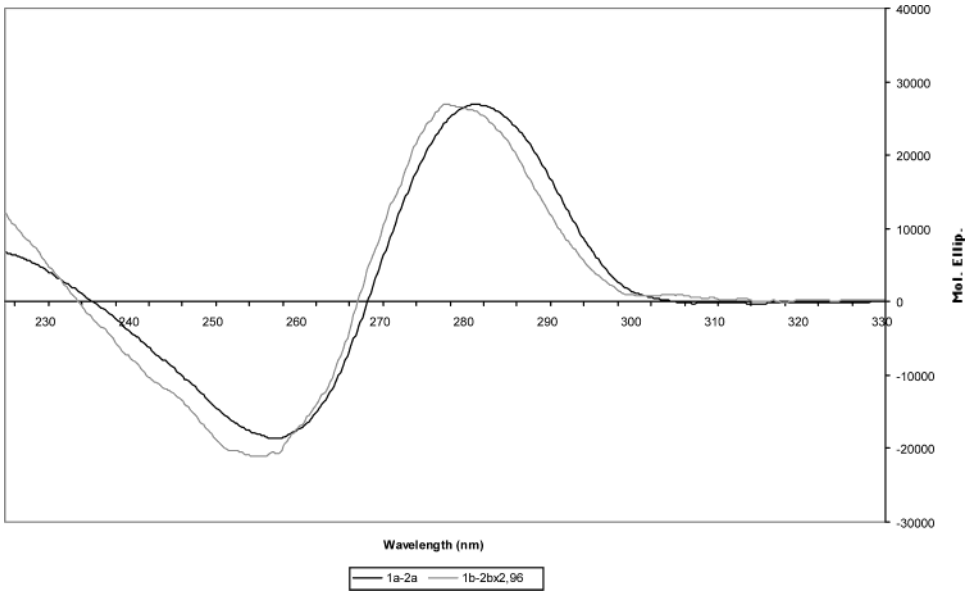

FIGURE 4. CD difference spectra between **1a** and its corresponding monomer **2a** and between **1b** and its corresponding monomer **2b** multiplied by a factor of 3 at 20 °C. Other conditions are those of Figure 2.

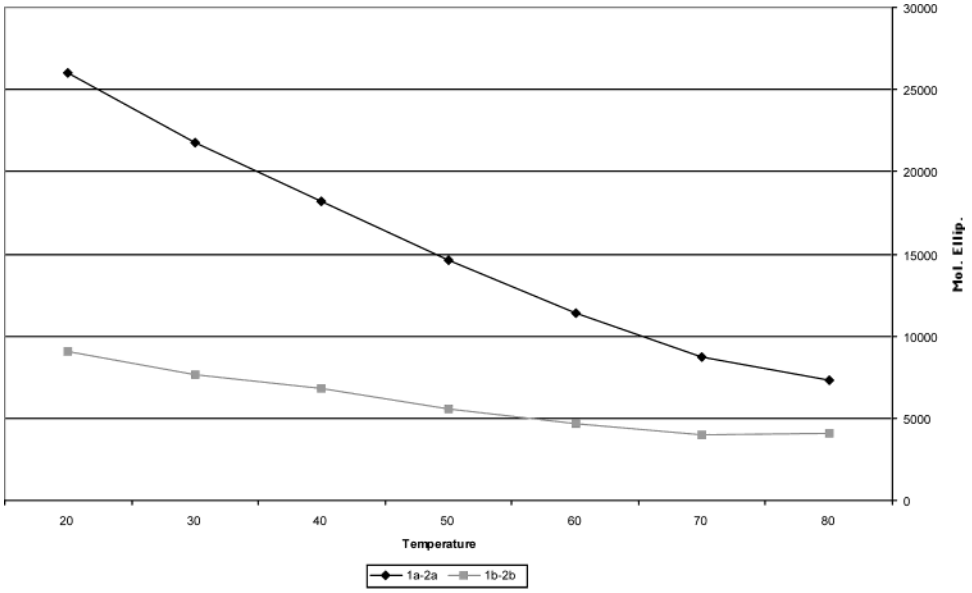

FIGURE 5. CD melting curves obtained by following the CD difference intensity between the dimer and the monomer at 279 nm for **1a** and 278 nm for **1b**.

for **1a** or **1b**, thus precluding the determination of the extent of stacking.[30] However, if at 80 °C the dimers have almost reached a completely unstacked conformation, this shows that if **1a** is more stacked than **1b**, it does not reach the 50% stacked population at 20 °C since the sigmoidal part of the curve is not reached.

