Pure & Appl. Chem., Vol. 67, No. 1, pp. 183–190, 1995.
Printed in Great Britain.

DNA, sunlight and skin cancer

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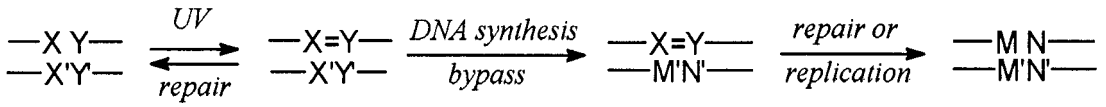
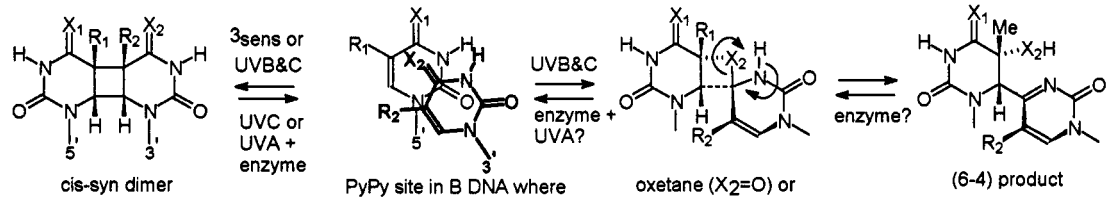
Abstract. Mutations in the p53 tumor suppressor genes in the most common types of skin cancer occur almost exclusively at dipyrimidine sites and are highly characteristic of UVB light. The UVB in sunlight produces cis-syn dimers and (6-4) products at dipyrimidine sites, the latter of which are isomerized by UVA/B light to their Dewar valence isomers. Which of these photoproduct classes is primarily responsible for the mutagenic and carcinogenic effects of sunlight is unknown and to approach this problem we have been developing methods for the construction and study of DNA containing unique photoproducts. Our studies have led us to a new proposal for the origin of C→T mutations, the principal mutations induced by UVB light in many living systems and the major mutation found in the human p53 gene.

Introduction. Skin cancer is the most prevalent form of cancer in the United States and will affect approximately 1 in 4 Americans in their lifetime. There are three major types of skin cancer, basal cell carcinoma (500,000 cases/yr, 500 deaths/yr), squamous cell carcinoma (120,000 cases/year, 1,500 deaths /yr) and melanoma (32,000 new cases/year, 6,500/yr). These cancers arise in the corresponding cells of the epidermis which is on average about 100 microns thick, corresponding to the penetration depth of UVB light. The basal cells differentiate into squamous cells which then migrate to the surface over a period of a month and are sloughed off. The melanocytes produce melanosomes which produce the highly UV absorbing melanin. Of the three types of skin cancer, basal and squamous cell carcinomas correlate the best with exposure to sunlight. The correlation between UV damage to DNA and skin cancer comes from a number of important observations. The first evidence came from very early studies (late 30's) that demonstrated that the effectiveness of UV light in causing cell death or inducing mutations paralleled the absorption spectrum of nucleic acids and not proteins (for a review see ref. 1). It wasn't until the early

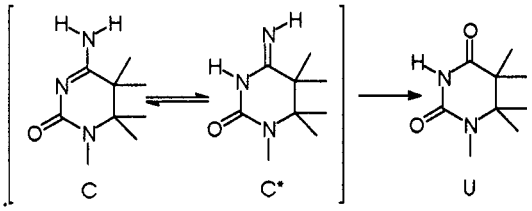


60's that the major photoproduct of DNA was isolated and identified as the cis-syn cyclobutane dimer of dipyrimidine sites (for a review of early work see ref. 2). The key piece of evidence linking photoproducts in DNA with skin cancer induction came from a seminal study on the genetic disease xeroderma pigmentosum (XP) which is associated with a 2000-fold higher incidence of skin cancer. Cleaver was able to demonstrate that cells of those afflicted with XP had defects in their ability to repair cis-syn dimers thus correlating a failure to repair DNA photodamage with skin cancer induction (3). Most recently it has been discovered that the p53 tumor suppressor gene of the most common types of skin cancer, basal cell and squamous cell carcinomas, contain a very high percentage of C→T mutations at dipyrimidine sites, including the CC→TT mutation highly characteristic of UVB induction (4, 5). Dipyrimidine sites are also the principal target of UVB-induced DNA damage suggesting that the mutations result from DNA synthesis errors that occur during DNA synthesis past the photoproduct. The photoproducts and mechanisms by which these mutations arise are not well understood, primarily because of a lack of methods for preparing DNA substrates containing site-specific adducts for the required biological studies.

