



# Photochemistry of the Thio-analogue of the DNA (6-4) Photoproduct Dewar Valence Isomer: Structure of a New Photoproduct

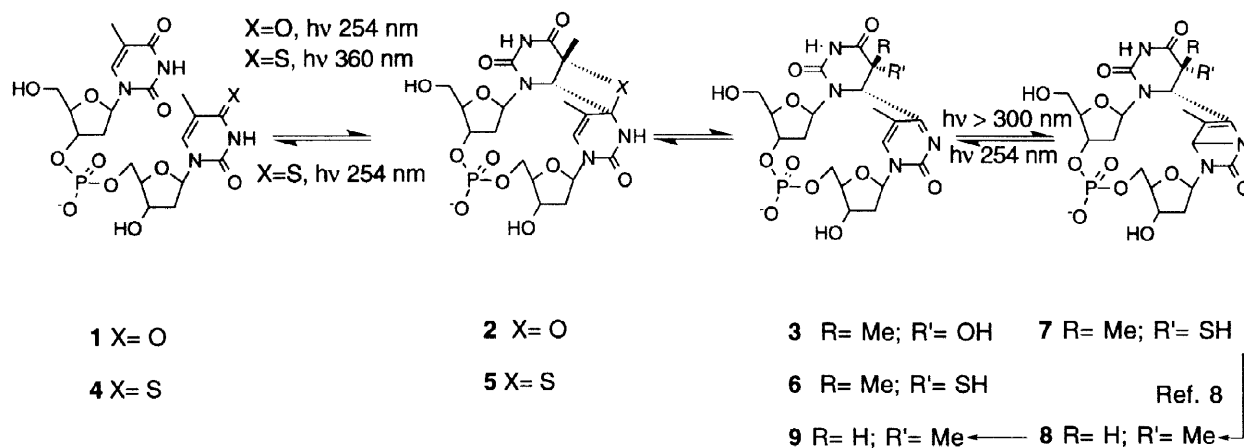
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**Abstract :** Short wavelength (254 nm) irradiation of the thio-analogue **7** of the Dewar valence isomer of the DNA (6-4) pyrimidine-pyrimidinone photoproduct led to the formation of a "tetramer" which has been given structure **10**. © 1997 Elsevier Science Ltd. All rights reserved.

Cyclobutane pyrimidine photoproducts (CPDs) and (6-4) pyrimidine-pyrimidinone photoproducts [(6-4)PPs] are the major mutagenic and carcinogenic photoproducts of DNA.<sup>1</sup> Presently, considerable efforts are being made to elucidate the repair mechanisms of UV induced DNA damages occurring at TpT (or TpdC) sites. It is now well established, in the case of CDPs, that one such a repair mechanism is carried out enzymatically by DNA photolyases in the presence of visible light.<sup>2</sup> More recently, during the isolation of photoreactivating enzymes, evidence has been provided for a repair pathway of DNA which can reverse the (6-4)PPs (**3**) to the parent dinucleotides TpT (**1**).<sup>3</sup> This photoenzymatic reversal of the (6-4) pyrimidine-pyrimidinone photoproduct **3** is thought to occur by means of a two step process.<sup>4</sup> Thus, the proposed mechanism postulates first the formation of an oxetane **2** to be further splitted to give two thymines. Interestingly, in a recent work Falvey *et al.* have described a model system in which splitting of an oxetan, closely related to (6-4) photoadducts, was triggered by a photoinduced electron transfer.<sup>5</sup>