These CD studies indicated that, at room temperature, the fraction of stacked species is higher in **1a** than in **1b**.

Discussion

In this study, we have observed, at the single-stranded dinucleotide level, that 2′-α-methoxylation of **1b** was accompanied by a stacking enhancement of thymines. 2′-*O*-methylation has been shown to enhance base stacking of dipyrimidine ribodinucleotides.[27,31] Similarly, 2′-hydroxylation was found to stabilize the stacked conformers of dipyrimidine dinucleotides.[31] However, no explanation was given to rationalize the relationship between 2′-*O*-methylation or hydroxylation upon stacking preferences. In fact, this could be a consequence of the C3′-endo sugar conformational preference, which is increased in the order 2′-deoxy[8] < ribo[7] < 2′-*O*-methylribo,[9] and that is the main difference between **1a** and **1b**.

Indeed, relationships between intramolecular base stacking within dinucleotides and the sugar pucker have been evidenced. For example, intramolecular base stack-

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ing induced by low temperature is known to favor the C3′-endo conformation in ribodinucleoside phosphates.[7] A similar conclusion was drawn from examination of the stacking/unstacking process of dinucleotides by molecular modeling.[32] However, to our knowledge, no precise mechanism has ever been truly proposed to account for these observations, and the intimate correlation process that links the C3′-endo conformation and the intramolecular base stacking needs to be assessed. The torsion angles along the sugar phosphate backbone are interdependent:[33] for instance, the endocyclic torsion angle ν_3 of the sugar about the C4′−C3′ bond and the backbone torsion angle δ are directly correlated, since they describe torsions about the same C4′−C3′ bond. Moreover, δ is correlated with several other torsion angles, one of which is ζ (O3′−P bond).[34] Interestingly, molecular modeling studies have proposed that ζ was the main angle involved at the onset of the stacking/destacking process of dinucleotides,[32,35,36] which is consistent with NMR studies.[8] In addition, calculations have predicted that the C2′-endo/C3′-endo sugar pucker interconversion is associated with the concomitant t/g-conformation interconversion of ζ corresponding to the unstacked/stacked state.[36] Finally, when the C3′-endo conformation is due to the presence of a 2′-methoxy group, the latter is so oriented (favorable interaction between the methoxy group and the adjacent ($n + 1$) sugar residue) to stabilize the g-conformation of ζ[37] and, therefore, the population of the stacked conformer. Thus, we propose that promotion of the C3′-endo pucker population in **1a** is likely to be correlated with a fractional change in the ζ torsion angle that results in enhancement of the stacked species compared to **1b**.

Another question that needs to be addressed concerns the real involvement of the ground-state stacked conformational preferences of **1a** for the observed faster photolysis rate compared to **1b**. To be the case, the stacking/destacking process has to be sufficiently slow compared to the photochemical reaction. According to the Franck−Condon principle, the excitation of a molecule is extremely rapid (10^{-15} s) compared to nuclear motion.[38] In addition, formation of CPD and (6-4) PP from TpT involves singlet states of the excited thymine, whose lifetime is on the order of 10^{-12} s.[39] Thus, excitation of an unstacked structure would lead to deactivation before conformational motion can reorient the bases so that the photochemical reaction occurs. Therefore, CPD and (6-4) PP formation is highly likely to derive from the excitation of stacked complexes[39] and the ground-state stacked conformational preferences are indeed responsible for the observed photolysis rate differences. In accordance with our conclusions, it has been demonstrated, through variable-temperature experiments or with solvents that disrupt base stacking, that in single-stranded di- and oligonucleotides, base stacking favors photoproduct formation.[40]

Finally, the proposed similar stacked geometries of **1a** and **1b** determined from CD studies are also supported by a molecular modeling analysis (data not shown)[41] and explain the qualitative similarities of their photochemistry.

Therefore, in comparison to **1b**, the C3′-endo sugar preference of **1a** imposed by 2′-methoxylation results, through the ζ torsion angle, in an enhancement of the population of stacked species with retention of similar geometry that in turn increases the photolysis efficiency.