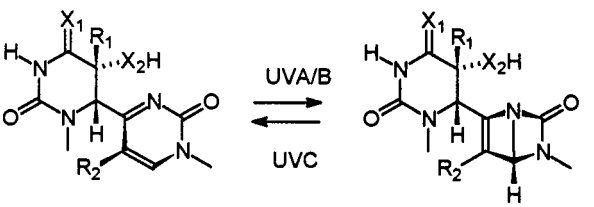
Structure-Activity Relationships in UV Mutagenesis. The major photoproduct of DNA and the first to be characterized is the cis-syn cyclobutane thymine dimer that results from a photo [2+2] cycloaddition reaction between adjacent thymines in a DNA strand (for a recent review of DNA photochemistry and photobiology see ref. 6). Its structure and stereochemistry derives from the favorable intrastrand base stacking and the *anti* glycosyl bond conformation of the nucleotides in duplex B form DNA, the most prevalent form of DNA inside the nucleus of cells. Most early studies on mutagenesis and skin cancer induction focused on the cis-syn thymine dimer because of a number of extremely useful physical and biological properties that made analysis of mutagenesis data extremely simple: (1) it is the major photoproduct of 254 nm irradiation of DNA and can be selectively formed by triplet sensitization with acetophenone, (2) it can be cleanly reversed to two thymines with a quantum yield approaching 1 by the action of the enzyme photolyase and visible light, (3) the dimers can be mapped at a sequence level by way

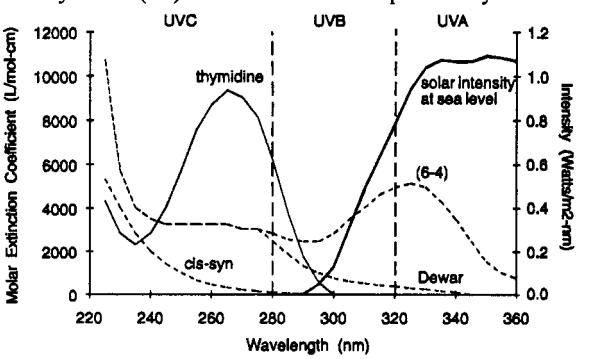


of the enzyme T4 denV endonuclease V which selectively cleaves DNA at the site of cis-syn dimers. Researchers made use of 254 nm light to induce the dimers and on enzymatic photoreversal to discriminate their biological effects from those of other photoproducts, such as the (6-4) product. The (6-4) product, so named because of the bond formed between the 6 and 4 positions of adjacent pyrimidines was first characterized in the late 60's and is proposed to arise via an alternate [2+2] cycloaddition reaction leading to a unstable oxetane or azetidine intermediate. Recently, evidence has been obtained to indicate that an enzyme exists that is capable of photoreverting the (6-4) photoproduct (7, 8), which is quite surprising considering the two step process that is involved in the formation of this product. It wasn't until the early 80's, however, that the role of cis-syn dimers and (6-4) products could be systematically studied by way of high resolution sequencing methods. Haseltine's group found that it was possible to identify and quantify (6-4) products at a sequence level by their susceptibility to cleavage under alkaline conditions, thereby enabling them to correlate the formation of these photoproducts with many mutation hotspots (9). Unfortunately, most studies aimed at correlating individual photoproducts with specific mutations have utilized heterogeneous mixtures of photoproduct-containing DNA substrates making it difficult to rigorously establish the precise structure-activity relationships. Making the correlation becomes even further complicated by the fact that photoproducts in which the 5,6 double bond of C becomes saturated are prone to tautomerize (C*) and deaminate to U at rates competitive with other biological processes (vide infra). By considering the chemical and biochemical pathways available to DNA photoproducts, it becomes clear that mutation hotspots must not only due to the influence of flanking sequence on the rate of formation of these photoproducts, but also on their further chemical transformation, their rate of repair and DNA synthesis bypass, and their coding properties. To begin to unravel the origin of uv-induced mutations, one needs to understand the photochemistry of DNA, and then to develop general methods for the sequence-specific introduction of these photoproducts and their secondary products into DNA substrates suitable for detailed physical and biological studies. With this goal in mind we set out in 1983 to study the photochemistry of DNA and to develop methodology suitable for preparing oligonucleotides containing site-specific photoproducts. These photoproduct-containing oligonucleotides would then be incorporated into: (a) short duplexes (8-10 base pair) for high field 2D NMR and x-ray crystallographic studies, (b) polymers for DNA bending and unwinding studies, (c) long DNA duplex fragments for in vitro repair studies, (d) template-primers (40-60 nucleotides) for in vitro replication studies, and (e) viral DNA (≈6,000 base pairs) for cell extract and in vivo mutation studies (for recent descriptions of our approach and progress see ref. 10, 11).