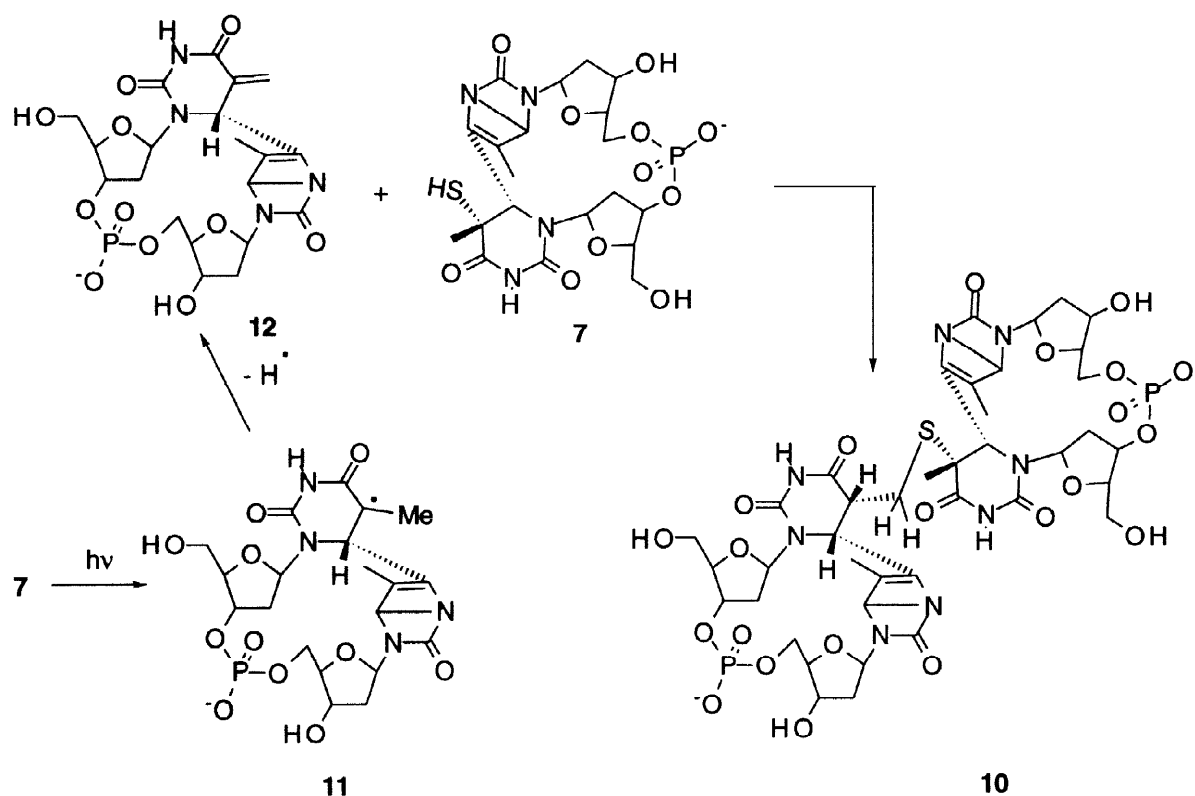


Recently, examination of this mechanism could benefit from the ready formation of the thio-analogue **5** of the postulated oxetan **2**.<sup>6</sup> Indeed, whereas the oxetan leading to (6-4) photoproducts appeared not to be stable above -80 °C<sup>1</sup> we observed, by irradiating thymidyl-3',5'-(4-thiothymidine) (**4**, Tps<sup>4</sup>T), the high yielding formation of thietane **5** which was found to interconvert with its corresponding s<sup>5</sup>-(6-4) pyrimidine-pyrimidinone photoproduct **6** at room temperature. Longer irradiation time led to the total conversion of compound **6** into its Dewar valence isomer **7**.<sup>6</sup> Finally, at the dimer level, the components of this system

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could be isolated and characterized, proving to be valuable model compounds for the photoenzymatic reversal reaction studies.<sup>7,8</sup>

Indeed, recent studies on the photochemical behaviour of the *s*<sup>5</sup>-Dewar photoproduct **7**, either embedded in an oligonucleotide<sup>7</sup> or at the dimer stage,<sup>8</sup> derived from the *s*<sup>5</sup>-(6-4) pyrimidine-pyrimidinone photoproduct **6**, led to divergent results. We observed that, in contrast to the *s*<sup>5</sup>-(6-4) pyrimidine-pyrimidinone adduct **6**, the corresponding *s*<sup>5</sup>-Dewar valence isomer photoproduct **7** could not be used to study the photoreversion of the (6-4)PPs of DNA since after a prolonged irradiation (254 nm) the compound was found to be essentially desulfurized to give presumably **8** which, subsequently, underwent a transformation into the stable pyrimidinone **9**.<sup>8</sup>



Scheme 2: For the sake of clarity, in the text each dimer of **10** are designed TpT (left side) and T'pT' (right side), respectively. Tp (or T'p) and pT (or pT') stand for the pyrimidine in the 5'- and 3'-end of their respective dimer.

