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Far UV Irradiation of a Bis-thymine PNA Dimer: Conformational Implications

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Abstract : *Short wavelength (254 nm) irradiation of the bis-thymine PNA dimer* ***5*** *led to the formation of the cis-syn cyclobutane pyrimidine dimer* ***6*** *as the major photoproduct.* © 1998 Elsevier Science Ltd. All rights reserved.

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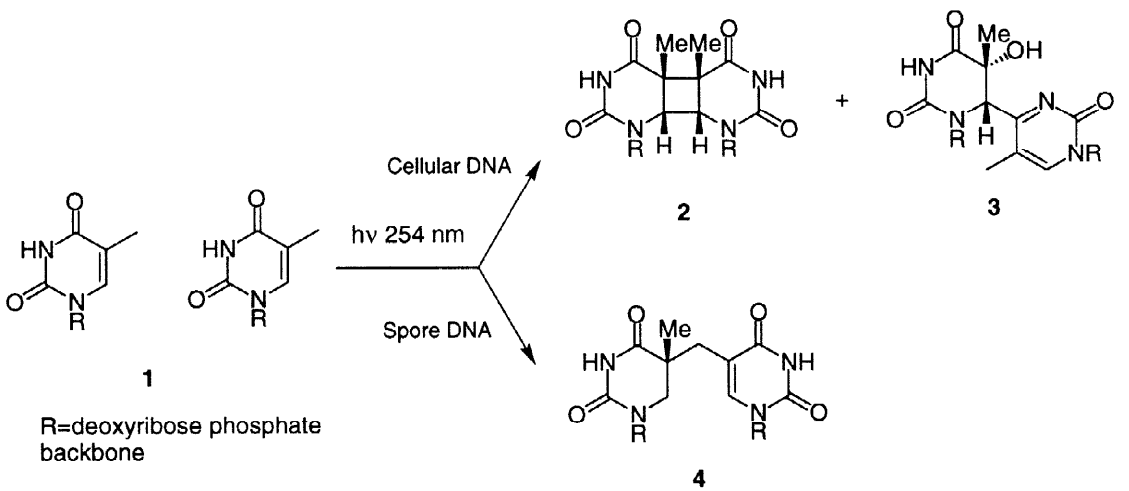
It is now well established that the DNA conformation significantly alters the photoreactivity and the type of UV-induced photoproducts.[1] As the most significant example, exposure of cellular B-DNA to UV light produces two major types of photoproducts at dipyrimidine sites (**1**): the cyclobutane pyrimidine dimers (CPD, **2**) and the (6-4) pyrimidine-pyrimidone photoproducts (**3**)[1] (Scheme 1). In contrast, UV irradiation of A-DNA in bacterial spores results exclusively in the formation of 5,6 dihydro-5-(α-thyminyl)thymine **4**, termed "spore photoproduct"[2] (Scheme 1).



1 **2** **3** **4**

Scheme 1

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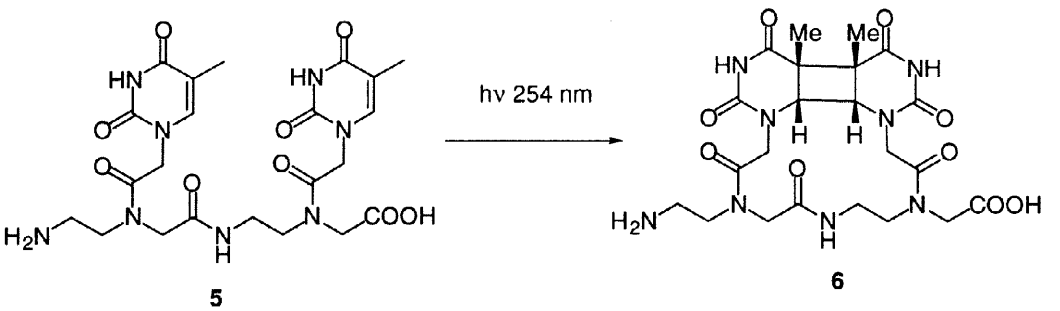
PNAs are highly promising nucleic acid analogues[3] in which the deoxyribose phosphate backbone has been replaced by a pseudopeptide chain. Although the solid and solution state conformation of mixed PNA duplexes or triplexes has been investigated[4a-c], little is known about the preorganization of PNA at the single-stranded level.[4d] Recently, we used a photochemical approach based on the remarkable sequence-dependent photoreactivity of 4-thiothymine observed in the dinucleotide series[5] to probe the conformation of PNA at the single-stranded dimer level[6,7] and showed that the backbone of PNA was structured, but differently from that of the corresponding dinucleotide.

Continuing our efforts at probing photochemically the structuration of PNA in solution, we now report our preliminary results on the photochemical reactivity of bis-thymine PNA **5**[8] under unsensitized irradiation conditions.



