dimethyl ester hydrochloride (Sigma Chemical Co.) was recrystallized from absolute methanol. Water was distilled from a copper-bottom still, passed through a Barnstead mixed-bed ion-exchange column, boiled for 20 min, and cooled suddenly. Heavy water (Stohler 99.8 atom % deuterium) was distilled and the 100–120 °C fraction was used. Ester substrates used in this study in protiated and deuterated forms (>99.5% labeled) were prepared from the appropriate purified phenol and labeled acetyl chloride as previously described.[4c]

Solutions of 1.0 M hydrazine monohydrochloride and semicarbazide hydrochloride were made and titrated with standard KOH solutions to give 80% free base. The pH of such a semicarbazide buffer is 4.46 and that of the hydrazine buffer is 8.90 or pD 9.5 in DOD. Serial dilutions of these buffers were made with a 1.0 M KCl solution and a deviation of pH ±0.03 was tolerated on dilution. Methoxyamine was used at 0.2 M concentration, ionic strength 0.4 M with KCl, and was 50% neutralized with a KOH solution to pH 4.80 (pD 5.30) shortly before use. A 1.6 M solution of DL-aspartic acid dimethyl ester was prepared, KOH was added to pH 4.80 (pD 5.30), and the solution was diluted with 1.6 M KCl to give concentrations 0.1–1.0 M at ionic strength 1.00 M after a 1:1 dilution with the methoxyamine stock solution. The reaction solutions contained 0.1 M methoxyamine at a fraction of 0.555 free base; the fraction of the DL-aspartic acid dimethyl ester in the acidic form was 0.99. The pH of reaction mixture was determined at 25 °C before and after each experiment. A Radiometer 26 pH meter was employed for pH measurements.

Kinetics. Rate measurements were made with a Cary 16 spectrophotometer interfaced to a Hewlett Packard 2100-A minicomputer or to a DEC-Heathkit H-11 microcomputer. First-order rate constants for reactions of hydrazine and semicarbazide were obtained by a weighted, nonlinear, least-squares fit of the data to an exponential function. Methoxyaminolysis was slow and was studied under zero-order conditions. Duplicate samples at 0.01 M initial concentration of phenyl acetate were followed for 2–3 h, corresponding to 1–5% reaction. A linear least-squares fit of absorbance A vs. time t gave dA/dt. The difference in extinction coefficients of phenol and phenyl acetate at 275 nm is 1340 M^{-1} cm^{-1}. Exact values of initial substrate concentrations, E_0, were obtained by hydrolysis of an aliquot of the phenyl acetate stock solution in methanol with 0.1 M NaOH. The absorbance of the liberated phenoxide ion was measured at 286.9 nm (ϵ 2544 M^{-1} cm^{-1}). The first-order rate constant was calculated from

$$k_0 = (dA/dt)/E_0(1340)$$

In all kinetic studies, rates for the protiated and deuterated substrates were measured in alteration with as little intervening time as possible. Identical solutions were employed with the two isotopic substrates in these paired experiments. Reaction temperatures were controlled by a Lauda K4/RD circulating water bath connected to the cell compartment and cuvet holder. Temperatures were monitored with a digital thermistor.

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Spectroscopy and Photochemistry of Thiouracils: Implications for the Mechanism of Photocrosslinking in tRNA

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Abstract: Nanosecond laser photolysis studies of 4-thiouridine (4t-Urd), 1,3-dimethyl-4-thiouracil (DMTU), and uracil (U) are described. Absorption spectra of the triplet states of these molecules are presented along with INDO/S calculations of ground-state and excited-state spectra. The excited triplet-state spectra of 4t-Urd and DMTU are similar, indicating that the T_1 state of 4t-Urd must be primarily in the thione tautomeric form. The intersystem crossing yields for 4t-Urd and DMTU are 0.9 ± 0.1 and 1.0 ± 0.1, respectively. Quenching of the lowest triplet state of 4t-Urd and DMTU by a number of quenchers is shown to proceed primarily by an electron-transfer mechanism. Separated electron-transfer products are observed on quenching of 4t-Urd and DMTU by the two amines diethylaniline (DEA) and triethylamine (TEA). This suggests that photocrosslinking of 4t-Urd with cytidine in tRNA may occur by a mechanism which involves an initial electron-transfer quenching reaction.

