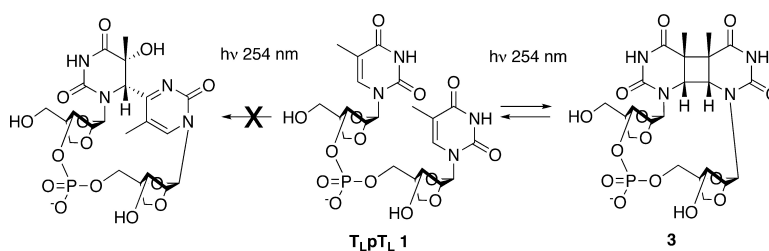Communication

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The Sugar Conformation Governs (6-4) Photoproduct Formation at the Dinucleotide Level

Céline Desnous,[†] B. Ravindra Babu,[‡] Céline Moriou,[†] Javier Ulises Ortiz Mayo,[†] Alain Favre,[§] Jesper Wengel,[‡] and Pascale Clivio*,[†,#]

Institut de Chimie des Substances Naturelles, CNRS, 1 Avenue de la Terrasse, 91190 Gif sur Yvette, France, Nucleic Acid Center, Department of Physics and Chemistry, University of Southern Denmark, 5230 Odense M, Denmark, and Laboratoire de Photobiologie Moléculaire, Institut Jacques Monod, CNRS, Université Paris 6 et Paris 7, 2 Place Jussieu, 75251 Paris cedex 05, France

Received September 13, 2007; E-mail: pascale.clivio@univ-reims.fr

Exposure of DNA to solar UV light induces structural modifications mostly in the form of covalent bond formation between two adjacent pyrimidines. As a result, two major types of DNA photoproducts (PPs), cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone photoproducts ((6-4) PPs), are formed (Figure 1).[1] These DNA lesions are implicated in photoaging and skin cancer and induce differential biological effects.[2−4]

In addition to the intrinsic photophysical properties of the targeted nucleobases, the formation efficiency of photoproducts is also known to depend upon the local structure of DNA which can either promote or prevent the formation of a given PP.[5] Consequently, identification of the precise conformational parameters governing PP formation is central to understanding the DNA specific photoreactivity and therefore its subsequent biological effects. Thymidylyl(3′-5′)thymine (TpT, 2) represents the shortest and most photoreactive single-stranded DNA sequence able to mimic DNA photoreactivity by giving rise to dipyrimidine PPs.[6,7] In addition, in TpT, the furanose moieties exist in a dynamic equilibrium between C2′-endo (major) and C3′-endo (minor) conformations as observed in B-DNA.[8]
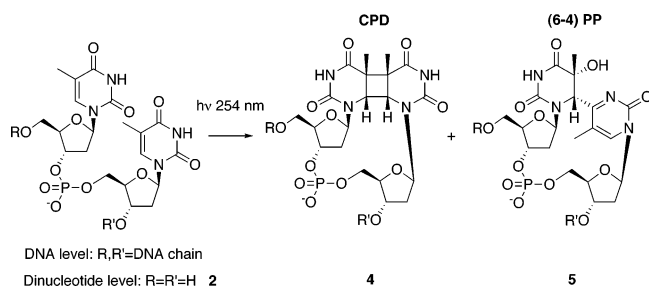

Figure 1. Chemical structure of CPD and (6-4) PP at dithymine site in DNA and in 2 (TpT).

The photochemistry of several TpT analogues has already been studied to tentatively decipher the relationships linking the sugar−phosphate backbone conformation to PP formation.[9−11] Whereas the yield of formation and the relative distribution of PPs have been shown to be clearly influenced by the dinucleotide backbone structure, only peptide nucleic acids (PNAs), DNA analogues with no sugar and no phosphate residues, have demonstrated a unique photoreactivity leading exclusively to CPD.[9] Independently, promotion of the population of the C3′-endo sugar conformers in TpT analogues has been demonstrated to increase the intramolecular base
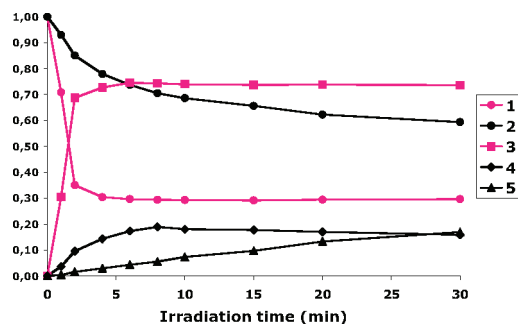
† Institut de Chimie des Substances Naturelles.
‡ Nucleic Acid Center.
§ Laboratoire de Photobiologie Moléculaire.
# Current address: FRE 2715 CNRS, Université de Reims Champagne-Ardenne, 51 rue Cognacq Jay, 51 096 Reims cedex, France.

Figure 2. Fractional amount of **1** and **2** and of their respective PPs as a function of irradiation time.

stacking ability and the quantum yield of formation of CPD and (6−4) PPs.[10,11] Therefore, finding a dinucleotide model whose sugar conformation could selectively and efficiently drive the thymine photoreactivity was a major challenge. We herein report the striking photochemical properties of **1**, the locked nucleic acid (LNA) dinucleotide analogue of **2**[12] in which the furanose moieties are locked in the C3′-endo conformation.

