Tetrahedron Letters, Vol. 33, No. 12, pp. 1615-1618, 1992
Printed in Great Britain

0040-4039/92 $3.00 + .00
Pergamon Press Ltd

Novel Insight into the Stereochemical Pathway Leading to (6-4) Pyrimidine-Pyrimidone Photoproducts in DNA

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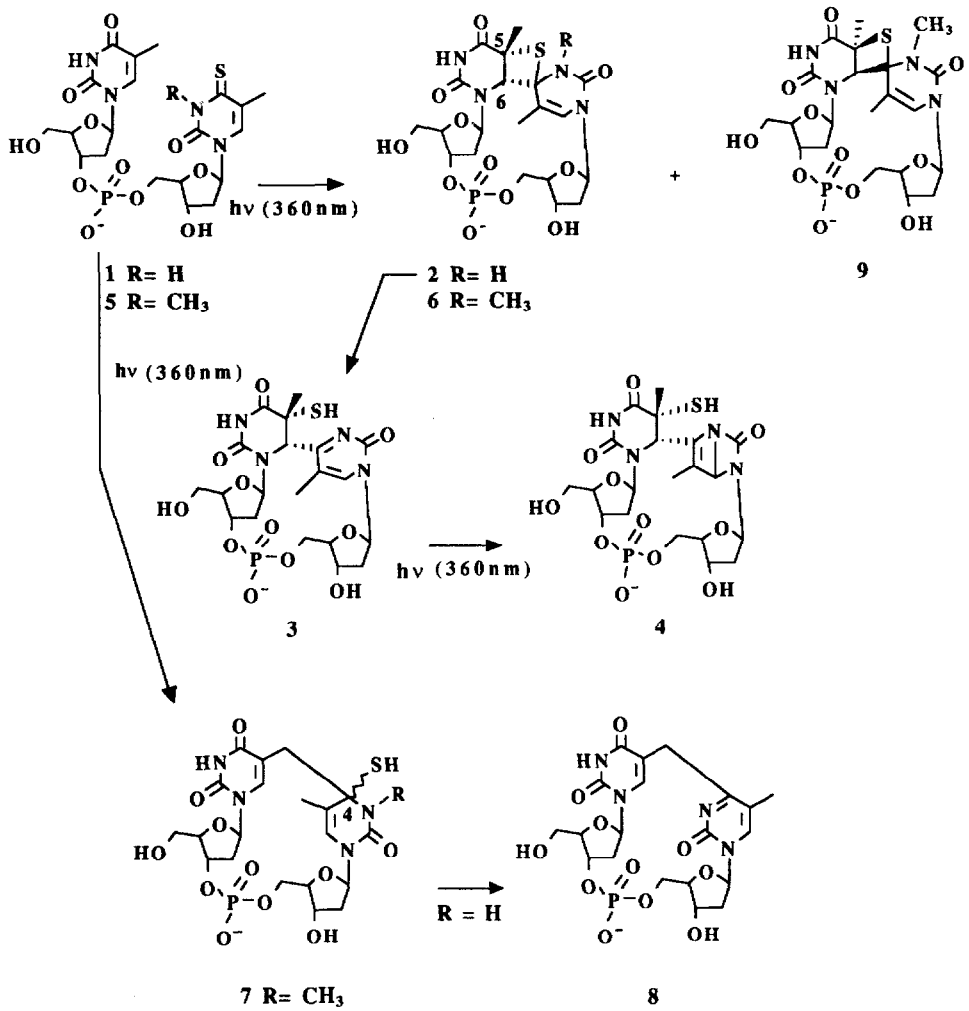
Key words: photochemistry; DNA; "6-4" lesions; UV light; skin cancer.

Abstract: Irradiation of the dinucleoside phosphate **5** led to three photoproducts which were fully characterized, the formation of thietane **9** provides evidence suggesting a novel minor pathway to "6-4" lesions in DNA.

The (6-4) pyrimidine-pyrimidone photoproducts which correspond to the second most abundant DNA photolesions produced by the ultraviolet portion of the solar spectrum may play a major role in mutagenesis and in inducing human skin cancer[1]. The first step in the formation of these lesions, occurring at bipyrimidine sequences, presumably involves a (2+2) cycloaddition between the C-5/C-6 double bond of a 5'-pyrimidine base and the C-4 carbonyl or imine of a 3'-pyrimidine base to give a four-membered heterocyclic intermediate (oxetane or azetidine).