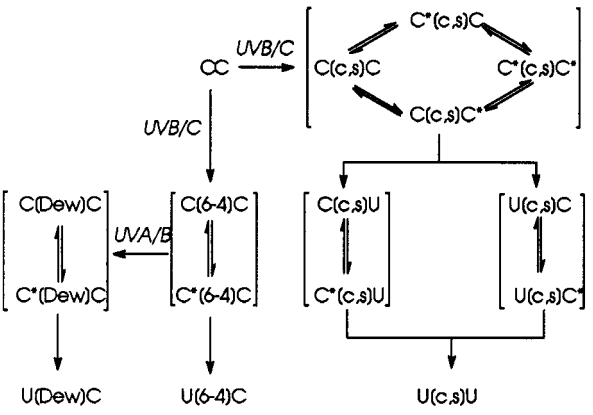


The Dewar Photoproduct of TpT. At the time we embarked on our studies, we decided to determine the absolute stereochemistry and conformation of the (6-4) photoproduct of TpT which was unknown at the time. In studying the literature on the preparation and properties of this compound we discovered that in 1964 Johns and coworkers had reported that what was later established to be the (6-4) product of TpT was not photostable and could be converted by 313 nm light to a product referred to as TpT3, and back again at 254 nm (12). Other literature also suggested that (6-4) products were converted to other products upon irradiation at 313 nm and could be correlated with the reversal of some of the lethal effects of 254 nm light on certain bacteria termed Type III photo-reactivation (for a detailed discussion see ref. 13). After isolating the (6-4) product and carrying out extensive spin decoupling and NOE experiments we decided to expose the (6-4) product to 313 nm light and determine the structure of the further photoproduct. Initially, I thought that the photoproduct might simply be a photohydrate of the pyrimidinone ring but certain spectral features were not supportive of such an assignment. When searching the literature for relevant spectral data on photohydrates I ran across a paper by Nishio and coworkers on the photoisomerization of a pyrimidone to its Dewar valence isomer (14), something that I hadn't considered at all. The possible role of Dewar valence isomers in nucleotide chemistry had been anticipated by Shugar and coworkers who had proposed such an intermediate in a photochemical ring opening reaction of 2,6-dimethyl-4-aminopyrimidine (15) and has most recently been proposed to be an intermediate in similar reaction of cytidine (16). To examine the possibility that the product was a Dewar valence isomer, the FAB MS was acquired in negative ion mode and indicated that the photoproduct was indeed an isomer of the (6-4) product. It was the high frequency carbonyl band in the IR spectrum and an extremely high ^{1}H-^{13}C coupling constant for a key C-H bond, however, that rigorously established that TpT3 was the Dewar valence isomer of the (6-4) product (17). Because sunlight at sea level is principally composed of wavelengths > 290 nm (6-4) products induced by the shorter wavelengths will become isomerized to the Dewar products, leading us to conclude that Dewar product may play an important role in the induction of skin cancers by sunlight. Evidence for production of the Dewar product in the DNA of living cells comes mainly from radioimmune assays developed by Mitchell and coworkers (for a review of the photochemistry and photobiology of (6-4) products see ref. 18). Based on available photophysical data, the half life of the (6-4) product in sunlight can be estimated to be about 4 hours, which is about equal to the half life for repair of both the (6-4) and Dewar product in vivo. The photoisomerization of (6-4) to Dewar photoproducts could be one explanation for Type III photoreactivation noted above. Based on modeling studies with the Dewar photoproduct which indicated that the Dewar isomer was less distorting to DNA structure than the (6-4) product, we proposed that the Dewar product might be less of a block to DNA polymerases, and hence less lethal, but perhaps more mutagenic (13). It is thus possible that photoisomerization of (6-4) products to their Dewar isomers could explain the anecdotal correlation between severe sunburn in youth with skin cancer formation in later years. The fact that isomerization of the (6-4) product takes place only with wavelengths centered around 320 nm, points to the inappropriateness of using 254 nm radiation to study the mechanism of skin cancer induction by sunlight.