Conclusion

In this work, we have synthesized **1a**, the dinucleotide analogue of **1b** containing two 2′-α-methoxy groups, and shown, as anticipated, that the two modified sugar substitutions induce a strong enhanced conformational *bias* toward the C3′-endo pucker. As a consequence, this conformational change increases the population of the stacked species of **1a**, which in turn significantly accelerates the photochemical reactivity. Exhibition of an overall similar geometry of the stacked conformers of **1a** and **1b** explains the qualitative similarity in photoproduct formation.

Interestingly, under our photolysis conditions, **1a**, which exhibits through its C3′-endo sugar pucker a conformation reminiscent of A-DNA, did not give rise to the spore photoproduct (SP), whose formation occurs when DNA adopts an A conformation. So far, in the context of polymer by opposition to the base or nucleoside series, SP has only been obtained by UV exposure of dry or frozen-state DNA, whose conformation most resembles that of A-DNA or when this required A conformation is induced by a bacterial spore protein association.[42,43] It is noteworthy that irradiation of **1b** under conditions known to allow the formation of SP in DNA never led to this type of photoproduct.[44] This could indicate that a specific structural context of adjacent thymines, which is allowed in DNA and not at the dinucleotide level, might be necessary for SP formation. On the other hand, the absence of SP may simply be ascribed to the presence of water molecules, since it has also been proposed that the degree of hydration was the critical factor for SP formation instead of the A conformation.[45]

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Experimental Section

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*,5-dimethyluridine (5). Dry 2,2′-*O*-anhydro-1-(β-D-arabinofuranosyl)-5-methyluracil (**4**;[16] 130 mg, 0.54 mmol), obtained by repeated coevaporation with anhydrous pyridine, was dissolved in anhydrous pyridine (6 mL). To this solution was added dimethoxytrityl chloride (403 mg, 1.19 mmol, 2.2 equiv) and DMAP (6 mg). The solution was stirred at room temperature overnight and then evaporated. The residue was dissolved in CH_2Cl_2 and washed with 5% aqueous $NaHCO_3$. The aqueous phase was extracted four times with CH_2Cl_2, and the organic layers were combined, dried with Na_2SO_4, and evaporated. The oily residue was coevaporated twice with toluene and triturated with diethyl ether. The liquid was removed by decantation, the solid residue was dissolved in CH_2Cl_2 (1 mL), and 50 mL of diethyl ether was added. The solution was kept at 5 °C for 1 h, and then the organic phase was decanted, affording a precipitate (292 mg) that was dried under vacuum and used as such in the next step. To magnesium methoxide prepared from magnesium turnings (25 mg, 1.038 mmol) in dry MeOH (1 mL) were added the above-mentioned precipitate (94 mg) and DMF (3 mL). The resulting suspension was heated at 100 °C for 2.5 h. After elimination of the solvent by filtration, the residue was suspended in CH_2Cl_2 and stirred at room temperature for 2 h. The residue obtained after filtration of the suspension and concentration of the filtrate was purified by silica gel chromatography. Elution with 0−2% CH_2Cl_2/MeOH afforded in 68% yield compound **5** as a foam whose ^{1}H NMR data were identical with those in the literature.[12]

3′-*O*-Acetyl-2′-*O*,5-dimethyluridine (7). To a solution of 5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*,5-dimethyluridine (**5**; 240 mg, 0.418 mmol) in anhydrous pyridine (30 mL) was added acetic anhydride (3.9 mL, 41.8 mmol). The mixture was stirred at room temperature overnight and then concentrated. The residue was dissolved in MeOH, coevaporated with MeOH, and concentrated to dryness to give an oil. This latter was dissolved in anhydrous CH_2Cl_2 (80 mL), and trifluoroacetic acid (2.4 mL) was added. The solution was stirred at room temperature for 30 min, treated with MeOH (3 mL), and diluted with CH_2Cl_2 (40 mL). After washing with 5% aqueous $NaHCO_3$ and then with brine, the aqueous phase was extracted five times with CH_2Cl_2. The organic layers were combined, dried with Na_2SO_4, and evaporated. The residue was purified by silica gel chromatography using a gradient of MeOH in CH_2Cl_2 (2−3%) as the eluent to afford **7** as a white foam (93 mg, 71% yield based on **5**, two steps): ^{1}H NMR ($CDCl_3$; 250 MHz) δ 7.44 (s, 1H), 5.69 (d, 1H, ^3J(H,H) = 5.6 Hz), 5.34 (dd, ^3J(H,H) = 5.1, 4.0 Hz, 1H), 4.30 (t, ^3J(H,H) = 5.4 Hz, 1H), 4.19 (m, 1H), 3.95 (dd, ^2J(H,H) = 12.5, ^3J(H,H) = 1.9 Hz, 1H), 3.79 (dd, ^2J(H,H) = 12.4, ^3J(H,H) = 2.0 Hz, 1H), 3.43 (s, 3H), 2.16 (s, 3H), 1.92 (s, 3H); ^{13}C NMR ($CDCl_3$; 75.5 MHz) δ 171.1, 164.5, 151.2, 138.3, 112.0, 91.0, 83.9, 81.4, 71.3, 62.4, 59.7, 21.5, 13.1; HRMS (CI) $(M + H)^+$ calcd for $C_{13}H_{19}N_2O_7$ 315.1192, found 315.1185.