Near-ultraviolet light has been shown to inhibit the growth of *E. Coli*[1-3] with an action spectrum having a maximum at 330nm. The action spectrum is similar to the absorption spectrum of 4-thiouridine (4t-Urd)[1], a somewhat uncommon tRNA nucleoside base. It has been shown that near-UV photolysis of tRNAs leads to a decrease in their activity due to crosslinking of the 4t-Urd that is in the 8 position of tRNA with a cytidine that is in the tRNA 13 position.[4-7] Given that the action spectra of photocrosslinking and growth inhibition are similar, it is believed that

the photocrosslinking reaction is responsible for the near-UV inhibition of *E. coli*. Furthermore, in both solution and in vitro intact tRNA, 4t-Urd exhibits a weak phosphorescence.[8,9] Quenching of the phosphorescence of 4t-Urd in tRNA leads to a concomitant decrease in crosslinking, implying that the 4t-Urd triplet is the photoactive state.[9]

Despite the central importance of the 4t-Urd triplet state in the photoreaction of tRNA, no comprehensive spectroscopic study of this excited state has appeared. Thus, we have explored the ground- and excited-state spectroscopy of 4t-Urd and related compounds. Among other properties of this state, we have investigated the extent of tautomerization in the triplet state and whether tautomerization is important in its photoreactions. While 4t-Urd only exists in the thione form in its ground state, the ground state of the closely related 2-thiouracil exists in an equilibrium

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between both the thione and the thiol form.[10-12] Thus, it is not obvious in what tautomer the triplet of 4t-Urd will exist.

To determine the structure of the triplet of 4t-Urd, we measured the absorption spectra of the lowest triplet states of 4t-Urd and 1,3-dimethyl-4-thiouracil (DMTU), an analogue which cannot tautomerize. We also studied uracil (U), as it has been studied
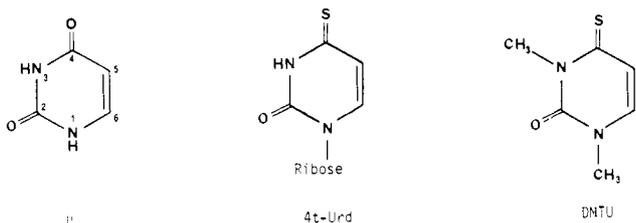
to a much greater extent than 4t-Urd and thus allows us to more easily compare our methods with others.

In each molecule, we have investigated ground- and excited-state absorption spectra both experimentally and theoretically. We found that INDO/S calculations give good descriptions of the excited-state absorptions and that the substitution of a sulfur for the oxygen at the 4-position of uracils significantly affects the triplet-state spectra. We also explored the effect that sulfur substitution has on the photophysics and photochemisty of these pyrimidinones. In particular, we have observed for the first time transient products from both oxidative and reductive electron-transfer triplet quenching of 4t-Urd and DMTU. The significance of the electron-transfer reactions is discussed below with regards to how electron-transfer quenching is the likely initial step in the photocrosslinking reaction of tRNA.

Experimental Section

Materials. U obtained from ICN Pharmaceutical and 4t-Urd obtained from Sigma were used as received. DMTU was synthesized by using the method of Elion and Hitchings[13] and was recrystallized 3 times from water. The final product exhibited the appropriate electronic absorption spectrum and a melting point of 129–130 °C. Water was doubly distilled, and 2-methyltetrahydrofuran (2Me-THF) from Aldrich was distilled from molecular sieves. EPA was made from ether/isopentane/ethanol (5:5:2 by volume). These and all other solvents were the highest purity commercially available and were dried with molecular sieves before use. MV(BF$_4$)$_2$ (MV = methylviologen) was made by metathesis of MV(Cl)$_2$ with Ag(BF$_4$) and was recrystallized from acetonitrile/ether. Naphthalene and *trans*-stilbene were sublimed, and diethylaniline and triethylamine were vacuum-distilled before use.