To investigate the mechanism of (6-4) photoproduct formation, we have recently devised a model system based on the photochemisty of the dinucleoside phosphate thymidylyl(3'-5')-4-thiothymidine (**1**). When irradiated, this produced a moderately stable thietane intermediate **2**[2], whose stereochemistry was determined to be C-5 *R* and C-6 *S* in confirmation of all other known (6-4) bipyrimidine adducts[3]. In order to understand better the stereochemical course of the first step of this photochemical pathway, reminiscent of the DNA photodamaging process, we have endeavoured to stop the reaction at the thietane stage and avoid its further transformation into secondary products such as the (6-4) pyrimidine-pyrimidone **3** and its Dewar isomer **4**.



We reasoned that this should be possible if the N^3 position of 4-thiothymidine was methylated as in thymidylyl(3'-5')-3-N-methyl-4-thiothymidine (**5**)[4] abbreviated[6] Tpm^3s^4T. We also anticipated that the other photochemical route to give **8** from **1** would be either inhibited or yield the primary photoproduct **7**. We herein report the successful trapping of the primary photoproducts of **5**. These were characterized as the foreseeable adducts **6** and **7** together with thietane **9** which, compared to **6**, shows inverse configurations at carbons C-5 and C-6. Its formation places in evidence another practicable stereochemical pathway to "(6-4) lesions" in DNA which, to our knowledge, has never been proposed.

The three photoproducts **6**, **7** and **9**, formed by irradiation of **5** in water, were isolated after reverse phase HPLC in 25, 24 and 2% yield, respectively. Compounds **6** and **9** were identified as thietane derivatives whereas adduct **7** resulted from hydrogen abstraction from the methyl of the 5'-base and addition of the resulting radical onto carbon C-4 of the 3'-base. The formation of **6** and **7** paralleled that of **2** and **8** when starting from **1**[2].

Fab MS data (positive mode) indicated that photoproducts **6** and **9** have the same molecular weight as **5**. Both their ^{1}H NMR spectra display characteristic H-6 proton signals[7]. Shielding of the -pm^{3}s^{4}T base H-6 proton of **6** and **9** (δ: 6.44 and δ: 6.48 ppm, respectively) compared to **5** (δ: 7.74 ppm) indicates the lack of conjugation of the 3'-pyrimidine base. The large upfield shift experienced by the H-6 proton of the Tp- part of each thietane (δ: 5.18 ppm for **9** and δ: 4.97 ppm for **6**) with respect to **2** (7.63 ppm) is consistent with the saturation of the 5'-pyrimidine as well as the absence of any UV. absorption above 260 nm in their u.v. spectra.

Comparison between the ^{1}H NMR spectra of **6** and **9** showed that they mainly differ by the chemical shift of their Tp- part H-1' proton signal which is shifted 1.22 ppm upfield in the case of **9** compared to **6** (5.09 ppm vs 6.31 ppm) supporting the inversion of the configuration at positions C-5 and C-6 of the Tp- part.

Finally, configurational assignments at carbon C-5 and C-6 of the Tp- unit of **6** and **9** were deduced from 2D phase sensitive NOESY spectra. In both cases, observation of an NOE between the H-6 proton and the methyl protons of the Tp- part established their cis relationship. The 3' -glycosyl conformations of **6** and **9** are anti as demonstrated from the observed cross peaks between the H-6 proton of the base and the H-2' and H-3' protons of its corresponding sugar. In the case of **6**, the H-6 Tp- proton gave an NOE with both H-2' and H-3' of its sugar and with the methyl protons of the -pm^{3}s^{4}T base. These correlations confirm an anti orientation of the 5'-glycosidyl bond and determine the C-5 *R* and C-6 *S* configuration, respectively. This stereochemistry results from a cycloaddition between the pyrimidinyl units in an anti glycosyl conformation. In the case of **9**, the H-6 Tp- proton gave an NOE with H-1' of its deoxyribose and the N^3-methyl protons of the -pm^{3}s^{4}T base which indicated a syn orientation of the 5'-glycosidyl bond and then a C-5 *S* and C-6 *R* configuration, respectively. Thus, the C-5 *S* and C-6 *R* configurations establishes that the cycloaddition to give **9** proceeds with a syn glycosyl conformation for the 5'-unit and an anti glycosyl conformation for the 3'-unit.