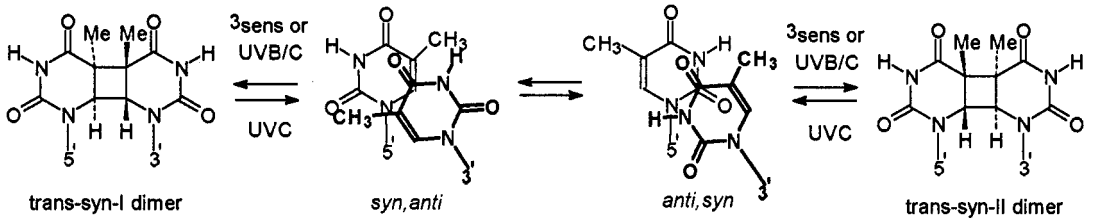




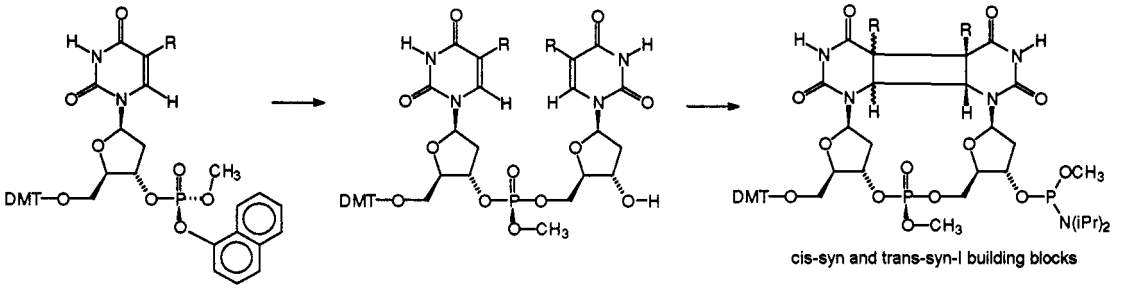
Overall Photochemical Picture for Dipyrimidine Sites. If one now considers all the primary and secondary photochemistry that can occur at all dipyrimidine sites and the tautomerization and deamination of C-containing photoproducts, one can immediately appreciate the complex and dynamic nature of uv-induced damage. Illustrated to the right is the reaction scheme for a CC site following irradiation with sunlight. The rate of deamination of C-containing dimers has been estimated to be on the order of a day for cis-syn dimers (19), and about a week for (6-4) products (20). At the moment the influence of sequence context or local environment on the rates and equilibirum constants are unknown.



Cis-Syn and Trans-Syn-I Phosphoramidite Photoproduct Building Blocks. Having now a more complete picture of the photochemistry of dipyrimidine sequences we focused initial efforts on the photoproducts of TT sites because of the problems attendant with competitive deamination of photoproducts in which the 5,6 double bond of cytosine has become saturated. Because the cyclobutane dimers of TT sites can be formed selectively by triplet sensitization, we first explored the synthesis of a building block for the cis-syn dimer of TpT that could be incorporated into oligonucleotides by automated DNA synthesis. An early attempt to make a building block for a cis-syn dimer of TpT utilizing phosphotriester methodology appeared to be successful but its use was never reported again (21). Rather than pursue the same methodology we opted for the much more efficient and rapid phosphoramidite technology. To prepare the required building block we subjected an analog of TpT in which the phosphate was protected as a methyl phosphotriester and the 3'-OH as a TBDMS group to acetophenone sensitized irradiation. Because our synthetic route resulted in an epimeric mixture of methylphosphotriesters that



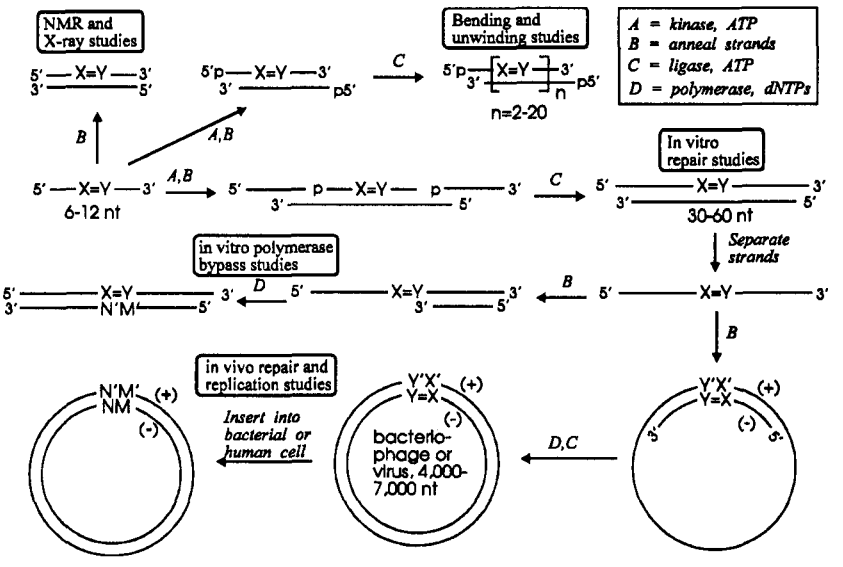
could not be readily separated, irradiation led to a mixture of starting material plus diastereomeric pairs of cis-syn and trans-syn-I dimers which could only be completely separated by a tedious combination of silica gel and reverse phase chromatography. Once separated they were converted to the phosphoramidite building blocks in three steps (22, 23). The trans-syn-I dimer results from photodimerization of conformation of TpT in which the glycosyl bond of the 5'-T is syn. Recently we have succeeded in preparing diastereomerically pure photoprecursor from diastereomerically pure α- napthol phosphate esters (24). Early attempts to sensitize the dimerization of the 5'-DMT derivative were unsuccessful, but more recently we have found that this works reasonably well and can be carried out on the DMT-derivative with