Experimental Methods. Transient spectra were taken by using a Quanta-Ray DCR-1 Nd:YAG laser as the pump source (fwhm = 7 ns, λ = 355 or 266 nm). The probe beam (fwhm = 5 μs) was produced by an EG&G short arc xenon flashlamp. After passing through the sample, the probe beam was directed through a 0.45 M monochromator and detected with either an EMI D279 or an EMI 9876QP photomultiplier. The signal was then digitized by a Biomation 6500 transient recorder (2 ns per point) and sent to a Z-80-based microcomputer for signal averaging and analysis.

Solutions were deoxygenated by bubbling with either Ar or N$_2$O for at least 10 min. Since U, 4t-Urd, and DMTU are all somewhat photosensitive, care was taken to ensure that the sample degraded by no more than 5% as determined by the decrease of the transient absorption at the triplet maximum. At room temperature, this was done by either repeatedly changing the solution in the sample cuvette or by flowing the sample. At 77 K, where the sample was dissolved in a glass, care was taken to move to a fresh portion of the sample after a small number of laser shots. For 4t-Urd and DMTU at room temperature, the transient absorption was short-lived (τ < 300 ns), and the signal was taken to be the maximum absorbance change after photolysis. For U at room temperature and for 4t-Urd and DMTU at 77 K, the transient did not decay appreciably on our detection time scale. Signals in these cases were taken to be the average of the transient absorbance for the 25 data bins between 250 and 300 ns after photolysis. All points in the transient spectra are

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Table I. Ground-State Absorption Spectrum of Uracil

transition S$_0$ →		10^3E, cm^{-1} (f)	
		calcd	obsd(H$_2$O)
S$_1$	nπ*	32.3 (0.001)	
S$_2$	nπ*	39.3 (0.000)	
S$_3$	ππ*	42.5 (0.400)	38.9 (0.18)
S$_4$	ππ*	50.2 (0.242)	50.0 (0.29)
S$_5$	ππ*	52.0 (0.172)	

Table II. Ground-State Absorption Spectra of 4-Thiouracil Derivatives

transition S$_0$ →		10^3E, cm^{-1} (f)		
		calculated 1Me-4-TU	observed	
			4t-Urd(H$_2$O)	DMTU(H$_2$O)
S$_1$	nπ*	23.1 (0.000)	25.0^a	
S$_2$	ππ*	31.5 (0.433)	30.2 (0.38)	30.5 (0.37)
S$_3$	nσ*	37.0 (0.011)		
S$_4$	πσ*	38.3 (0.001)		
S$_5$	ππ*	41.3 (0.023)		
S$_6$	nπ*	42.5 (0.001)		
S$_7$	ππ*	43.7 (0.121)	40.8 (0.17)	41.3 (0.17)
S$_8$	nπ*	45.1 (0.001)		
S$_9$	σπ*	48.0 (0.009)		

^{a} From ref 12. This transition is reported for 4t-Urd in CH$_3$CN, with ε = 700 M^{-1} cm^{-1}. There is no report in the literature for a similar transition in DMTU, but DMTU does exhibit an obvious weak absorption shoulder in this region of the spectrum.

from the average of at least 40 measurements.

