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DNA–Protein Cross-Linking: Model Systems for Pyrimidine–Aromatic Amino Acid Cross-Linking

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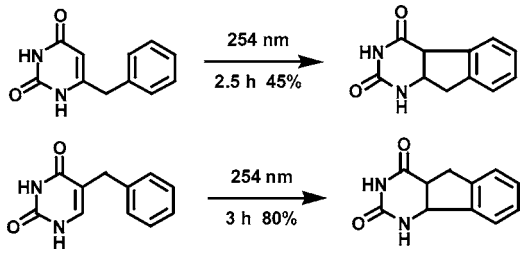
ABSTRACT

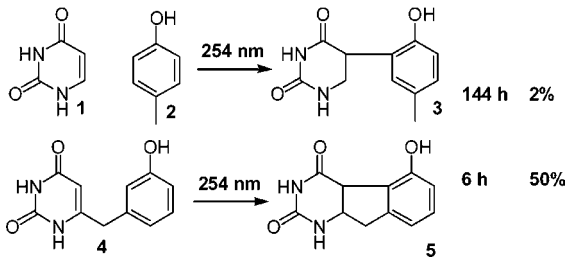


We have synthesized simple model systems to explore the possibility of photo-cross-linking between the pyrimidine bases and the side chains of the aromatic amino acids. Thymine/phenylalanine and thymine/tyrosine models gave cross-links, and thymine/tryptophan models gave complex mixtures; the cytosine/phenylalanine model was unreactive. The quantum yields for the model cross-linking reactions were 18–46 times smaller than those for thymine dimer formation. Biphotonic excitation contributes little to the yield of these reactions.

UV irradiation of protein−nucleic acid complexes results in the cross-linking of the protein to the nucleic acid. The use of this reaction to study protein nucleic acid interactions has been limited because it is very inefficient and is accompanied by extensive protein and nucleic acid damage.[1] The resulting complicated reaction mixtures also make it difficult to identify specific amino acid/base cross-links; Ser-T, Cys-T, Lys-T, and Tyr-T are the only cross-links so far characterized.[2−4] Essentially, nothing is known about the cross-linking mechanisms. Here, we describe simple model systems to explore the cross-linking of tyrosine, phenylalanine, and tryptophan to the pyrimidine bases.

Our model-building strategy involved attaching the pyrimidine base to the tyrosine, phenylalanine, and tryptophan side chains, using a short linker to mimic the proximity and orientation in DNA−protein complexes. We first synthesized the uracil−tyrosine model **4**. Irradiation of **4** resulted in the formation of the cyclization product **5**. This reaction was much more efficient than the corresponding intermolecular reaction of uracil **1** and cresol **2** (Scheme 1) and is analogous to the previously reported tyrosine/thymine photolysis which yielded a similar adduct but also in low yield.[5]

Scheme 1



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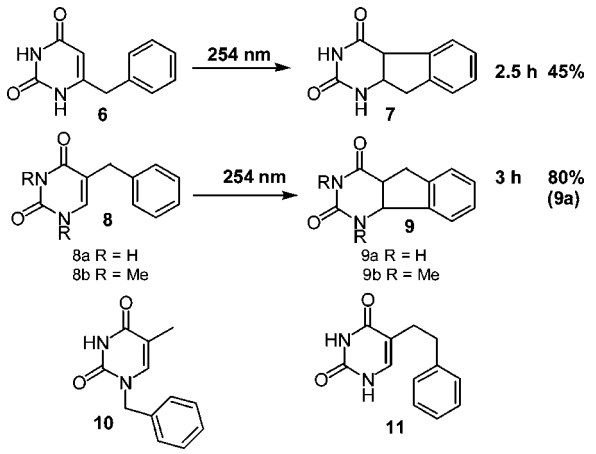
6-Benzyluracil **6** and 5-benzyluracil **8** were next synthesized to explore thymine/phenylalanine cross-linking. Irradiation of **6** and **8** resulted in the corresponding cyclization products **7** and **9** (Scheme 2). The structures of **7** and **9** were

Scheme 2



fully characterized by ^{1}H NMR, ^{13}C NMR, and MS. These adducts suggest possible structures for the cross-linking of phenylalanine to DNA, a reaction reported twenty years ago for which the adduct structure was not determined.[6] Structures **7** and **9** revealed that both carbons 5 and 6 of the pyrimidine can participate in cross-link formation. Compounds **10** and **11** failed to undergo photocyclization, suggesting that the orientation of the base and the amino acid side chain is critical for cross-linking. There was no change in the quantum yield for the conversion of **8** to **9** in the presence and absence of oxygen (Table 1), suggesting

Table 1. Quantum Yields (QY) for the Photoreactions of Compounds **5**, **7**, and **9** and Thymine Photodimer (T=T)

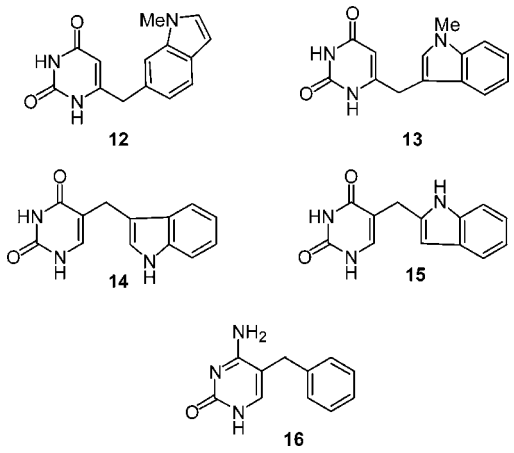
entry	products	conditions	QY × 10^3
1	**5**	degassed	1.19
2	**9a**	degassed	1.60
3	**9a**	aerated	1.60
4	**9a**	aerated	2.63 (laser)[a]
5	**9a**	O_2 saturated	1.58
6	**9b**	aerated	0.79
7	**7**	aerated	1.02
8	**7**	aerated	2.24 (laser)[a]
9	T=T	aerated	46.7[b]

[a] Laser peak intensity ~4 × 10^9 W/cm^2; CW intensity ~4 × 10^{-3} W/cm^2. See text for details. [b] See the last paragraph.

that the cross-linking reaction involves singlet state chemistry.

