Synthesis and Photochemical Cleavage of Cis-Syn Pyrimidine Cyclobutane Dimer Analogs

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The synthesis of cis-syn cytosine–cytosine and cytosine–thymine cyclobutane dimer analogs are described. In contrast to the thymine and uracil cyclobutane dimers, the cytosine-containing dimers have significant absorption bands in the UV-B region of the spectrum. These dimers can be reverted back to their monomeric form (split) by irradiation with UV light. Additionally, the dimers can be split by photochemical electron transfer. Use of excited state electron donors *N,N*-dimethylaniline and *N,N,N,N*-tetramethylbenzidine gives high yields of the monomeric pyrimidines—even at high conversions. Use of excited-state electron acceptors, 2-anthraquinonesulfonate and 9,10-dicyanoanthracene also resulted in efficient splitting. However the conversion-corrected chemical yields for these splitting reactions is much lower, due to the formation of a large number of side products.

Damage of the DNA molecule caused by ultraviolet (UV) radiation has received renewed attention. This is in part due to concerns about the depletion of stratospheric ozone which is expected increase the flux of UV radiation at the earths surface over the next decade.[1] This in turn is predicted to lead to higher rates of human skin cancer.[2] Cis-syn thymine–thymine cyclobutane dimers have long been considered to be the main cause of UV-induced mutations. These dimers are the photoproducts formed in the highest yield upon UV irradiation of DNA.[3] Consequently, there have been numerous studies on the photochemistry of their formation,[3-5] their repair,[3,5-16] and their role in genetic mutations.[17-20]

However, recent work suggests that the less frequent cytosine–thymine and cytosine–cytosine cyclobutane dimers are more responsible for the lethal effects of UV light.[21-24] There are currently two proposed mechanisms by which cytosine containing dimers can direct misincorporation of a base during DNA replication. First, loss of the 5,6 double bond upon dimerization of cytosine makes the exocyclic NH_2 group (on C4) more susceptible to tautomerization.[25] The resulting imino tautomer has the same hydrogen bonding pattern as thymine. Consequently, this can lead to incorporation of an A rather than a G in the daughter strand during DNA replication.[21] Second, cytosine dimers are known to be quite susceptible to hydrolysis.[26,27] This reaction converts the cytosine to a uracil (which reads as T during replication). The net genetic result from either of these pathways is a C → T mutation in the progeny. In fact, C → T mutations have been linked to a number of UV induced carcinogenic transformations.[28,29]

Compared with the thymine–thymine photodimers, relatively little is known about the cytosine-containing cis-syn dimers. One reason for this is that there are few reliable large scale synthetic routes to such compounds. Previous work has shown that photolysis of cytidine–thymidine dinucleotides can provide small amounts of cytosine-containing dimers.[30-32] However, the aforementioned tautomerization/hydrolysis pathway makes it difficult to isolate large quantities of dimers in high purity. Photolysis of the cytosine bases and their methyl derivatives in free solution provides mixtures of the trans-anti and trans-syn homodimers.[33] Unfortunately, none of the

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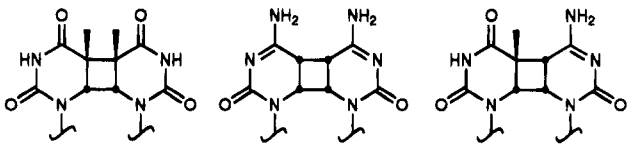
Chart 1



Cis-Syn Thymine Dimer | Cis-Syn Cytosine Dimer | Cis-Syn Cytosine-Thymine Dimer

desired cis-syn cyclobutane dimers appear to be available from this procedure. Varghese has reported that irradiation of frozen solutions of cytidine produces a substance containing one or more cyclobutane dimers.[34] Again, the facile hydrolysis reaction makes this an impractical method for large scale synthesis.

The research described herein was undertaken with the goal of further characterizing the chemical behavior and physical properties of cytosine cyclobutane dimers. Of special interest is the susceptibility of such dimers to repair by DNA photolyase. The latter are photochemical enzymes that are capable directly repairing pyrimidine cyclobutane dimers using UV and visible light.[10,35] A number of model studies using thymine and uracil dimers have supported a photochemical electron transfer mechanism for this process. Both photochemical electron acceptors and photochemical electron donors have been shown to be capable of mediating the photoreversal of thymine dimers.[6,7,12,14,15] Spectroscopic studies done on the enzyme itself also support the electron transfer pathway.[36-38]

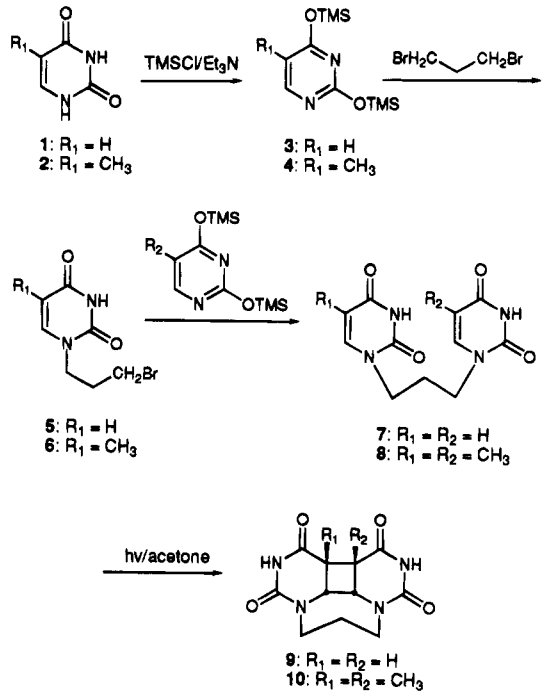
Considerably less attention has been paid to the cytosine-containing dimers. Kim and Sancar have reported that DNA photolyase enzymes are capable of mediating photoreversal of the cytosine–cytosine and cytosine–thymine cyclobutane dimers.[39] However, these substrates are repaired with lower efficiency than that observed for the thymine–thymine dimers.