Theoretical Methods. Experimentally determined spectra were compared with molecular orbital calculations of uracil and 1-methyl-4-thiouracil (1Me-4-TU) using the all-valence-electron INDO/S method.[14-17] Two-center repulsion integrals were evaluated by an empirical Mataga–Nishimoto–Weiss formula. Transition energies and intensities were obtained from CI wave functions constructed from a basis of 197 configurations including all single excitations between the 14 highest occupied MOs and the 14 lowest unoccupied MOs. Atomic coordinates were taken from published crystal structures.[18,19]

Results and Discussion

Ground-State Spectra. Table I compares data on the transitions observed in the ground-state spectrum of U with the results of INDO/S calculations. Two nπ* transitions are calculated to lie significantly below the lowest ππ* transition. However, these transitions have never been observed in absorption, circular dichroism, or magnetic circular dichroism spectra either in solution or in single crystals.[12,20] Photophysical experiments show that the lowest excited singlet state of U is either a ππ* or a nearby nπ* state depending on the solvent. However, an nπ* singlet state much lower in energy than the lowest ππ* singlet is not consistent with the excited-state dynamics.[21] Thus, it appears that the INDO/S method underestimates the energy of the nπ* states relative to ππ* states. Others have previously noted that INDO/S and other similar semiempirical methods underestimate the energies of nπ* states in pyridone and pyrimidinone type molecules.[22-24] The results shown in Table I indicate, however, that the ππ* transitions are described well by the INDO/S method.

Table II compares the calculated ground-state electronic spectrum of 1Me-4-TU with observed transitions in 4t-Urd and DMTU. Again, the positions of the two strong ππ* transitions are accurately calculated. A weak nπ* transition is predicted

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Table III. Calculated Energies of the Five Highest HOMOs and Five Lowest LUMOs at the SCF Level
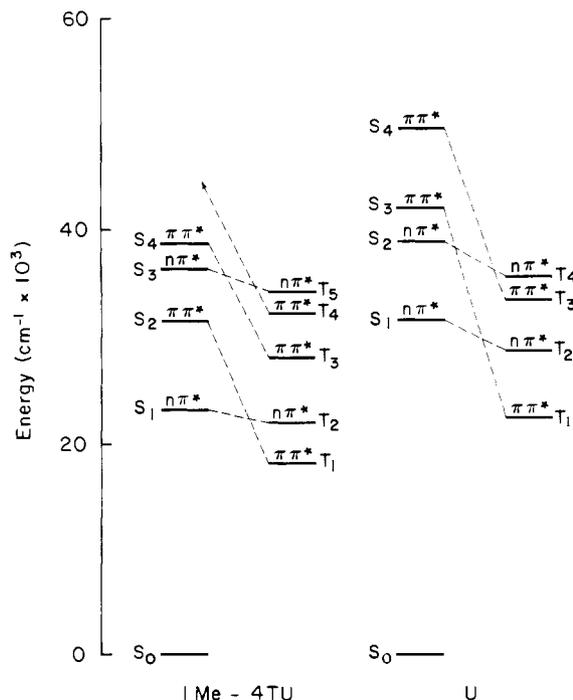
orbital designation[a]	$10^3 E$, cm^{-1}	
	U	1Me-4-TU
	LUMOs	
σ^*	27.87	23.26
π^*	21.51	18.87
σ^*	21.07	18.65
π^*	7.46	5.71
π^*	-0.66	-5.04
	HOMOs	
π	-76.15	-66.93
n	-80.75	-67.59
π	-85.36	-80.10
n	-87.56	-87.34
π	-107.96	-103.36

[a] No difference in the relative ordering of the orbitals is calculated to occur between U and 1Me-4-TU. However, for some molecular orbitals, there is a significant difference in the relative contributions of the atomic orbitals.

significantly below the lowest $\pi\pi^*$ transition, at 23 100 cm^{-1}. This is close to an observed weak transition found to lie near 25 000 cm^{-1} which has been assigned as nπ^*.[12] Previous work with thioketones has shown that sulfur substitution drastically lowers the energy of the lowest nπ^* state.[25-27] Thus, it appears that INDO/S more accurately places the nπ^* states in this sulfur-substituted pyrimidinone.