a free 3'-OH. When using the diastereomerically pure phosphotriester precursor we discovered that a third cyclobutane dimer was formed in very low yield and identified it as the trans-syn-II isomer that arises from photodimerization of TpT in a 5'-anti, 3'-syn glycosyl conformation (25). We also detected small amounts of this product in the sensitized irradiation of TpT itself. The photoproducts of the protected TpT derivatives were then converted in one step to their phosphoramidites which could then be used to incorporate the cis-syn dimer into oligonucleotides of up to 50 or more nucleotides by solid phase DNA synthesis. The cis-syn dimers were found to be stable to the standard deprotection conditions for automated DNA synthesis. Phosphoramidite photoproduct building blocks corresponding to the deamination products of the cis-syn dimers of TpdC (24) and dCpT have also been prepared. All of our attempts to date to produce cis-syn dimers of various protected derivatives of TpdC, however, have only yielded the trans-syn-I dimers as the major products.

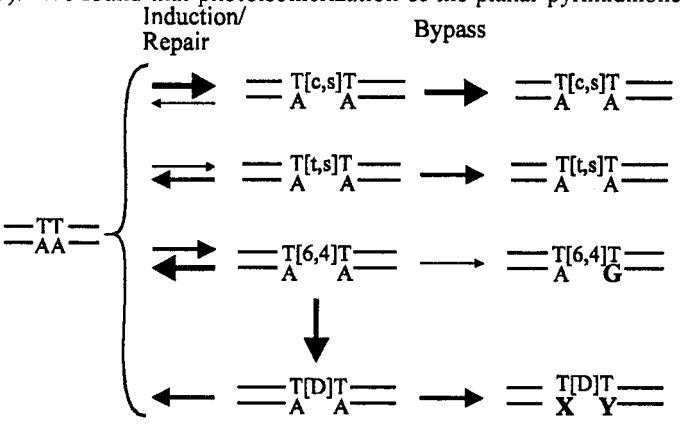
Preparation of (6-4) and Dewar Product-Containing Oligonucleotides. All attempts to produce a (6-4) product building block via direct irradiation of methylphosphate protected TpT with 254 nm light under a number of conditions failed to give detectable amounts of the product. We therefore decided to take the direct approach of irradiating an oligonucleotide and isolating the (6-4) product-containing oligonucleotide by HPLC, a procedure first shown feasible by Demidov for tri and tetranucleotides (26) and later utilized by Lawrence and coworkers (27). To insure that enough product would be produced in the purity desired we first decided to irradiate the hexamer d(AATTAA) containing a unique TT sequence. We could isolate the (6-4) product containing duplex in approximately 3% yield and >98% purity by irradiation with 254 nm light in greater than 95% as estimated by NMR following purification by C-18 HPLC (28). A small amount of Dewar product was also produced, probably by the small amount of 313 nm light produced by the low pressure mercury lamp. The (6-4) product-containing hexamer could then be converted to the Dewar photoproduct-containing hexamer in virtually quantitative yield by irradiation with Pyrex and Mylar filtered medium pressure mercury arc light.

Preparation of Photoproduct-Containing DNA substrates for Physical and Biological Studies. Having developed methods for preparing site-specific photoproduct-containing oligonucleotides we then needed to develop methods for incorporating them into the various types of substrates needed for the structure-activity studies (for a more detailed description see ref. 10, 11). For NMR and x-ray crystallographic studies, the photoproduct-containing oligonucleotides are assembled into mini DNA duplexes by annealing them to their complementary strands. For bending and unwinding studies these mini photoproduct-containing duplexes are polymerized by enzymatic ligation with T4 DNA ligase following phosphorylation of their 5'-ends with T4 kinase. Large DNA fragments containing site-specific photoproducts are prepared by enzymatic ligation of the photoproduct-containing oligonucleotides to other oligonucleotides via a ligation scaffold and used for in vitro repair studies. The photoproduct-containing strand is separated from the ligation scaffold and the photoproduct-containing single strand and is used as a template for in vitro DNA polymerase bypass experiments. The same template can also be used to prime DNA synthesis of the



second strand of a bacteriophage or virus which could then be used to study repair and replication past DNA photodamage in living cells or in cell extracts.