A sample of **7** was deprotected (1/1 MeOH/concentrated aqueous ammonia) to give **2a**,[12] whose ^{1}H NMR spectrum was recorded in D_2O: ^{1}H NMR (D_2O; 300 MHz) δ 7.7 (s, 1H), 5.99 (d, ^3J(H,H) = 4.4 Hz, 1H), 4.36 (t, ^3J(H,H) = 5.5 Hz, 1H), 4.13−4.01 (m, 2H), 3.86 (m, 2H), 3.50 (s, 3H), 1.90 (s, 3H).

2′-*O*,5-Dimethyluridylyl(3′-5′)-2′-*O*,5-dimethyluridine (1a). To a solution of compound **7** (16 mg, 0.05 mmol) in anhydrous CH_3CN (1 mL) was added under argon a solution of tetrazole (12 mg, 0.165 mmol) in anhydrous CH_3CN (1 mL) followed by a solution of **6**[12] (58 mg, 0.075 mmol) in anhydrous CH_3CN (2 mL). The mixture was stirred under argon at room temperature for 30 min, and then a solution of iodine (38 mg, 0.15 mmol) in THF/H_2O/2,6-lutidine (2/1/1; 0.5 mL) was added. After it was stirred for 20 min at room temperature, the mixture was evaporated and the residue was dissolved in CH_2Cl_2 (1 mL). A saturated aqueous solution of $Na_2S_2O_3$ was dropped into it until decolorization, followed by H_2O to obtain the two-phase system. The separated aqueous layer was extracted several times with CH_2Cl_2. The combined organic layers were dried with Na_2SO_4 and evaporated. The residue was chromatographed on silica gel (particule size 6−35 μm) using a gradient of MeOH in CH_2Cl_2 (1−4%) as eluent to afford the fully protected dimer **8**. Compound **8** was dissolved in MeOH/concentrated aqueous ammonia (1/1; 4 mL), and the solution was stirred at room temperature overnight. The residue obtained after evaporation was dissolved in 80% aqueous acetic acid (4 mL). The resulting solution was stirred at room temperature for 6 h, evaporated, and coevaporated with water. Water and CH_2Cl_2 were added, and the aqueous phase was separated, washed with CH_2Cl_2, and evaporated. Chromatography was performed on silica gel using a gradient of MeOH in CH_2Cl_2 (20−25%) containing concentrated aqueous ammonia (5%) as eluent to afford, after dissolution in water, filtration through cotton, and evaporation, compound **1a** as a white glassy material (8 mg, 26% yield based on **7**, four steps): ^{1}H NMR (D_2O; 500 MHz) δ 7.93 (s, 1H, H6 -pTmo[a]), 7.85 (s, 1H, H6 Tmop-[a]), 6.09 (d, ^3J(H,H) = 3.2 Hz, 1H, H1′ -pTmo), 5.95 (d, ^3J(H,H) = 2.4 Hz, 1H, H1′ Tmop-), 4.64 (ddd, ^3J(H,H) = 4.9, 7.4, 8.3 Hz, 1H, H3′ Tmop-), 4.49 (dd, ^3J(H,H) = 5.2; 6.2 Hz, 1H, H3′ -pTmo), 4.34−4.27 (m, 3H, H4′ Tmop-, H4′ -pTmo, H5′ -pTmo[b]), 4.24 (dd, ^3J(H,H) = 2.4, 4.9 Hz, 1H, H2′ Tmop-), 4.17 (dt, ^2J(H,H) = 12.0, ^3J(H,H) = 3.0 Hz, 1H, H5" -pTmo[b]), 4.05 (dd, ^3J(H,H) = 2.4, ^2J(H,H) = 13.3 Hz, 1H, H5′ Tmop-[c]), 4.03 (dd, ^3J(H,H) = 3.2, 5.5 Hz, 1H, H2′ -pTmo), 3.91 (dd, ^2J(H,H) = 13.3, ^3J(H,H) = 3.1 Hz, 1H, H5′ Tmop-[c]), 3.64 (s, 3H, OCH_3), 3.57 (s, 3H, OCH_3), 1.90 (s, 6H, CH_3 Tmop-, CH_3 -pTmo); ^{13}C NMR (CD_3OD; 75.5 MHz) δ 167.5, 167.2, 152.7, 152.5, 137.9, 137.4, 112.6, 112.1, 88.4, 88.2, 84.3, 84.0, 83.4, 82.6, 71.8, 68.9, 64.7, 60.2, 59.3, 58.7, 12.9, 12.7; ^{31}P NMR (D_2O; 243 MHz) δ −0.61; HRMS (FAB) $(M + H)^+$ calcd for $C_{22}H_{32}N_4O_{14}P$ 607.1653, found 607.1654.

