Remarkable Photoreversal of a Thio Analog of the Dewar Valence Isomer of the (6−4) Photoproduct of DNA to the Parent Nucleotides

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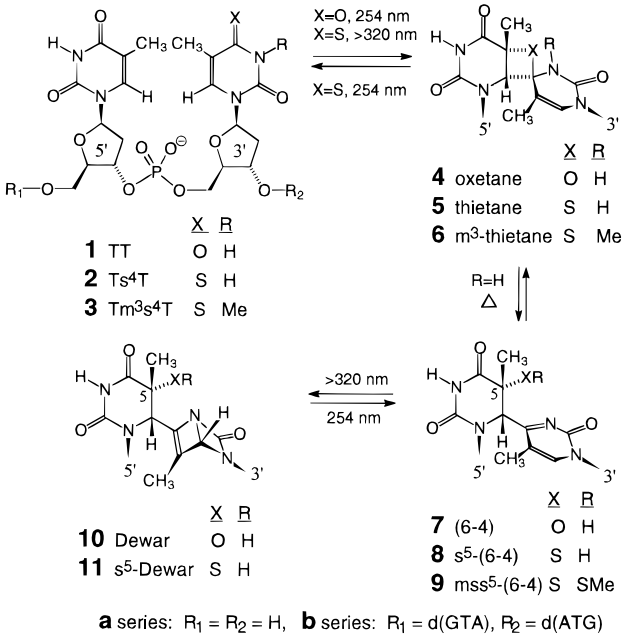
Cyclobutane dimers and (6−4) photoproducts of dipyrimidine sites are the major mutagenic and lethal UV photoproducts of DNA.[1] Until recently, cyclobutane dimers were the only dinucleotide photoproduct known to be directly repaired by photoenzymatic reversal to the parent nucleotides, a process carried out in *Escherichia coli* by the enzyme photolyase in the presence of visible light.[2] In most organisms, visible light does not eliminate (6−4) photoproducts from DNA,[1c,2] although high doses of 300−350 nm light convert the (6−4) product to its Dewar valence isomer (Scheme 1).[3] Recently, there has been a report that *Drosophila melanogaster* cell free extracts restored the biological activity of (6−4) product-containing DNA in a fluorescent light-dependent reaction, concomitant with the loss of (6−4) product antigenic sites and associated alkali-labile sites, suggesting the existence of a (6−4) photoproduct photolyase.[4] Further studies of the extract established that a restriction site inactivated by the (6−4) product of TT could be reactivated with greatest efficiency at 400 nm, confirming the hypothesis that the (6−4) product was indeed being reverted to the parent nucleotides (**7** → **1**).[5] It was proposed that photoenzymatic reversal of the (6−4) product of TT proceeds through the same oxetane intermediate (**4**) proposed to be involved in its formation, by way of both photo-induced electron transfer and thermal steps.[5] Unfortunately, the oxetane intermediate involved in the formation of the (6−4) product does not appear to be stable above −80 °C, making it difficult to study.[6] Recently, stable oxetane adducts between acetophenone or benzaldehyde and *N*,*N*-dimethylthymidine have been shown to reverse by photo-induced electron transfer, lending further support to one class of mechanisms for the enzymatic reaction.[7] What has been lacking, however, is a stable analog of the oxetane intermediate in DNA that could be used for investigations of the enzymatic reaction. In contrast to the presumed oxetane intermediate, its sulfur analog, a thietane, is reported to be stable and to interconvert with the corresponding s^5-(6−4) product.[8] We therefore reasoned that the s^5-(6−4) product might be a useful model substrate for studying the photoenzymatic reaction mechanism. Herein, we report that an attempt to produce the s^5-(6−4) product in an oligonucleotide by photoreversal of its s^5-Dewar valence isomer with 254 nm irradiation unexpectedly led instead to the parent oligonucleotide. We also demonstrate that the likely thietane intermediate, which is in equilibrium with the s^5-(6−4) isomer, also reverses under the same conditions.