Herein, we report the preparation of cis-syn cyclobutane dimers of cytosine–cytosine and cytosine–thymine which have been modified in order to stabilize them to the hydrolysis reaction. We show that as with the thymine dimers, these species can be split (dedimerized) using low wavelength UV light. We will also show that the cytosine-containing dimers are capable of being split using electron donor and electron acceptor photosensitizers.

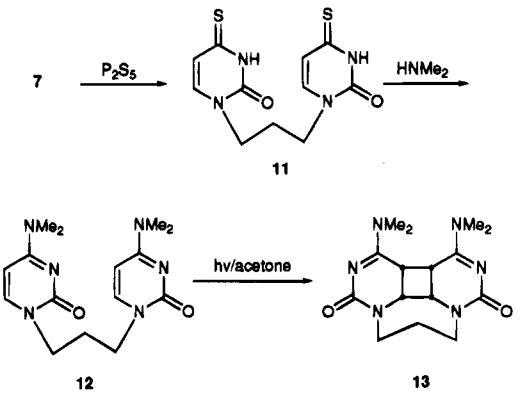
Results and Discussion

1. Synthesis. Scheme 1 illustrates the synthesis of linked T<>T and U<>U pyrimidine dimers **9** and **10**, respectively. The linked uracil and thymine dimers were prepared so their photochemical behavior could be compared with the cytosine-containing systems. The synthetic strategy relies on the formation of the bis-trimethylsilyl pyrimidine derivatives **3** and **4**, followed by stepwise addition to 1,3-dibromopropane to form the

Scheme 1



Scheme 2



trimethylene bridge at N1 between the two pyrimidines. The synthesis of these compounds follows the general procedure of Golankiewicz and co-workers and is described in detail elsewhere.[40] However, this methodology is not suitable for the preparation of linked cytosine-containing dimers as reaction of N_4-acetylbis(trimethylsilyl)cytosine with 1,3-dibromopropane leads to decomposition of the starting materials. Scheme 2 shows the preparation of the cytosine homodimer DMC<>DMC **13**. Treatment of linked uracil dimer **7** with phosphorus pentasulfide according to the method of Golankiewicz and Langer[41] gives linked 4-thiouracil **11**. Reaction of crude **11** with dimethylamine in a sealed tube gives N1-linked DMC monomer **12**. Acetone-sensitized photolysis of the latter gives DMC<>DMC **13** in 25% isolated yield. In this case, the yield is limited by low conversion of the starting material. Attempts at irradiation beyond ca. 30% conversion resulted in slow decomposition of both starting material and products.

For the preparation of DMC<>T dimer **17** (Scheme 3), compound **5** was converted to **14** in 80% yield by reaction

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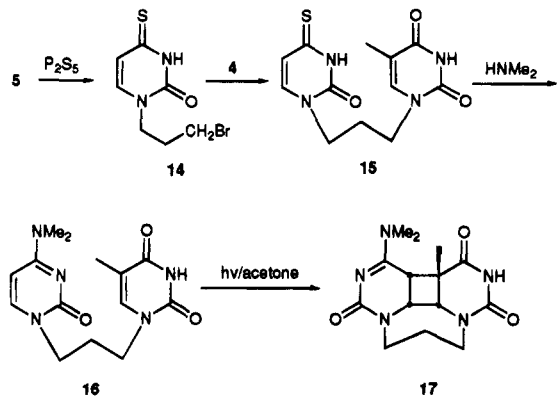
Scheme 3



with P_2S_5 in dioxane. No thiolation of the alkyl bromide side chain or alkylation of the thiocarbonyl product by the alkyl bromide side chain was observed. Reaction of **14** with bis(trimethylsilyl)thymine[42] in dioxane gives **15**, which was reacted in its crude state with dimethylamine in a sealed tube to give **16** after purification by C18 reverse phase chromatography. Acetone-sensitized photolysis of **16** gave **17** in 50% isolated yield.

Linking the pyrimidines with a *N*1-trimethylene-*N*1 chain enforces cis-syn dimerization. The NMR spectra of the dimers showed that only a single diastereomer was formed in each case. We assign the stereochemistry to the cis-syn (syn, H-H) isomer. This is based on an earlier X-ray crystallography study of T<>T dimers[43] and the close correspondences of the NMR spectra of DMC<>T and DMC<>DMC. Further confirmation of the stereochemistry comes from the nonequivalence of the protons on C2 of the methylene bridge. The possible stereoisomers of DMC<>DMC are shown in Figure 1. For the cis-syn (meso) diastereomer, the H_A on C2 of the bridge is held cis to the dihydropyrimidine rings and H_B is held trans. These would be expected to have different chemical shifts. For the trans-anti (racemic pair) diastereomer the C_2 symmetry element puts both protons into an identical environment. These would be expected to have the same chemical shift. The observed ^{1}H NMR shows two well-resolved multiplets for the bridge-C2 protons, one at 1.45 ppm and one at 2.38 ppm. The T<>T and U<>U homodimers as well as the DMC<>T heterodimer show analogous patterns (Table 1). Thus we conclude that all of the dimers synthesized for this study are of the cis-syn configuration.

The paucity of work done on cytosine-containing cyclobutane dimers is, in part, due to their instability in aqueous solution. The exocyclic NH_2 group at C4 can be hydrolyzed to a carbonyl group via its imine tautomer. We attempted to prepare the corresponding unmethylated C<>C dimer **19**. Following an earlier procedure,[41] the N1-linked cytosine monomers **18** were successfully obtained. However attempts at their acetone-sensitized dimerization gave only U<>U **9**. Presumably the dimers formed, but the exocyclic NH_2 group was hydrolyzed during the purification (Scheme 4). However, a previous study showed that methylating the exocyclic NH_2 group on cytosine greatly increases the stability of its trans-syn photodimer to hydrolysis.[33] Therefore the *N*4,*N*4-dimethyl derivative was prepared and used in the preliminary studies.