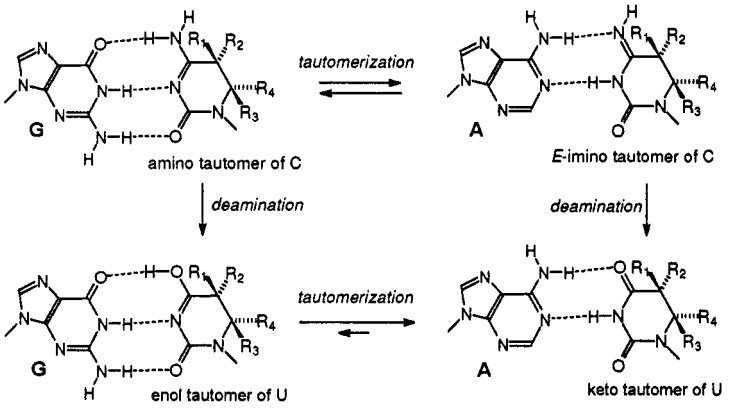
Structure-Activity Relationships of Photoproducts of TpT Sites. Most of our studies to date have focused on the photoproducts of TpT sites with the idea that they should serve as adequate models for the four major classes of photoproducts of all dipyrimidine sites. It is perhaps most useful at this point to analyze the results of our structure-activity studies along with those of others with regard to the factors that contribute to the overall mutagenicity of UV light by considering the various processes that ensue following irradiation of DNA. The first factor is the rate of production of a photoproduct at a given site. Clearly the more photoproduct formed the greater the chance that it has to lead to a mutation. In this regard the cis-syn, trans-syn-I and (6-4) products are produced directly by sunlight at relative rates of approximately 1 : 0.1 : 0.01 in duplex DNA (2). The second factor that modulates the amount of photoproduct available for a mutagenic process is its rate of further chemical transformation. Of the three direct photoproducts of TpT, only the (6-4) is unstable and is converted to the Dewar valence isomer with a half life of about 4 hours in sunlight. Despite its highly strained structure, the Dewar product appears to be quite stable at neutral pH, but its stability in vivo is unknown at the moment. The third factor, which also modulates the amount of photoproduct is the rate of repair of the DNA photoproduct. In vivo repair studies have suggested that cis-syn dimers are repaired with half lives of about 24 hours, whereas, (6-4) and Dewar photoproducts are more rapidly repaired with half lives of approximately 3-4 hours (18). These half lives represent averages for photoproducts in highly heterogeneous environments comprising both inactive and actively transcribed genes. The relative rates of repair, however, are similar to those that we have found in collaborative studies with Sancar's group for the repair of DNA fragments containing site-specific DNA photoproducts by the *E. coli* uvr(A)BC excinuclease. This enzyme system was found to incise cis-syn, trans-syn, (6-4) and Dewar photoproducts with initial rates of 1:7:9:9 (29). The rates of incision roughly paralleled the recognition of these photoproducts by the uvrA subunit which bound with relative affinities of 1:10:9:4, and which was remarkably similar to that observed for the human DNA damage binding protein which bound with relative affinities of 1:8:9:4 (30). The fact that the binding constant for the Dewar photoproduct is less than for the (6-4) product in both cases is rather exciting in that it supports earlier molecular modeling work of ours that suggested that the Dewar photoproduct is less distorting to B DNA structure than the (6-4) product and might therefore be less easily recognized by repair enzymes than the (6-4) product (13). We found that photoisomerization of the planar pyrimidinone ring of the (6-4) product to the Dewar structure makes the product more compact, and hence more like B DNA. A fourth factor to consider is the rate at which a particular DNA polymerase can bypass a photoproduct. In this case we have only found one enzyme that can synthesize past all four classes of DNA photoproducts, and this is an exonuclease deficient polymerase from T7 bacteriophage which bypasses the cis-syn, trans-syn-I, (6-4) and Dewar photoproducts with relative rates of 1 : 0.03 : 0.006 : 0.1 under otherwise identical conditions (31). Again the finding that the Dewar product was more easily bypassed than the (6-4) product was in accord with the same modeling results that led us to predict that it should be less easily repaired. We have also found that the cis-syn dimer is also more easily bypassed than the trans-syn-I dimer by the mammalian polymerase δ, the enzyme involved in DNA replication (32).

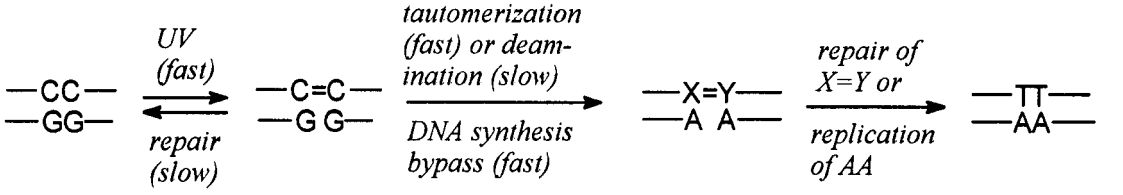


Mutagenic Potential of DNA Photoproduct Classes. Together, all the available data strongly suggests that the cis-syn dimer class has the highest mutagenic potential because it is produced in the highest yield, is repaired the slowest, and is bypassed the fastest. This is entirely in accord with what we know about its