Scheme 1



a series: $R_1 = R_2 = H$, **b** series: R_1 = d(GTA), R_2 = d(ATG)

The s^5-Dewar product, **11b**, was prepared by irradiating d(GTATs^{4}TATG)[9] in an argon-purged aqueous solution at 0 °C for 15 min with >320 nm light and identified by the similarity of its UV and ^{1}H NMR spectra to that of the Dewar product of d(GTATTATG), **10b**, that was prepared in a similar fashion from the corresponding (6−4) product, **7b**.[10] In an attempt to produce the s^5-(6−4) product of d(GTATs^{4}TATG), **8b**, the s^5-Dewar product **11b** was irradiated with 254 nm light at room temperature, conditions which are known to revert the Dewar product of TT, **10a**, to the (6−4) product, **7a**.[3a,11] Irradiation produced two new products, one of which (Peak II), unexpectedly coeluted with d(GTATs^{4}TATG) and had a UV absorption maximum at 333 nm that is characteristic of s^4T (Figure 1). When the irradiation was monitored by ^{1}H NMR, signals characteristic of the s^5-Dewar product **11b** (5.30 and 1.65 ppm) disappeared, and those characteristic of d(GTATs^4-TATG) (8.08, 7.44, 5.91, and 1.91 ppm) appeared over 2 h. Signals at 7.70 and 2.35 ppm, characteristic of the (6−4) product of d(GTATTATG) **7b**, were not observed at any stage of the irradiation. The second product (Peak I) lacked an absorption maximum above 300 nm that is also characteristic of the (6−4) product and was not converted to d(GTATs^{4}TATG) by further irradiation at 254 nm, suggesting that it was not the thietane (vide infra).

To investigate whether the thietane could be an intermediate in the photoreversal reaction, use was made of a report that irradiation of d(Ts^{4}T) with 366 nm light leads to a 3:1 equilibrium mixture of the thietane and (6−4) products, **5a** and **8a**,[8a] whereas d(Tm^{3}s^{4}T) leads to the noninterconverting thietane **6b**.[8b] Irradiation of d(GTATs^{4}TATG) with 366 nm light produced three principal products, the major of which appeared to be the thietane **5b** in equilibrium with an undetectable amount

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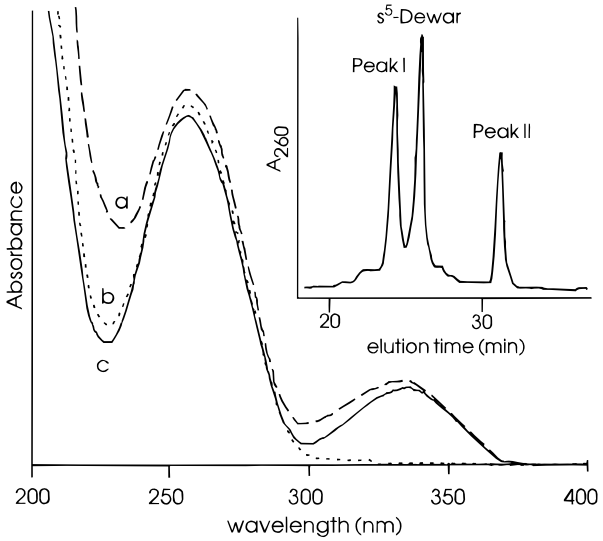
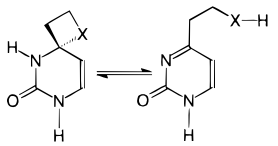


Figure 1. UV spectra of (a) Peak II, (b) the s^5-Dewar product **11b**, and (c) d(GTATs^{4}TATG), **2b**. Inset, C-18 HPLC trace of the reaction mixture resulting from exposure of the s^5-Dewar product **11b** to approximately 0.8 mW of 254 nm radiation for 30 min.