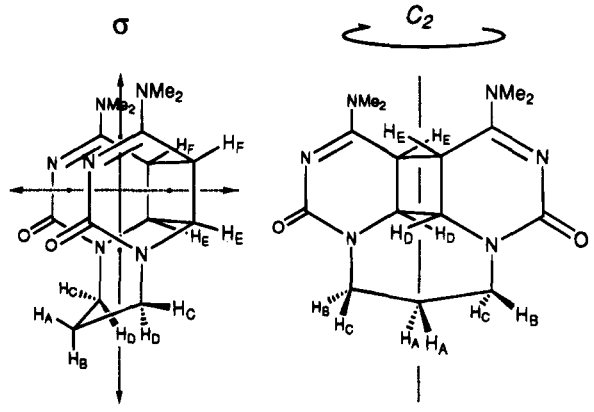


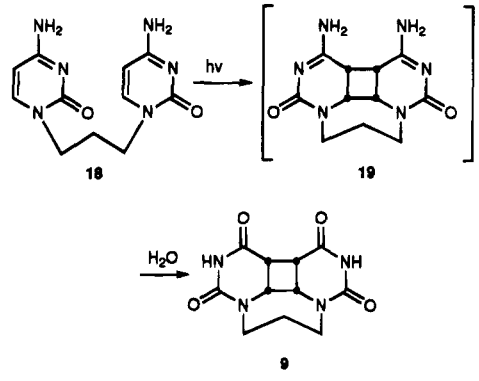
Figure 1. The cis-syn (left) and trans-syn (right) stereoisomers of DMC<>DMC dimer **13**. For the cis-syn compound, the two protons on C2 of the trimethylene bridge (H_A and H_B) are nonequivalent and thus should give two distinct peaks by ^{1}H NMR. For the trans-syn compound, these two protons are equivalent by symmetry (H_A) and thus should appear as one peak by ^{1}H NMR.

Table 1. Preparative Yields and 400 MHz ^{1}H NMR Data for Linked Pyrimidine Dimers

dimer	preparative yield, %	δH_A (ppm)[a]	δH_B (ppm)[a]
T<>T (**10**)	71	1.45–1.51	1.83–1.89
U<>U (**9**)	36	1.44–1.49	1.81–1.85
DMC<>T (**17**)	50	1.33–1.39	1.81–1.89
DMC<>DMC (**13**)	25	1.42–1.48	2.30–2.41

[a] All NMR spectra were acquired using d_6-DMSO as the solvent except for DMC<>DMC, which was acquired using CD_3OD as the solvent.

Scheme 4



The UV absorption spectra of the dimers are of particular interest because they provide information on the susceptibility of the dimers to direct photoreversal. The singlet state energies of the dimers, which can be estimated from onset of UV absorption, are critical to distinguishing between energy transfer and electron transfer pathways. The UV spectra of the DMC<>DMC, DMC<>T along with their corresponding monomers are shown in Figure 2. The absorption maxima and the molar absorptivities are shown in Table 2.

An interesting feature of these spectra is that the cytosine-containing dimers have significant absorptions in the UV-B region (280–320 nm), both showing λ_{max} at 272 nm. In fact, there is considerable overlap between the monomer and dimer absorption spectra. Similar

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(43) Frank, J. K.; Paul, I. C. *J. Am. Chem. Soc.* **1973**, *95*, 2324–2332.

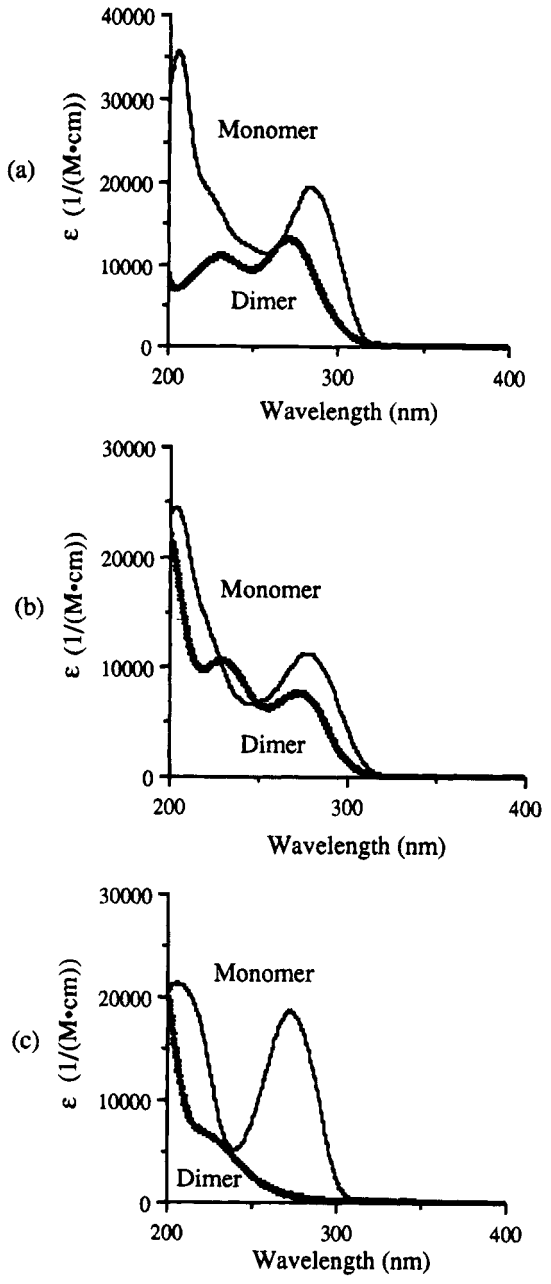

Figure 2. Normalized pyrimidine UV spectra in H_2O. (a) DMC–DMC monomer **12** (light) and dimer **13** (bold); (b) DMC–T monomer **16** (light) and dimer **17** (bold); (c) T–T monomer **8** (light) and dimer **10** (bold).

spectra have been reported by Wang et al. for the trans-syn dimers of cytosine.[33] These workers attributed the high wavelength maxima to the amino tautomers. The corresponding *N*4-imino tautomers were shown to have absorption bands that are considerably blue-shifted, and recent work by Lemaire and Ruzsicska concurs with this result.[32] Thymine and uracil dimers, in contrast, show only tail absorption in the UV-B region. It would follow from this that even though they split very efficiently upon direct irradiation[3], one of the reasons thymine dimers form readily in DNA is that their absorption spectra differ significantly from the monomers. Consequently, they can be formed using UV-B light, but UV-C (240–280 nm) is necessary for the observation of direct (i.e. unmediated by sensitizers or enzyme) photoreversal. In contrast it appears that cytosine dimers do absorb UV-B and it is possible that direct photoreversal may occur at