structure from NMR, melting temperature and bending studies. We have found that the cis-syn dimer only bends DNA by 7° (33), and in accord with prior work from Kaptein's lab (34), does not perturb the thermodynamics of duplex formation or the conformation of the flanking nucleotides significantly (35). As a result one might expect that the cis-syn dimer is not easily detected by repair enzymes and is readily bypassed by DNA polymerases because it does not perturb DNA structure significantly. This contrasts with the physical and biological properties of the trans-syn-I dimer which we know to bend DNA by about 22° (33) and to significantly perturb the thermodynamics of duplex formation (35), presumably in part due to the fact that the 5'-T is in a syn conformation which puts the methyl group in the Watson-Crick base pairing interface. This would explain why the trans-syn-I dimer is so readily detected and repaired, and such a block to DNA synthesis by polymerases. To be highly mutagenic, however, a photoproduct must be mutagenic in addition to having high mutagenic potential. By sequencing the bypass products of a cis-syn dimer flanked by A's produced by polymerase I of *E. coli* (36), we discovered that A's were almost exclusively introduced. This also turned out to be the case for bypass of the dimer by exo$^-$ T7 polymerase (37), and the polymerase involved in bypassing DNA damage in *E. coli* under SOS conditions (38, 39). By contrast the (6-4) and Dewar products, which have lower mutagenic potential, are mutagenic in *E. coli* under SOS (27). The non-mutagenic nature of the bypass of the cis-syn dimer of TT can be readily understood when one realizes that the Watson-Crick base pairing properties of the original two T's have not been altered by formation of a cyclobutane dimer. It is precisely this realization that led us to the deamination-bypass mechanism for the origin of C→T mutations at dipyrimidine sites.

Deamination and Tautomer-Bypass Mechanisms for UV-Induced C→T Mutations. We were aware at the time of our studies with the cis-syn dimer of TpT that the cytosine in cyclobutane dimers deaminates to U much more rapidly than does C because of a loss of ring aromaticity, and that U is identical in structure to T except that it lacks a methyl group. The deamination rate has been estimated to be about one day in vivo (19), which is competitive with the repair rate of dimers in mammalian cells and the rate of cell division. This led us to propose what we term the deamination-bypass mechanism for the origin of UV-induced C→T mutations at dipyrim-idines (24, 36, 39). In this mechanism, deamination of a cyclobutane dimer at a C-containing dipyrimidine sequence results in the formation of a U-containing dimer which would code for the introduction of an A during DNA synthesis. The newly synthesized A-containing strand would then code for the introduction of a T in place a C that was originally there. We have recently shown that the deaminated product of the cis-syn dimer of TC is indeed replicated in *E. coli* as though it were a TT site (39). Another possible mechanism to account for C→T mutations is based on evidence that the tautomeric forms of C are more prevalent in dimers, again because saturation of the 5,6 double bond abolishes the aromaticity of the ring.





This realization led to a much earlier proposal that we term the tautomer-bypass model in which A's are directed for incorporation during synthesis by the *E*-imino tautomer of C in the dimer (40, 41). Of course it has been suggested that the intrinsic bias of many polymerases for introducing A opposite non-

instructional sites, such as those lacking a base (abasic site) may also explain the origin of X→T mutations (for a review see ref. 42). All these proposals have yet to be tested rigorously and are the subject of much of our current research.

Acknowledgments. The majority of this work was supported by PHS Grant R01-CA40463 awarded by the National Cancer Institute, DHHS.

References.