of the (6−4) product **8b**. This assignment was based on the lack of a significant absorption peak above 300 nm and the presence of a single set of discernable NMR peaks, one of which was a singlet at 5.98 ppm which is similar to that of 6.3 ppm observed for the 3′-TH6 signal of **5a**.[8a] Furthermore, increasing the pH to 11 led to the reversible formation of an absorption peak at 328 nm, characteristic of the (6−4) product, as was observed to occur for the mixture of thietane and (6−4) products of d(Ts^{4}T), **8a**.[8a] Addition of methanethiolsulfonate at neutral pH, which was reported to trap the thiol group of **8a**,[8a] similarly led to a 328 nm absorption maximum within 20 s of mixing, along with loss of the singlet at 5.98 ppm and appearance of one at 7.86 ppm which is similar to those observed for the 3′-TH6 signal of (6−4) products (8.0 ppm for **8a** and 7.70 ppm for **7b**). The thietane product **5b** and its noninterconvertible N^3-methyl analog **6b**[12] were both found to reverse almost quantitatively to the corresponding parent octamers within 15 min upon 254 nm irradiation under the same conditions used to photorevert the s^5-Dewar product **11b**. Irradiation of the s^5-Dewar or s^5-(6−4) products, **8b** or **11b**, at pH 11 did not lead to photoreversal, nor did irradiation of the thiol trapped s^5-(6−4) product, **9b**.

One mechanism for the 254 nm induced reversal of the s^5-Dewar product **11b** to the parent octamer at room temperature, is that the s^5-Dewar product is first photoreverted to the s^5-(6−4) product **8b**, which thermally isomerizes to the thietane **5b**, which in turn is photoreverted to the parent octanucleotide **2b**. Because no peaks assignable to the thietane or s^5-(6−4) product could be detected by NMR during irradiation at room temperature, conversion of the (6−4) product to the thietane and subsequent photoreversal would both have to be fast, which is consistent with the thiol-trapping experiment and the efficient photoreversal of the thietane product. In accord with this mechanism, 254 nm irradiation of the s^5-Dewar product **11b** for 15 min at −42 °C in 80:20 ethanol:water did not lead to the parent oligonucleotide, but led instead to a small amount of a peak coeluting with the thietane product **5b**, which presumably arose from the thermally trapped s^5-(6−4) product **8b**. Warming of the irradiation mixture to room temperature, followed by reirradiation at −42 °C for 15 min, led to a small amount of the parent oligonucleotide as would be expected if the thietane were indeed now present.

Unlike the cis−syn cyclobutane dimer of TT[11] and the oxetane formed between dimethylthymine and benzaldehyde,[7] there is no physical evidence that the (6−4) product can be reversed by direct irradiation, presumably because of the high energy of the oxetane intermediate. Whereas AM1 calculations[14] predict that the difference in the heat of formation between the thietane **12b** and its ring-opened form **13b** is relatively small, in accord with experiment, the difference between the oxetane **12a** and its ring-opened form **13a** is rather large. The calculations suggest that if the oxetane were an intermediate in the enzymatic reaction, it would have to be substantially stabilized, or that photoreversal takes place through some other intermediate.



12 **13**

	X	ΔH (kcal)
a	O	-25.2
b	S	-1.5

The reversibility of the s^5-Dewar and thietane photoadducts by direct irradiation to the parent nucleotides may be a useful property for characterizing and reversing photocrosslinks of thiolated bases used to map RNA tertiary structure.[15] Currently, the thietane-containing oligonucleotides are being investigated as stable substrate analogs of the putative oxetane intermediate for further physical and enzymatic studies of this fascinating photoreversal reaction.

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Supporting Information Available: Experimental procedures and chromatographic and spectroscopic data (15 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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(12) **6b** was obtained by >320 nm irradiation of d(GTATm^{3}s^{4}TATG) which was prepared by automated DNA synthesis utilizing the CED phosphoramidite of m^{3}s^{4}T that was synthesized in one step from 5′-(dimethoxytrityl)-N^3-methyl-4-thiothymidine.[13]

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