Table 2. UV Spectral Properties of Linked Pyrimidine Monomers and Dimers

compound	λ_{max} (nm)[a]	ϵ (M^{-1} cm^{-1})
DMC–DMC monomer (**12**)	205	35 800
	283	19 600
DMC–DMC dimer (**13**)	230	11 100
	272	13 100
DMC–T monomer (**16**)	206	24 300
	278	13 500
DMC–T dimer (**17**)	232	9 000
	272	7 700
T–T monomer (**8**)	206	21 400
	272	18 700
T–T dimer (**10**)	220 (sh)	7 000
U–U monomer (**7**)	208	17 400
	268	19 500
U–U dimer (**9**)	218 (sh)	5 700
C–C monomer (**18**)	275	14 900

[a] In H_2O.

significant rates for these species. In the following subsection, the direct photosplitting of these dimers is discussed.

Based on the absorption onset, the singlet energies (E_s) of both DMC<>DMC and DMC<>T are estimated to be 100 ± 5 kcal/mol. Some of the low wavelength (UV-B) repair reactivity in *E. coli* photolyase has been traced to a specific tryptophan residue in the apoprotein (Trp-277).[44] Model studies also have shown that tryptophan is capable of photosensitizing the splitting of thymine dimers.[7,12] These processes have been suggested to occur through an electron transfer pathway. Traditionally, energy transfer has been excluded because it was thought that the singlet energy of the thymine dimers was too high (E_s ca. 100 kcal/mol)[45] to be populated by most of the photosensitizers (this is certainly true for the dihydroflavin chromophore (E_s = 60 kcal/mol)). On the other hand tryptophan has E_s = 95 kcal/mol.[46] On the basis of this evidence, we propose that a singlet energy transfer mechanism for tryptophan-mediated splitting can not be ruled out—at least for the cytosine-containing dimers. Further explorations of the tryptophan mechanism should prove interesting.

2. Direct Irradiation of Dimers and Monomers. It is well established that thymine dimers can be both formed and split (monomerized) by UV light (eq 1).[3]

$$\text{Thy<>Thy} \underset{h\nu}{\overset{h\nu}{\rightleftharpoons}} \text{Thy} + \text{Thy} \qquad (1)$$

Experiments were carried out to determine the efficiency of dimerization and monomerization of our model systems by direct irradiation with UV light. Table 3 shows that irradiation of the N1-linked monomers using the unfiltered output of a medium-pressure Hg lamp results in clean formation of the dimers in high yields when corrected for conversion. Likewise, irradiation of the dimers under the same conditions results in formation of the corresponding monomers, again in high conversion-corrected yields.

Table 3 also shows that for most of the systems studied, a clean photostationary state was achieved. For these linked pyrimidine systems, a photostationary state occurs when the rate of photodimerization is equal to the rate

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Table 3. Photostationary Ratios, Direct Splitting Yields, and Relative Quantum Yields for Linked Pyrimidines

model system	photostationary ratio (monomer:dimer)	dimer direct splitting % yield (% conversion)	$\frac{\Phi_{dimer}}{\Phi_{mon}}$	E_{strain} (kcal/mol)
T–T (**8/10**)	1:4	100 (95)	1	51.7
U–U (**7/9**)	2:1[a]	75 (63) 6 (98)	0.9[a]	39.4
DMC–T (**16/17**)	3:2	100 (14) 80 (60)	0.5	40.2
DMC–DMC (**12/13**)	32:1	100 (95)	0.02	45.2
C–C (**18/19**)	–	–	–	38.3

[a] A stable photostationary state was not achieved.

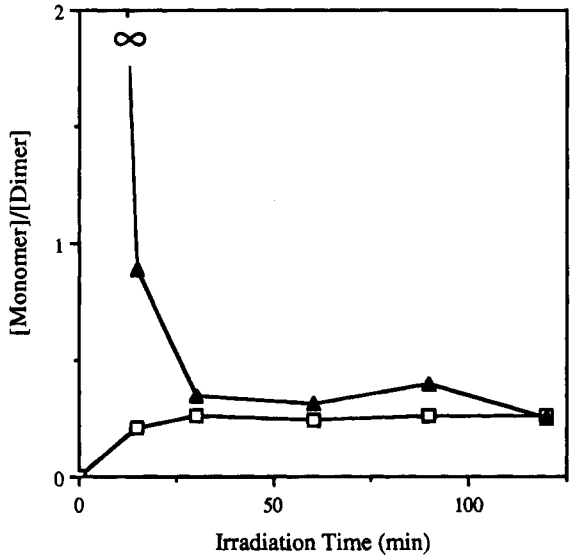

Figure 3. Graph of [monomer]/[dimer] vs irradiation time with an unfiltered 450 W Hg lamp starting with linked thymine monomer 8 (triangles) and linked thymine dimer 10 (squares).

of photosplitting. For each of the systems, the same dimer/monomer ratio is achieved regardless if the dimer or the monomer is used as the starting material. This is illustrated in Figure 3 for the thymine system, and all of the other dimer/monomer pairs show similar behavior.