1. Doudney, C. O. (1976) in *Photochemistry and Photobiology of Nucleic Acids*, Vol. II (Wang, S. Y., ed), pp. 309-374, Academic Press, New York.
2. Patrick, M. H., and Rahn, R. O. (1976) in *Photochemistry and Photobiology of Nucleic Acids*, Vol. II (Wang, S. Y., ed), pp. 35-95, Academic Press, New York.
3. Cleaver, J. E. (1968) *Nature* **218**, 652-656.
4. Brash, D. E., Rudolph, J. A., Simon, J. A., Lin, A., McKenna, G. J., Baden, H. P., Halperin, A. J., and Ponten, J. (1991) *Proc. Natl. Acad. Sci. U.S.A.* **88**, 10124-10128.
5. Ziegler, A., Leffell, D. J., Kunala, S., Sharma, H. W., Gailani, M., Simon, J. A., Halperin, A. J., Baden, H. P., Shapiro, P. E., Bale, A. E., and Brash, D. E. (1993) *Proc. Natl. Acad. Sci. U.S.A.* **90**, 4216-4220.
6. Cadet, J., and Vigny, P. (1990) in *Bioorganic Photochemistry*, Vol. 1 (Morrison, H., ed), pp. 1-272, John Wiley & Sons, USA.
7. Todo, T., Takemori, H., Ryo, H., Ihara, M., Matsunaga, T., Nikaido, O., Sato, K., and Nomura, T. (1993) *Nature* **361**, 371-374.
8. Kim, S. T., Malhotra, K., Smith, C. A., Taylor, J. S., and Sancar, A. (1994) *J. Biol. Chem.* **269**, 8535-8540.
9. Lippke, J. A., Gordon, L. K., Brash, D. E., and Haseltine, W. A. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 3388-3392.
10. Taylor, J.-S. (1994) *Acc. Chem. Res.* **27**, 76-82.
11. Taylor, J.-S. (1990) *J. Chem. Educ.* **67**, 835-841.
12. Johns, H. E., Pearson, M. L., LeBlanc, J. C., and Helleiner, C. W. (1964) *J. Mol. Biol.* **9**, 503-524.
13. Taylor, J.-S., Garrett, D. S., and Cohrs, M. P. (1988) *Biochemistry* **27**, 7206-7215.
14. Nishio, T., Katoh, A., Omote, Y., and Kashima, C. (1978) *Tetrahedron Lett.*, 1543-1544.
15. Wierzchowski, K. L., Shugar, D., and Katritsky, A. R. (1963) *J. Am. Chem. Soc.* **85**, 827-828.
16. Shaw, A. A., and Shetlar, M. D. (1990) *J. Am. Chem. Soc.* **112**, 7736-7742.
17. Taylor, J.-S., and Cohrs, M. P. (1987) *J. Am. Chem. Soc.* **109**, 2834-2835.
18. Mitchell, D. L., and Nairn, R. S. (1989) *Photochem. Photobiol.* **49**, 805-819.
19. Ruiz-Rubio, M., and Bockrath, R. (1989) *Mutat. Res.* **210**, 93-102.
20. Douki, T., Voituriez, L., and Cadet, J. (1991) *Photochem. Photobiol.* **53**, 293-297.
21. Hayes, F. N., Williams, D. L., Ratliff, R. L., Varghese, A. J., and Rupert, C. S. (1971) *J. Am. Chem. Soc.* **93**, 4940-4942.
22. Taylor, J.-S., and Brockie, I. R. (1988) *Nucleic Acids Res.* **16**, 5123-5136.
23. Taylor, J.-S., Brockie, I. R., and O'Day, C. L. (1987) *J. Am. Chem. Soc.* **109**, 6735-6742.
24. Taylor, J.-S., and Nadji, S. (1991) *Tetrahedron* **47**, 2579-2590.
25. Kao, J. L.-F., Nadji, S., and Taylor, J.-S. (1993) *Chem. Res. Toxicol.* **6**, 561-567.
26. Demidov, V. V., and Potaman, V. N. (1984) *J. Chromatogr.* **285**, 135-142.
27. LeClerc, J. E., Borden, A., and Lawrence, C. W. (1991) *Proc. Natl. Acad. Sci. U.S.A.* **88**, 9685-9689.
28. Smith, C. A., and Taylor, J.-S. (1993) *J. Biol. Chem.* **268**, 11143-11151.
29. Svoboda, D. L., Smith, C. A., Taylor, J.-S., and Sancar, A. (1993) *J. Biol. Chem.* **268**, 10694-10700.
30. Reardon, J. T., Nichols, A. F., Keeney, S., Smith, C. A., Taylor, J.-S., Linn, S., and Sancar, A. (1993) *J. Biol. Chem.* **268**, 21301-21308.
31. Smith, C. A. (1993) Ph.D. dissertation, Washington University.
32. O'Day, C. L., Burgers, P. J., and Taylor, J.-S. (1992) *Nucleic Acids Res.* **20**, 5403-5406.
33. Wang, C.-I., and Taylor, J.-S. (1993) *Chem. Res. Toxicol.* **6**, 519-523.
34. Kemmink, J., Boelens, R., Koning, T., van der Marel, G. A., van Boom, J. H., and Kaptein, R. (1987) *Nucleic Acids Res.* **15**, 4645-4653.
35. Taylor, J.-S., Garrett, D. S., Brockie, I. R., Svoboda, D. L., and Telser, J. (1990) *Biochemistry* **29**, 8858-8866.
36. Taylor, J.-S., and O'Day, C. L. (1990) *Biochemistry* **29**, 1624-1632.
37. O'Day, C. L. (1989) Ph.D. dissertation, Washington University.
38. Banerjee, S. K., Christensen, R. B., Lawrence, C. W., and LeClerc, J. E. (1988) *Proc. Natl. Acad. Sci. U.S.A.* **85**, 8141-8145.
39. Jiang, N., and Taylor, J.-S. (1993) *Biochemistry* **32**, 472-481.
40. Bockrath, R., and Cheung, M. K. (1973) *Mutat. Res.* **19**, 23-32.
41. Person, S., McCloskey, J. A., Snipes, W., and Bockrath, R. C. (1974) *Genetics* **78**, 1035-1049.
42. Strauss, B. S. (1991) *Bioessays* **13**, 79-84.