The 254 nm photoreactivity of **1** was compared to that of **2** using our recently published hplc-based method.[11] After 2 min of irradiation, 65% of **1** had reacted compared to only 15% of **2** (Figure 2). This clearly evidenced the dramatically increased photoreactivity of **1**, compared to **2**, induced by the conformational locking of the sugar moieties. Even more remarkably, the 254 nm irradiation of **1** afforded a single PP (**3**) (Scheme 1) in contrast to **2** in the limit of ^{1}H NMR and UV detection. Absence of UV absorption above 240 nm in the UV spectrum of **3** was indicative of a cyclobutane-type structure. Examination of the ^{1}H NMR spectrum of a crude irradiation mixture of **1** confirmed the presence of the characteristic features of a CPD structure: the absence of H6 signals and the presence of two shielded methyl signals below 1.5 ppm (Figure S9, Supporting Information).[13] The cyclobutane nature of **3** is also fully consistent with the steady-state reached after 5 min of irradiation (Figure 2), CPD being known to photoreverse at 254 nm.[6] After purification, CPD **3** was isolated in 53% yield together with 19% of recovered **1**. The stereochemistry of **3** was deduced from NOEs to be cis−syn as in the case of the TpT-derived major CPD (**4**) (Figure S13). The quantum yield of formation (Φ) of **3** was evaluated to be $(6.8 \pm 0.8) \times 10^{-2}$ mol/einstein using a previously described procedure[11] which considers partial photo-reversion. Compared to the TpT series,[14] this reveals a formation efficiency of CPD in the LNA series ca. 6 times higher.

To understand the unprecedented photoreactivity of **1**, we investigated its stacking properties by circular dichroism (Supporting Information). Taking into account the monomer contribution, the
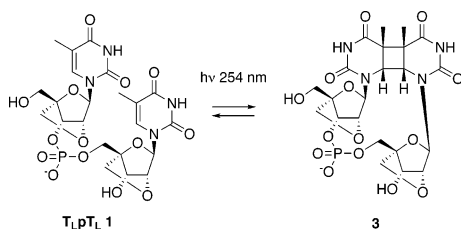
Scheme 1

T_LpT_L **1**   **3**

shape of the CD spectrum of **1** at 20 °C is very similar to the one of **2** with, however, a slight red-shift (2 nm) of λ_{max} (281 nm) and λ_{min} (257 nm) and a roughly 3.7-fold increase in $[\theta]$ at λ_{max} (Figure 3). Therefore, the overall stacking geometry appears similar in the two dinucleotides with **1** exhibiting a roughly 4-fold propensity to base stack than **2**.[15]

The strong base-stacking propensity of **1**, whose sugar moieties exclusively exist as C3′-endo conformers substantiates our previous statement that the percentage of C3′-endo conformers is one of the major factors influencing the dinucleotide stacking ability.[10a,11] In addition, the absence of trans−syn CPD formation from **1** is in line with strong base-stacking interactions.[7,16]

During the first 2 min of irradiation, compound **1** appears (5.7 ± 1)-fold more photoreactive than standard TpT (using the quantum yields ratio). This result is in line with the increased stacked efficiency of **1**, compared to **2**, and further confirms the relationship between stacking and overall photoreactivity.[10,11] In the closely related 2′-α-OMe series, the 5′-end and the 3′-end C3′-endo sugar conformer population is 75% and 66%, respectively (versus 30% and 37%, respectively, in **2**).[10a] However in this series, if the base stacking ability and photoreactivity are simultaneously increased, compared to TpT, the increase in C3′-endo population does not significantly alter PP distribution.[10] In contrast, in the case of **1** where both sugar puckers are fully constrained in the conformationally rigid C3′-endo sugar pucker, (6-4) PP formation is impeded. Consequently, it is likely that the C2′-endo sugar conformation is necessary for (6-4) PP formation. Moreover, this study demonstrates for the first time that in the dinucleotide series, CPD and (6-4) PP result from distinct photoreactive stacked nucleobase patterns. In addition, since the CD λ_{max} and λ_{min} in the LNA and the 2′-α-OMe
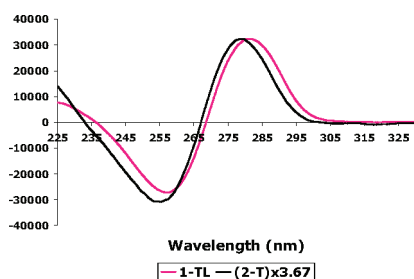

Figure 3. CD difference spectra between **1** and its corresponding mononucleoside thymidine LNA (TL) and between **2** and thymidine (T) multiplied by a factor of 3.67 at 20 °C, in a buffer containing 0.01 M Na phosphate, 0.1 M NaCl, pH 7.0.

series[10a] coincide, indicating a very close overall geometry of stacked species, it can be concluded that (6-4) PP formation results from minor stacked species.

Our results clearly bring a new enlightenment to the field of DNA photoproduct mechanistic studies. In addition, they provide an easy and efficient access to a cis−syn CPD analogue for biological studies after incorporation into oligonucleotides. This study also points up the identical and unique photochemical behavior of the bis thymine LNA and PNA dinucleotide analogues, demonstrating their similar base-stacking patterns and therefore similar preorganized conformation. This could bring new insights in the comprehension of their unprecedented thermal stabilities toward complementary DNA and RNA, which have allowed the development of numerous applications in biotechnology and therapeutics.[17]

Supporting Information Available: Synthesis procedures, experimental conditions for UV irradiation, HPLC, CD spectrum of **1** at various temperatures, chromatogramms of the photolysis of an equimolecular mixture of **1** and **2**, ^1H NMR spectrum of the photolysate of **1** and 1D and 2D NMR spectra of **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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