The linked uracil system was the only example for which a clean photostationary state could not be achieved. Although both photodimerization and photosplitting were observed, a number of side products were observed by HPLC. Previous studies have shown that uracil is more susceptible to photohydration than is thymine or methylated cytosine.[47]

Equation 2 shows that at the photostationary state, the monomer:dimer ratio depends on their molar absorptivity ($\epsilon_{monomer}$ and ϵ_{dimer}, respectively) as well as the quantum yields for dimerization and splitting (Φ_{dimer} and Φ_{split}, respectively). However, this relationship only holds true for irradiation at a single wavelength. From the spectra in Figure 2 it can be seen that the ratio $\epsilon_{monomer}/\epsilon_{dimer}$ depends on λ. Thus, the photostationary state composition depends on the wavelength of the light source. To get a wavelength independent measure of the efficiency of dimerization vs monomerization, one needs to calculate $\Phi_{dimer}/\Phi_{monomer}$. This ratio is a measure of the excited-state lifetimes of the dimers and monomers (τ_{dimer} and $\tau_{monomer}$), and the rate constants for dimerization and splitting (k_{split} and k_{dimer}) (eq 3). If the light source emits at multiple wavelengths, the convolution of the source intensity distribution $I(\lambda)$with the absorption profile of the substrates, $\epsilon(\lambda)$, can be substituted into eq 2 to give eq 4. Therefore, using the monomer:dimer ratios from Table 3 and the intensity distribution of the light source from the literature,[46] the quantum yield ratios, $\Phi_{dimer}/\Phi_{monomer}$, were calculated.

$$\frac{[dimer]}{[monomer]} = \frac{\epsilon_{monomer}\Phi_{dimer}}{\epsilon_{dimer}\Phi_{split}} \tag{2}$$

$$\frac{\Phi_{split}}{\Phi_{dimer}} = \frac{k_{split}\tau_{dimer}}{k_{dimer}\tau_{monomer}} \tag{3}$$

$$\frac{[dimer]}{[monomer]} = \frac{\int_{200}^{400} I(\lambda)\epsilon_{monomer}(\lambda)d\lambda\Phi_{dimer}}{\int_{200}^{400} I(\lambda)\epsilon_{dimer}(\lambda)d\lambda\Phi_{split}} \tag{4}$$

It should be pointed out that this equation assumes that the quantum yield of dimer formation and dimer splitting is constant at different wavelengths. Previous studies have indicated that the quantum yield of pyrimidine dimer formation in DNA has little if any wavelength dependence.[3] Work is currently underway in our laboratory to determine the absolute quantum yields of these processes.

Table 3 lists the calculated quantum yield ratios for each system. The thymine–thymine, uracil–uracil, and the thymine–cytosine systems were found to have similar quantum yield ratios, between 0.5 and 1.0. The cytosine–cytosine system, on the other hand, shows a much lower tendency to form dimers, having a quantum yield ratio of 0.02. To investigate the origin of this reduced efficiency of dimerization for the dimethylcytosine system, strain energies of the dimer and monomers (E_{dim} and E_{mon}, respectively) were calculated using MM2 molecular mechanics.[48] The amount of strain energy that was introduced into the system upon dimerization (E_{strain}) was calculated using eq 5 and is listed in Table 3. These calculations suggest that for the cytosine containing systems there is about 7 kcal/mol of strain energy introduced into the dimer by methylation of the exocyclic nitrogens on cytosine (E_{strain}(DMC–DMC) – E_{strain}(C–C)).

$$E_{strain} = E_{dim} - E_{mon} \tag{5}$$

However, for the thymine system, the amount of steric strain introduced upon dimerization exceeds that of the DMC–DMC system by 6.5 kcal/mol, suggesting steric strain alone cannot account for the reduced efficiency of dimerization for the dimethylcytosine system. Thus, the low quantum yield ratio for the dimethylcytosine system must be, at least in part, due to an electronic effect. Whether this effect has a significant consequence on the formation of cytosine dimers *in vivo* warrants further investigation.

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Scheme 5

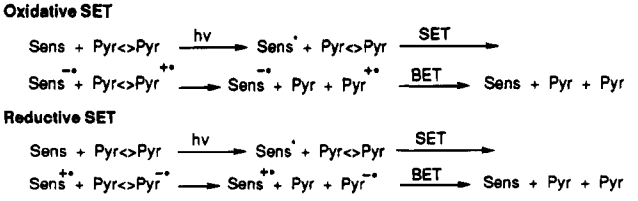


Table 4. Reductive Cleavage of Linked Pyrimidine Dimers

compound	sensitizer/solvent	% yield (% conversion)
DMC<>DMC (**13**)	DMA/H_2O[a]	100 (44)
	TMB/CH_3CN[b]	100 (87)
DMC<>T (**17**)	DMA/H_2O[a]	95 (43)
T<>T (**10**)	DMA/H_2O[c]	100 (45)
U<>U (**9**)	DMA/H_2O[c]	92 (74)

[a] Irradiated with λ = 330 nm light. [b] Irradiated with λ = 330 nm light. [c] Irradiated with λ > 300 nm light.

Table 5. Oxidative Cleavage of Linked Pyrimidine Dimers

compound	sensitizer/solvent	% yield (% conversion)
DMC<>DMC (**13**)	AQS/H_2O[a]	18 (36)
		20 (88)
	DCA/CH_3CN[a]	26 (31)
DMC<>T (**17**)	AQS/H_2O[a]	33 (21)
		22 (82)
T<>T (**10**)	AQS/H_2O[a]	64 (10)
		67 (60)
U<>U (**9**)	AQS/H_2O[b]	93 (35)
		54 (100)

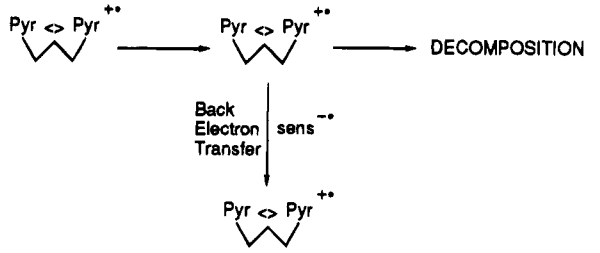
[a] Irradiated with λ > 330 nm light. [b] Irradiated with λ > 300 nm light.

3. Sensitized Splitting: Model Studies of DNA Photolyase. The catalytic step in DNA photolyase-mediated splitting of pyrimidine dimers is widely thought to occur through an excited state electron transfer.[10] The two pathways in Scheme 5 illustrate this in a general way. In the oxidative pathway, the photosensitizer (i.e. the enzyme) absorbs a photon and then abstracts an electron from the substrate (i.e. the pyrimidine dimer). The substrate radical cation cleaves and the charge-bearing fragment reoxidizes the sensitizer anion radical. The reductive pathway follows an analogous sequence. In this case, the initial electron flow is from the sensitizer to the substrate.