These results can be directly compared to those observed in the case of cyclobutane pyrimidine photodimer formation, the other major lesion occurring in DNA. In UV exposed DNA and dinucleotide models, the predominant dimer (cis-syn cyclobutane) results from the dimerization of two adjacent pyrimidinyl units both in anti glycosyl conformation, whereas the minor adduct is generated from an

intermediate having a 5'-unit in a syn glycosyl conformation[1b,8]. Hence the stereochemistry of the major adducts in both types of lesion (cyclobutane and "6-4 lesions") is the one that would be predicted to occur in B-form DNA where the glycosyl conformations are anti, thus precluding the formation of the minor adducts which might preferentially be produced in single stranded native and denaturated DNA[9].

References

(1) (a) D. L. Mitchell, and R. S. Nairn, *Photochem. Photobiol.*, 1989, **49**, 805; (b) J. Cadet, and P. Vigny, *The Photochemistry of Nucleic Acids*, Ed. H. Morrison, Wiley and Sons, New York, 1990, **1**, 79; (c) J. E. LeClerc, A. Borden, and C. W. Lawrence, *Proc. Natl. Acad Sci. USA*, 1991, **88**, 9685.
(2) P. Clivio, J.-L. Fourrey, J. Gasche, and A. Favre, *J. Amer. Chem. Soc.*, 1991, **113**, 5481.
(3) (a) R. E. Rycyna, and J. A. Aldefer, *Nucleic Acids Res.*, 1985, **13**, 5949; (b) J. S. Taylor, D. S. Garrett, and M. J. Wang, *Biopolymers*, 1988, **27**, 1571; (c) J. S. Taylor, H. F. Lu, and J. J. Kotyk, *Photochem. Photobiol.*, 1990, **50**, 161.
(4) Dideoxynucleotide **5** was prepared from 5'-O-(4,4'-dimethoxytrityl)thymidin-3'-yl H-phosphonate and 3'-O-acetyl-3-N-methyl-4-thiothymidine using published procedures[5].
(5) (a) P. J. Garreg, J. Redberg, J. Stawinski, and R. Strömberg, *Chemica Scr.*, 1986, **26**, 59; (b) B. Froelher, P. Ng, and M. D. Matteuci, *Nucleic Acids Res.*, 1986, **14**, 5399; (c) R. Strömberg, and J. Stawinski, *Nucleic Acids Res., Symposium Series*, 1987, **18**, 185.
(6) W. E. Cohen, N. J. Leonard, and S. Y. Wang, *Photochemistry and Photobiology of Nucleic Acids*, Ed. S. Y. Wang, Academic Press, New York, 1976, **2**, 403.
(7) All resonance assigments were performed using 2D- COSY and NOESY techniques.
(8) T. M. G. Koning, J. J. G. van Soest, and R. Kaptein, *Eur. J. Biochem.*, 1991, **195**, 29.
(9) Selected MS, UV and NMR (400 MHz, D_2O) data for compounds **5**, **6**, **7** and **9**:

-**5**: fab(+) 577 (M+1); uv λ_{max} 330 and 267 nm; ^{1}H nmr (ppm): 7.74 (H-6, -pm^{3}s^{4}T); 7.63 (H-6, Tp-); 6.29 (H-1', -pm^{3}s^{4}T); 6.12 (H-1', Tp-); 3.69 (N-Me); 2.10 (Me,-pm^{3}s^{4}T); 1.85 (Me, Tp-).
-**6**: fab(+) 577 (M+1); ^{1}H nmr (ppm): 6.44 (H-6, -pm^{3}s^{4}T); 6.33 (H-1', -pm^{3}s^{4}T); 6.31 (H-1', Tp-); 4.97 (H-6, Tp-); 3.34 (N-Me); 2.24 (Me, -pm^{3}s^{4}T); 1.83 (Me, Tp-).
-**7**: fab(+) 565 (M-34+Na$^+$); uv λ_{max} 267 nm; ^{1}H nmr (ppm): 7.27 (H-6, Tp-); 6.68 (H-6, -pm^{3}s^{4}T); 6.36 (H-1', Tp-); 6.19 (H-1', -pm^{3}s^{4}T); 2.94 (N-Me); 3.20-2.58 (CH_2, J= 14.6 Hz); 1.97 (Me, -pT).
-**9**: fab(+) 577 (M+1); ^{1}H nmr (ppm): 6.48 (H-6, -pm^{3}s^{4}T); 6.22 (H-1', -pm^{3}s^{4}T); 5.18 (H-6, Tp-) ; 5.09 (H-1', Tp-); 3.47 (N-Me); 2.04 (Me, -pm^{3}s^{4}T); 1.94 (Me, Tp-).

(Received in France 8 January 1992)