To probe the photoreactivity of tryptophan with thymine, compounds **12**−**15** were synthesized (Scheme 3). Photolysis

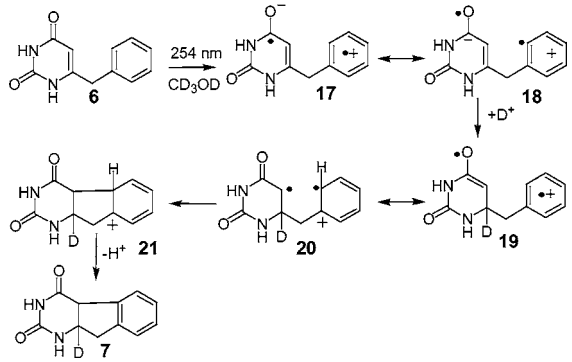
Scheme 3



of these compounds under anaerobic conditions gave complex reaction mixtures, and none of the expected photocyclization products were detected. This was surprising: because indoles are better reducing agents than benzene, we anticipated that the trapping of the short-lived pyrimidine excited state would have been more efficient with those systems.

The cytosine−phenylalanine model **16** was also studied. This compound is unreactive under the photolysis conditions used for the photocyclization of **6** and **8**.

Scheme 4



We propose the mechanism outlined in Scheme 4 for the photocyclization of **6**. Electron transfer from the phenyl group to the photoexcited pyrimidine would give the radical ion pair **17**. Protonation of the pyrimidine radical anion at C6 would give **19**. Analogous pyrimidine radical anion protonation reactions have been previously described.[7,8]

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Radical coupling would give **21**, and rearomatization by deprotonation would complete the reaction. This proposal is supported by the observation of deuterium incorporation from solvent at C6 of the dihydropyrimidine. A similar mechanistic analysis can be used to explain the formation of **5** and **9**.

It has been reported that irradiation with a high-intensity UV laser greatly increases the efficiency of protein−nucleic acid cross-linking because of a biphotonic excitation mechanism.[9] However, it is difficult to investigate cross-linking of proteins to nucleic acids in the presence of competing protein and nucleic acid degradation. Our models provide an ideal system in which to evaluate the importance of a biphotonic excitation mechanism because of their cleaner photochemistry. Using the frequency-tripled output of a Ti:sapphire regenerative amplifier (265 nm, 150 fs pulselength), we measured the intensity-dependent yields for the photoreactions presented in Scheme 2 (Figure 1). For peak UV intensities in the range of $\sim 10^7-10^{10}$ W/cm^2, fits of the data to a power law relationship (yield $= a \times$ intensity^b, where a and b are constants) suggest that the photochemical conversion of 8 to 9 is predominantly monophotonic ($b = 1.03$) whereas conversion of 6 to 7 has only a small biphotonic component ($b = 1.16$). The quantum yield for each reaction at the lowest laser intensities (see Supporting Information) is close to that measured with continuous-wave (CW) irradiation, ruling out the possibility that the intensity dependence is artificially low because of saturation effects.[10] NMR analysis was used to confirm that the products of laser irradiation are the same as those of CW irradiation. At best, the laser quantum yields are enhanced by only a factor of 2−3 over the CW values (Table 1) despite the difference of $\sim 10^{12}$ W/cm^2 in peak intensity, further suggesting that biphotonic excitation is relatively unimportant for these photoreactions within the intensity range investigated.

In conclusion, we have synthesized simple model systems to explore the possibility of photo-cross-linking between the pyrimidine bases and the side chains of the aromatic amino acids. Thymine/phenylalanine and thymine/tyrosine models gave cross-links and thymine/tryptophan models gave complex mixtures; the cytosine/phenylalanine model was unreactive. The quantum yields for the model cross-linking reactions were 18−46 times smaller than the quantum yield for thymine dimer formation, suggesting that UV damage to DNA will be a serious problem in the analysis of cross-linked protein−DNA complexes. We find that biphotonic excitation contributes little to the yield of these reactions, even when using high-intensity irradiation.

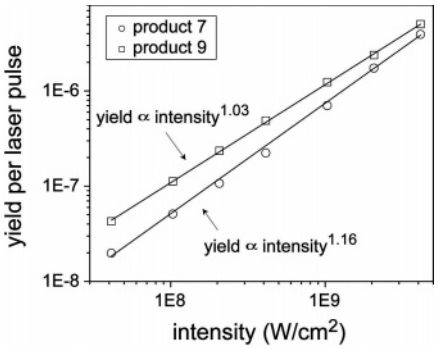

Figure 1. Product yield (expressed as a fraction of the starting material) per laser pulse for reactions in Scheme 2 as a function of UV intensity. Solid lines are the result of fits to a power law relationship, as described in the text.

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Supporting Information Available: Experimental details for the preparation and characterization of compounds **3**−**16**, for the photoreactions, and for the quantum yield determinations are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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