To evaluate the efficiency of these processes for our dimer systems, the reactions of the pyrimidine dimers with excited-state electron donors were examined and the results are compiled in Tables 4 and 5. For the reductive experiments, both *N,N*-dimethylaniline (DMA) and *N,N,N,N*-tetramethylbenzidine (TMB) were used. In all cases, nearly quantitative yields of the monomers are obtained. DMA has a singlet energy of 90 kcal/mol.[16] This is 5 kcal/mol below the lower limit estimated for the cytosine-containing dimers (see previous section), and consequently singlet energy transfer would be expected to be negligible. However, to further ensure that only electron transfer pathways were operative, some experiments were also carried out using TMB. The latter has a much lower singlet energy (E_s = 83 kcal/mol)[16] while maintaining a low excited state oxidation potential. This sensitizer was also found to efficiently split the dimers, thus supporting reductive electron transfer as the mechanism of dimer cleavage.

Both of these sensitizers are very powerful reducing agents in their excited states. DMA has an excited-state oxidation potential (E_{ox}*) of −3.0 V (vs SCE) and the corresponding value for TMB is −3.2 V.[16] These are both predicted to be stronger excited-state reductants than the dihydroflavin chromophore present in many photolyases. In free solution dihydroflavin is calculated to have E_{ox}* = −2.6 V. Previous work from our laboratory has shown that thymine dimers have reduction potentials of approximately −2.6 V.[16] The reduction potential of the cytosine−cytosine dimers is not known. However, Kim and Sancar have shown that the these substrates quench approximately 50% of the dihydroflavin fluorescence when bound to *E. coli* photolyase.[39] Based on this result, it was inferred that the initial electron transfer step occurs with reasonable efficiency. (Low efficiency of splitting was attributed to slower bond scission.) If this is true, the reduction potential of the cytosine−cytosine dimer must be reasonably close to the dihydroflavin, and certainly TMB and DMA should be capable of reducing these substrates.

Scheme 6



It is also known that excited state electron acceptors are capable of splitting of thymine dimers in solution.[6] Radical cation pericyclic processes, such as retro [2 + 2] reactions of cyclobutanes, are much more widely characterized than the corresponding radical anion processes.[49] Probably for this reason, earlier workers considered oxidative electron transfer a more probable pathway for photolyase than the reductive pathway.[45,50]

To determine if oxidative electron transfer would lead to the splitting reaction, the reactions of the dimers with excited-state electron acceptors was examined. Both anthraquinone sulfate (AQS) and 9,10-dicyanoanthracene (DCA) were used as photosensitizers. Both of these are strong excited state oxidants having oxidation potentials of +2.0 V (DCA)[51] and +1.8 V (AQS).[52] In all cases AQS was effective at splitting the dimers.

For most of the dimer systems, the conversion-corrected yields were rather low. Specifically, for dimers **13** and **17**, a large number of side products were observed by HPLC after irradiation. AQS-sensitized photolysis of the monomers gave mixtures with almost identical chromatograms. This is not surprising. Pyrimidine cation radicals are known to be highly reactive, undergoing deprotonation, hydration, and addition of O_2.[3] It seems reasonable to assume that the side products are due to reaction of the cytosine cation radical formed after splitting that occur in competition with back electron transfer from the sensitizer (Scheme 6).

The relative rates of the monomer cation radical decomposition reactions and back electron transfer would

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(52) Mariano, P. S.; Stavinoha, J. L. In *Synthetic Organic Photochemistry*; Horspool, W. M., Ed.; Plenum Press: New York, 1984; pp 145–257.

therefore be expected to depend on the sensitizer and the medium. For this reason some experiments were also carried out using DCA as a sensitizer. Because of solubility constraints these were carried out using acetonitrile. Even with this different sensitizer and solvent, low mass balances were still obtained. From the oxidative results, it can be surmised that any photolyase enzyme that utilizes such a pathway would have to also protect the incipient monomer cation radical from destructive side reactions.

Conclusions

The above results show that the cytosine-containing cis-syn dimers have relatively strong absorption bands in the UV-B region of the spectrum. They can be reverted back to their monomeric forms by direct photolysis. As with thymine, a photostationary state between monomers and dimers is reached after prolonged irradiation. The dimers can also be split by excited state electron transfer reactions. Use of excited state electron donors gives the monomers in high chemical yields with few or no side products. Use of excited state electron acceptors also results in splitting, but the chemical yields are much lower due to decomposition of the monomers.

Experimental Section

General. Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. All distillations were performed under a dry N_2 atmosphere unless otherwise stated. 1,3-Dibromopropane was distilled from P_2O_5 prior to use. Anhydrous 1,4-dioxane was purchased from Aldrich Chemicals.

Preparative photolysis reactions were performed using an Ace-Hanovia 450 W medium pressure Hg vapor lamp. Filters used were Corex (λ > 270 nm), flint (λ > 300 nm), or uranium (λ > 330 nm).

General Procedure for Irradiation of Pyrimidine Monomer and Dimer Solutions. A quartz test tube was charged with a solution (either H_2O or CH_3CN) of the monomer or dimer being studied (0.5–2 mM). An excess of the sensitizer of interest was added (if used) and the tube was sealed with a septum and purged with N_2 for 10 min. The solution was irradiated with a 450 W Hg vapor lamp fitted with the appropriate filter for that experiment (flint, uranium, or none). For single wavelength irradiations, the solution was irradiated with a Xe arc lamp through a monochrometer set to 330 nm. Reaction progress was followed by HPLC analysis.

HPLC Analysis of Reaction Solutions. After irradiation, samples were analyzed by HPLC and the peak areas of known products were correlated to concentration curves derived from authentic samples. For the U–U, T–T, and DMC–T systems, an analytical C_{18} reversed phase column was used with a 9:1 H_2O:CH_3CN mobile phase. For the DMC–DMC system, an analytical amino-modified silica gel column (Microsorb-MV) was used with a MeOH:Et_2O mobile phase (2:3 until the monomer comes off and then 9:1). Products were detected by a UV detector set at 222 nm for the U–U and T–T systems and 275 nm for the DMC–T and DMC–DMC systems.