Herein, in continuation of our study of the photochemical behaviour of the *s*<sup>5</sup>-Dewar photoproduct **7**, we report the structural elucidation of an unexpected photoproduct **10** which was isolated in about 7% yield after preparative HPLC purification.<sup>9</sup> The MALDI-TOF mass spectrum of the new product exhibited a quasi molecular ion at  $m/z$  1089.3 corresponding to M-1 suggesting the compound to result from the coupling of two dinucleoside phosphates **7** ( $M=562$ ) with the elimination of the elements of  $H_2S$ . The key structural arguments to confirm this initial interpretation were deduced from the <sup>1</sup>H nmr data. The <sup>1</sup>H nmr spectrum of **10** showed three methyl singlet signals only. One methyl at 1.70 ppm was suggested to be attached to a  $sp^3$  quaternary carbon, while the two others signals at 2.13 (pT') and 2.15 ppm (pT), respectively, could be ascribed to two vinylic methyls. These spectral observations suggested that one methyl group had given rise to a methylene whose two protons appeared as two well separated signals at 2.62 (dd,  $J=13.5$  and 4.6 Hz) and 3.45 (dd,  $J=13.5$  and 10.5 Hz) ppm, respectively. The multiplicity of these methylene protons indicated that they were attached to a methine carbon.

The lower field region (between 6.35 and 4.90 ppm) of the  $^1\text{H}$  nmr spectrum of **10** showed seven signals (8 protons) of high structural significance. The two H-1' of Tp and T'p appeared as a multiplet centered at 6.31 ppm (2H), while those of the pT parts were observed as typical triplets (5.65 and 5.63 ppm,  $J = 7$  Hz). On the other hand two signals, appearing as sharp singlets at 5.37 and 5.36 ppm, could be considered as characteristic of the H-6 protons of the Dewar valence isomers of both pyrimidones.<sup>10</sup> Moreover, the presence of the Dewar structure in compound **10** confirmed our earlier findings indicating that this motif is relatively stable under 254 nm irradiation conditions.<sup>8</sup> Finally, the signals of the two other H-6 protons of the Tp and T'p parts were detected as a doublet (5.13 ppm,  $J = 6.1$  Hz) and a singlet (4.92 ppm), respectively, confirming the saturation of these two pyrimidines and in agreement with the absence of UV absorption above 230 nm. In the case of the doublet the corresponding proton is coupled with the H-5 of Tp which gave a multiplet at 3.51 ppm. All these attributions have been confirmed by 2D nOe<sup>11</sup> which also provided other key arguments in favour of structure **10**. Thus, the strong interaction, which was revealed between the higher shielded methylene proton and the methyl of T'p, was decisive for our structural attribution. Finally, the relative configuration for the C-5 and C-6 centers of Tp was determined on the basis of the value ( $J = 6$  Hz) of the coupling constant between the corresponding H-5 and H-6 which is in favour of a cis relationship.<sup>8</sup> This indicates that as in the case of **8** the configuration at C-5 of Tp has been inverted.