5 **6**

Scheme 2

Analysis of the ^1H NMR spectrum of the crude irradiation mixture showed that irradiation of an aqueous solution of **5** at 254 nm[9] had led to the formation of a major photoproduct **6**[10] that composed *ca* 50% of the mixture after half-consumption of **5** and that did not belong to the (6-4)[11] or spore photoproduct series[12] since no extra protons appeared above 7 ppm. This was confirmed by the lack of new UV absorption above 240 nm on the 2D chromatogram of the crude irradiation mixture. The mass spectrum (LSIMS) of **6**, isolated after preparative HPLC[13] displayed a molecular ion at *m/z* 557 corresponding to M+Li. Absence of UV absorption above *ca* 240 nm and presence, on the ^1H NMR spectrum (Figure 1), of two methyl singlet signals at 1.57 and 1.45 ppm and two H6 protons at 4.44 and 4.04 ppm confirmed a cyclobutane type structure.[14] The vicinal coupling constant between the two H6 protons of 5.3 Hz suggested a cis-syn stereochemistry of the cyclobutane.[14a] This was unambiguously confirmed by a phase sensitive NOESY spectrum[15] in which strong intra- and interresidue correlations of comparable importance were observed (Figure 2). The difference between the cross-section at the methyl resonances indicated that there is a preferred puckered conformation of the cyclobutane ring-system.[14b]

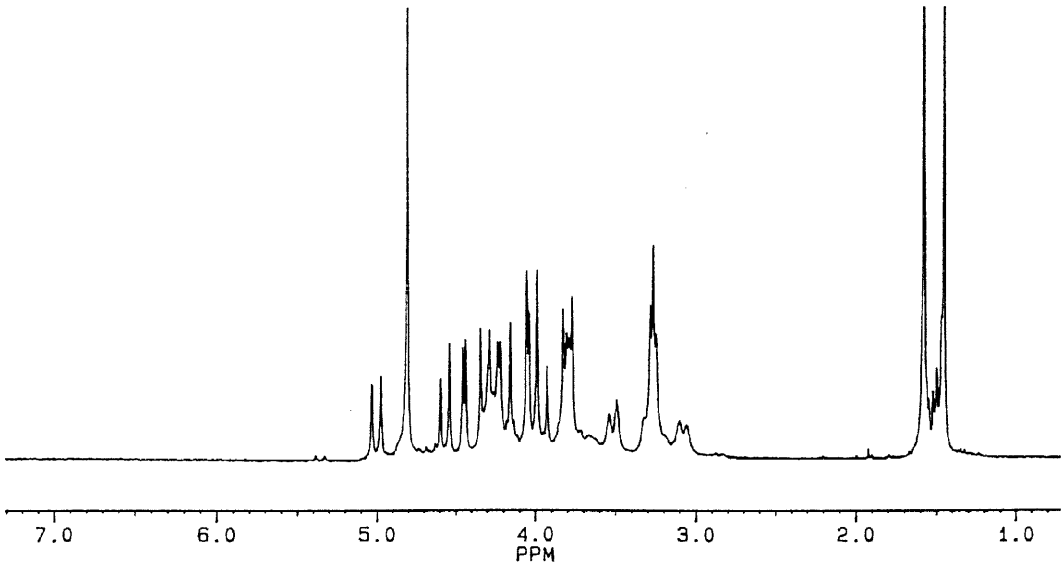


Figure 1: ^{1}H NMR spectrum (300 MHz, D_2O) of photoproduct **6**.

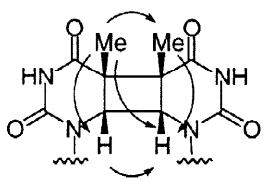


Figure 2: Selected nOes of **6**.

In conclusion, we have isolated and characterized the cis-syn cyclobutane pyrimidine dimer **6** as the major photoproduct from the photolysate of **5** whereas no trace of (6-4) or spore photoproducts were detected. Interestingly, 254 nm irradiation of TpT in aqueous solution produces 23% of cis-syn CPD **2** and 20% of (6-4) photoproduct **3**.[10,16] Our results indicate that the prerequired conformation needed for (6-4) adduct formation is not available in PNA and reinforce the notion of constrained flexibility or structuration of the PNA backbone even at the single stranded dimer level. Additionally, whereas our previous results in the 4-thiothymine PNA series leading exclusively to (α-thyminyl)thymine derivatives[6,7] and the P conformation observed for duplex or triplex PNAs[4a-c] led us to expect some spore photoproduct formation, absence of any detectable 5,6-dihydro-5-(α-thyminyl)thymine adduct under our experimental conditions shows that at the single-stranded level, PNAs adopt a conformation very different from that of A-type stacking. Obtention of a major cyclobutane photoadduct shows that, at the single-stranded level, the base geometry within PNA is unique. This leads to a photochemical behavior partially reminiscent of the B-like conformation. Finally and very interestingly, compound **6** represents a readily available DNA-photolesion analogue. When embedded in an oligonucleotide, this analogue could be a valuable tool to study, at the molecular level, the importance of the sugar-phosphate backbone on the lesion recognition process by repair enzymes such as photolyases.

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8 Compound **5** was synthesized following procedures published ref 7. At rt in D_2O, compound **5** presented a mixture of several rotamers due to restricted rotation around tertiary amide bonds and consequently displayed a complex ^1H NMR pattern. ^{13}C NMR (75.5 MHz, D_2O): δ 173.2, 172.9, 172.1, 171.1 170.9, 170.8, 170.1, 169.7, 169.5, 167.2, 166.9, 152.5, 144.1, 143.7, 143.5, 143.3, 111.1, 110.9, 110.6, 50.7, 49.6, 49.3, 48.7, 48.5, 48.3, 47.6, 47.1, 46.4, 38.2, 37.7, 11.8; MS (LSIMS$^+$, NBA, LiCl) *m/z* 557 $(M+Li)^+$*m/z* 563 $(M+2Li-H)^+$*m/z* 569 $(M+3Li-2H)^+$

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10 ^{13}C NMR data for **6** (75.5 MHz, D_2O): δ 176.1, 175.4, 172.5, 171.2, 154.9, 63.8, 61.6, 53.4, 51.7, 50.9, 50.5, 47.8, 47.5, 46.3, 44.1, 38.5, 38.1, 18.0, 17.6

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