1-(3-Bromopropyl)uracil (5), 1,1′-Trimethylenebis(uracil) (7), and 1,1′-trimethylenebis(thymine) (8) were synthesized using the general procedure of Golankiewicz and co-workers.[40] **1-(3-Bromopropyl)thymine (6)** was prepared as previously reported.[53] **1,1′-Trimethylenebis(4-thiouracil) (11)** was synthesized using the procedure of Golankiewicz and Langer.[41]

1,1′-Trimethylenebis(uracil) Photodimer (9). A solution of 500 mg (1.9 mmol) of **7** in 1000 mL of H_2O was placed in an immersion well photolysis tube. A volume of 100 mL of spectroscopic grade acetone was added, and the solution was purged with N_2. The solution was irradiated with a 450 W medium pressure Hg vapor lamp through a Corex filter for 1 h. The solvent removed by rotary evaporation, and the remaining solid was recrystallized from water and collected by vacuum filtration to give 181 mg (36%) of **9** as a colorless solid: mp > 300 °C; ^{1}H NMR (d_6-DMSO) δ 10.28 (s, 2H), 4.18 (m, 2H), 4.08 (m, 2H), 3.82 (m, 2H), 2.76 (m, 2H), 1.82 (m, 1H), 1.46 (m, 1H); ^{13}C NMR (d_6-DMSO) δ 166.1, 150.8, 54.4, 46.7, 36.7, 24.1.

1,1′-Trimethylenebis(thymine) photodimer (10) was prepared according to the procedure of Leonard and coworkers.[54] ^{1}H NMR, mp, and UV-vis data matched that previously reported.[54] ^{13}C NMR (d_6-DMSO) δ 169.6, 150.8, 59.7, 46.5, 44.6, 23.4, 19.9; high resolution mass spectrum, m/z 292.1176 ($C_{13}H_{16}O_4N_4$ requires 292.1172).

1,1′-Trimethylenebis(N_4-dimethylcytosine) (12). A high pressure reaction vessel was charged with a 35 mL of EtOH, 1.1 g (3.7 mmol) of **11**, and 12 mL (181 mmol) of $NHMe_2$. The vessel was sealed and the solution was allowed to stir at 90 °C for 12 h. The solution was allowed to cool and the solvent was removed by rotary evaporation leaving a red solid which was dried under vacuum. The solid was dissolved in 40 mL of EtOH and filtered with 1 g of activated carbon. The filtrate was concentrated by rotary evaporation to 15 mL and added dropwise to 150 mL of boiling Et_2O. The resulting white precipitate was collected, suspended on alumina, applied to the top of an alumina column, and eluted with 50:1 $CHCl_3$:EtOH to give 650 mg (65%) of **12** as a white solid: mp 223–226 °C, ^{1}H NMR (CD_3OD) δ 2.08 (quintet, J = 7.0, 2H), 3.08 (s, 6H), 3.13 (s, 6H), 3.82 (t, J = 7.0, 4H), 6.06 (d, J = 7.5, 2H), 7.63 (d, J = 7.5, 2H); ^{13}C NMR (CD_3OD) δ 165.4, 158.8, 141.8, 93.8, 79.5, 38.2, 37.5, 29.8; IR (KBr) 2951 (w), 1650 (vs), 1540 (s), 1501 (m), 1384 (m), 1314 (m), 1236 (w); low resolution mass spectrum, m/z 318 (M^+, 18), 179 (100), 166 (52), 123 (26), 68 (64); high resolution mass spectrum, m/z 318.1824 ($C_{15}H_{22}O_2N_6$ requires 318.1804).

1,1′-Trimethylenebis(N_4-dimethylcytosine) Photodimer (13). An immersion well photolysis tube was charged with a solution of 50 mg (0.16 mmol) of **12** in 130 mL of spectroscopic grade acetone. The solution was purged of O_2 with N_2 and irradiated with a medium pressure mercury vapor lamp through a 290 nm cutoff filter (Corex) for 1 h. The solvent was removed by rotary evaporation leaving a residue which was dried under vacuum (0.1 mmHg) and dissolved in 3 mL of CH_3CN. TLC analysis (amino plates, 100% EtOH) shows **13** with an R_f of 0.31 by UV detection. The sample was applied to a 2 × 16 cm amino modified silica gel flash column packed in 1:1 Et_2O:EtOH and eluted with 1:1 Et_2O:EtOH until all of the starting material and byproducts came off and then 100% EtOH. The fractions containing **13** were pooled and the solvent removed by rotary evaporation to give 12.7 mg (25%) of **13** as colorless needles: mp 264–270 °C dec, ^{1}H NMR (CD_3OD) δ 1.45 (m, 1H), 2.38 (m, 1H), 2.71 (m, 2H), 3.03 (s, 6H), 3.07 (s, 6H), 4.20 (m, 2H), 4.27 (m, 2H), 4.46 (m, 2H); ^{13}C NMR (CD_3OD) δ 165.1, 160.6, 57.7, 49.3, 39.5, 38.1, 34.6, 24.4; IR (KBr) 2938, (w), 2365 (w), 2339 (w) 1612 (s), 1564 (vs), 1460 (s), 1424 (s), 1402 (s), 1315 (s); low resolution mass spectrum, m/z 318 (M^+, 7), 179 (100), 166 (60), 153 (24), 123 (39); high resolution mass spectrum, m/z 318.1814 ($C_{15}H_{22}O_2N_6$ requires 318.1804).