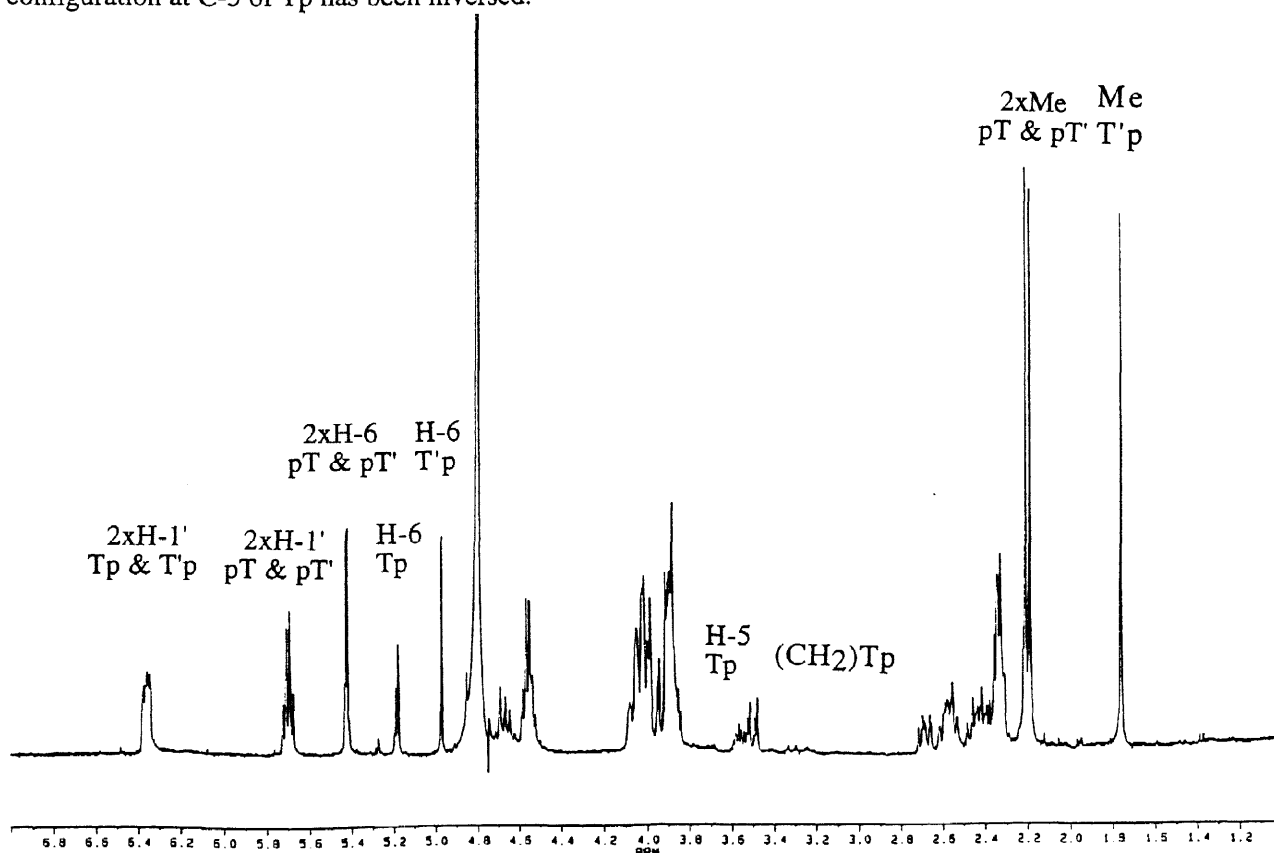


Figure:  $^1\text{H}$  NMR spectrum (400 MHz,  $\text{D}_2\text{O}$ ) of photoproduct **10**.

The mechanism of formation of **10** can be easily explained, in line with the previous observations which indicate **8** to be the initially main product of the irradiation of **7**. Thus, since **8** must derive from radical **11**, it means that the latter might undergo dismutation to give **8** and **12**. Finally **10** would result from a Michael addition of the thiol **7** upon the methylene of **12**. In this respect, it is worth to mention that in a related study in the *P*-methylphosphonate series, there is a precedent for such a reaction pathway.<sup>12</sup>

In conclusion, we have isolated and characterized, from the photolysate of compound **7**, a new photoproduct which was attributed structure **10** and which has conserved the two Dewar motifs of its parent compound **7**. From this observation it can be 1) concluded that **10** must have been formed at the early stage of the photolysis of **7** and 2) explained why the compound was isolated in low yield since prolonged irradiation triggers rearomatization of the Dewar isomer.<sup>8</sup> However, the importance of such an unusual photoproduct should not be overlooked and it cannot be only considered as a curiosity. Indeed, it is very likely that its formation involves a methacrylamide system as shown in structure **12** to behave as a Michael acceptor. This means that, in a biomolecular context, a similar addition might very likely take place with many types of nucleophiles being either nucleotide or amino acid functional groups. In such a case this would lead to a cross-linking reaction whose elucidation of the pathway is very important to precise at the molecular level the three-dimensional diagnostic value of photaffinity labeling experiments using thio-nucleosides.<sup>13</sup> Finally, addressing the problem of the reversibility of (6-4) pyrimidine-pyrimidinone photoproducts, the isolation of this new photoproduct indicates that a great care must be exercised when using analogues of potential intermediates involved in the formation of DNA photolesions. Interestingly, in this line of research, it is worthy of note that we have recently established that, in contrast to (6-4) photoproducts, their (5-4) analogues can reverse to their parent compounds under mild reaction conditions.<sup>14</sup>

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