Irradiation Conditions. For NMR analysis, a 1 mL D_2O solution of **1a** (OD_{260} = 22.9) in a quartz NMR tube was exposed for 7 h 30 min to the 254 nm light (2 × 15 W). A ^{1}H NMR spectrum was recorded after 1 h 30 min, 4 h 30 min, and 7 h 30 min. For HPLC analysis, an aqueous solution (500 μL; HPLC grade) of compound **1a** or **1b** (OD_{260} = 6.3 and 16.8 for **1a** and **1b**, respectively) or a 1/1 mixture of **1a** and **1b** (pH 6; OD_{260} = 6.3 for each solution) in a quartz cuvette was exposed for 2 h to the 254 nm light source. An aliquot of the solution was sampled at t = 0, t = 1 h, and t = 2 h and analyzed by reverse-phase HPLC.

HPLC Analysis. Ten to thirty microliters of the irradiation mixture was injected on a SYMMETRY C18 (5 μm, 100Å, 4.6 × 250 mm) column using a 50 mn, 1 mL/mn linear gradient of 0−15% CH_3CN in 0.05 M aqueous ammonium acetate (pH 6.8). A photodiode array detector was used. Calculations were made using peak areas measured at 230 nm.

Retention time (mn): **1a**, 40.1; **1b**, 34.5; **10a**, 22.0; **10b**, 15.1; **11a**, 20.8; **11b**, 11.2.

Circular Dichroism. Nucleosides and dinucleoside phosphates were dissolved in a phosphate buffer solution (0.1 M, pH 7, 1 M NaCl) using cells with 1 cm path lengths. The temperature was increased in 10 °C intervals from 20 to 80 °C. CD spectra were obtained in the 225−330 nm range at 0.2 nm intervals. The buffer baseline at each temperature was subtracted from the sample data and the resulting spectrum smoothed and converted in molar ellipticity per residue [θ]. Concentrations were based on the UV absorbance at 267 nm for thymidine **2b** and at 268 nm for methoxythymidine **2a**, assuming an extinction coefficient (ϵ) of 9.65×10^3 and $9.52\ 10^3\ M^{-1}\ cm^{-1}$, respectively:[46,12] concentration of **2b** 0.108 mM and concentration of **2a** 0.0921 mM. Concentrations of **1a** and **1b** were calculated using their optical density at 80 °C and considering that, at this temperature, stacking interactions can be neglected and, therefore, their extinction coefficients (per residue) are the same as those for their corresponding monomers: concentration of **1a** 0.0528 mM and concentration of **1b** 0.0717 mM.

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Supporting Information Available: Text giving general methods, figures giving ^{1}H (1D and COSY) NMR spectra of **1a**, the ^{1}H NMR spectrum of the crude irradiation mixture of **1a**, HPLC chromatograms of the photolysis of **1b**, **1a**, and a mixture of **1b** and **1a**. and peak areas of the HPLC chromatograms (230 nm) of the photolysis of a mixture of **1a** and **1b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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