1-(3-Bromopropyl)-4-thiouracil (14). To a solution of 2.5 g (10.3 mmol) of **5** in 70 mL of dry 1,4-dioxane was added 8.4 g (18.8 mmol) of P_2S_5. The solution was allowed to stir under N_2 for 5 h. The solution was poured into 100 mL of water and extracted with $CHCl_3$. The organic layers were combined and dried over $MgSO_4$, and the solvent was concentrated to 50 mL by rotary evaporation. The yellow liquid was applied to a silica gel column and eluted with 3:2 EtOAc:hex. The portions containing **14** (which were bright yellow) were combined, and the solvent was removed by rotary evaporation to yield 2.0 g (80%) of **14** as a yellow solid: mp 200–206 °C dec; ^{1}H NMR ($CDCl_3$) δ 2.29 (tt, J = 6.2, 6.6, 2H), 3.45 (t, J = 6.2, 2H), 3.98

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(t, J = 6.6, 2H), 6.40 (dd, J = 7.5, 2.1, 1H), 7.08 (d, J = 7.5, 1H), 9.83 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 189.9, 148.2, 139.6, 113.3, 48.2, 30.8, 29.5; IR ($CHCl_3$) 3370 (w), 2962 (w), 2261 (w), 1715 (s), 1622 (vs), 1443 (m), 1338 (m), 1254 (m), 1128 (m), 1085 (m); low resolution mass spectrum, m/z 250 (M + 2, 100), 248 (M^+, 99), 229 (7), 186 (6), 169 (38), 142 (60), 128 (37); high resolution mass spectrum, m/z 249.9604 ($C_7H_9ON_2{}^{81}BrS$ requires 249.9599).

1-[3-(Thym-1-yl)propyl]-4-thiouracil (15). A solution of 2.6 g (10.3 mmol) of **1-(3-bromopropyl)-4-thiouracil** and 7.3 g (48 mmol) of **4**[42] in 50 mL of dry 1,4-dioxane was allowed to stir under N_2 at reflux for 12 h. The dioxane was then removed by rotary evaporation while heating the solution in a water bath. This left crude **15** as a yellow solid and bis(trimethylsilyl)thymine as a liquid which was immediately decanted off. The remaining yellow solid was dissolved in boiling water, filtered, and allowed to precipitate upon cooling. The precipitate was collected and dried under vacuum to give 1.7 g of a yellow solid containing **15**, thymine, and some unidentified reaction side-products. The compound was carried on to the next step without further purification: ^{1}H NMR (d_6-DMSO) δ 11.23 (s, 1H), 10.80 (s, 1H), 7.58 (d, J = 1.3, 1H), 6.24 (dd, J = 7.3, 1.6, 1H), 3.70 (m, 4H), 1.91 (m , 2H), 1.7 (s, 3H); high resolution mass spectrum, m/z 294.0776 ($C_{12}H_{14}O_3N_4S$ requires 294.0787).

1-[3-(Thym-1-yl)propyl]-N_4-dimethylcytosine (16). A thick walled borosilicate glass tube was charged with 40 mL of EtOH, 925 mg (3.1 mmol) of crude **15**, and 13 mL (196 mmol) of $NHMe_2$. The reaction vessel was sealed and the solution was allowed to stir at 100 °C for 12 h. The solution was allowed to cool, and the solvent was removed by rotary evaporation. The resulting brown residue was dissolved in 50 mL of water and filtered with a small amount of activated carbon. The filtrate was collected and applied to a C18-reverse phase silica gel column. The product was eluted with 9:1 H_2O:CH_3CN until all of the thymine came off and then 6:4 H_2O:CH_3CN. The fractions containing **16** were pooled, and the solvent was removed by rotary evaporation. The remaining residue was triturated with 10 mL of acetone, dried, and collected to give 468 mg (49%) of **16** as a white solid: mp 238–242 °C (dec); ^{1}H NMR (d_6-DMSO) δ 1.72 (s, 3H), 1.85 (quintet, J = 7.0, 2H), 3.00 (s, 6H), 3.62 (t, J = 7.0, 2H), 3.66 (t, J = 7.0, 2H), 5.95 (d, J = 7.5, 1H), 7.52 (s, 1H), 7.67 (d, J = 7.5, 1H), 10.13 (br s, 1H); ^{13}C NMR (d_6-DMSO) δ 164.2, 163.5, 154.9, 150.8, 145.8, 141.2, 108.5, 90.7, 46.0, 44.9, 37.3, 36.3, 28.3, 11.8; IR (KBr) 3156 (w), 3031 (w), 1650 (vs), 1538 (m), 1382 (m), 1351 (m), 1307 (m), 1224 (w), 869 (w); low resolution mass spectrum, m/z 305 (M^+, 33), 179 (29), 166 (52), 153 (100), 138 (23), 123 (47); high resolution mass spectrum, m/z 305.1479 ($C_{14}H_{19}N_5O_3$ requires 305.1488).

1-[3-(Thym-1-yl)propyl]-N_4-dimethylcytosine Photodimer (17). To a flask containing 130 mL of refluxing acetone was added 50 mg (0.16 mmol) of **16**. The solution was allowed to stir at reflux for 15 min and then filtered (gravity filtration) to remove any insoluble particles. The solution was purged with N_2 and irradiated with a medium pressure Hg vapor lamp through a Corex filter for 30 min. The solvent was removed by rotary evaporation and the remaining residue dried under vacuum. The residue was suspended in 8 mL of EtOAc and centrifuged. The supernatant was removed and the remaining solid dried under vacuum and collected to give 25 mg (50%) of **17** as a colorless solid: mp 290–295 °C dec, ^{1}H NMR (d_6-DMSO) δ 1.37 (m, 1H), 1.65 (s, 3H), 1.89 (m, 1H), 2.54 (m, 1H), 2.69 (m, 1H), 2.90 (s, 3H), 3.01 (s, 3H), 3.87 (m, 2H), 4.07 (m, 2H), 4.15 (m, 1H), 10.32 (s, 1H); ^{13}C NMR (d_6 DMSO) δ 170.1, 163.0, 156.0, 150.5, 61.8, 52.2, 47.5, 46.8, 44.6, 38.1, 37.3, 25.1, 23.8; IR (KBr) 3431 (m), 3196 (m), 3067 (m), 2938 (m), 1671 (vs), 1626 (s), 1565 (vs), 1474 (m), 1300 (s), 1224 (m), 1102 (w), 943 (w), 829 (w), 769 (w); low resolution mass spectrum, m/z 305 (M^+, 69), 179 (31), 166 (66), 153 (100), 139 (21), 123 (48); high resolution mass spectrum, m/z 305.1471 ($C_{14}H_{19}O_3N_5$ requires 305.1488).

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Supplementary Material Available: ^{1}H NMR spectra for compounds **9**, **10**, **12**, **13**, **16**, and **17** (6 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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