An interesting difference in the spectra of U and 4t-Urd is that both the lowest nπ^* and $\pi\pi^*$ states are much lower in energy for 4t-Urd than for U. It is possible to understand this difference in transition energies by looking at the single configuration (SCF) level of calculation, since CI lowers all the excited-state energies but does not significantly change the state ordering. Table III presents the SCF energies of the five highest occupied molecular orbitals (HOMOs) and the five lowest unoccupied molecular orbitals (LUMOs). For 1Me-4-TU, the highest HOMO is raised by about 9200 cm^{-1} relative to its energy in U. The lowest LUMO energy is lowered by about 4400 cm^{-1} for 1Me-4-TU relative to U. Thus, most of the decrease of the excitation energies of the lowest excited states upon sulfur substitution is due to the higher energy of the HOMOs. This reflects the fact that the ionization potential of sulfur atoms in the gas phase is 26 200 cm^{-1} lower than that of oxygen atoms in the gas phase.[28]

Triplet-State Spectra. At room temperature in fluid solution, U has a very weak fluorescence with a lifetime of approximately 5 ps.[29-31] The intersystem crossing yield has been shown to be dependent on solvent, increasing from approximately 0.02 in water to 0.2 in acetonitrile.[32] This has been taken as an indication that the singlet-state ordering depends on the solvent. In nonprotic solvent, the ^{1}nπ^* state is lowest and there is facile intersystem crossing from it to the lower $^3\pi\pi^*$. In protic solvent, the ^{1}nπ^* state shifts to higher energy relative to the $^1\pi\pi^*$ state. The lowest singlet state then becomes the $^1\pi\pi^*$. Since the triplet nπ^* state will not be significantly lower than the nπ^* singlet, intersystem crossing can only be from the $^1\pi\pi^*$ to the $^3\pi\pi^*$ and the intersystem crossing yield is low. Regardless of the solvent, since the exchange splitting of a $\pi\pi^*$ state is much larger than the exchange splitting of an nπ^* state, we expect the lowest energy triplet to be the $\pi\pi^*$. This is confirmed by our calculation. Figure 1 shows the calculated

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Figure 1. Calculated energies of the lowest energy singlet states (S_n) and their correlated triplet states (T_n) for U and 1Me-4TU.
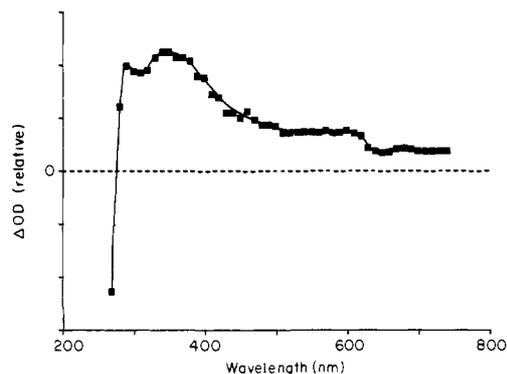
Figure 2. Transient absorption spectrum of an Ar-saturated CH$_3$CN solution of U at 295 K taken 250–300 ns after photolysis with a 7-ns, 266-nm laser pulse.

energies of the lowest singlet and triplet states of U. Even though INDO/S underestimates the energy of the lowest ^{1}nπ^* states in U, the singlet–triplet splitting of the $\pi\pi^*$ states is so great that the $\pi\pi^*$ triplet is predicted to be lowest in energy. Thus, transitions in the excited-state spectrum of the triplet state of U will originate from the $^3\pi\pi^*$.

Since the intersystem crossing yield of U is an order of magnitude higher in CH$_3$CN than in water, we obtained the triplet-state spectrum in this nonprotic solvent. The spectrum, displayed in Figure 2, agrees with a spectrum previously published in the 300–450-nm spectral region.[32] Our spectrum has been extended to cover the range of 270–750 nm. We determined the extinction coefficient of the triplet transition by comparing the relative size of the ground-state bleaching with the size of the transient absorption at the triplet absorption maximum. This measurement serves as an upper bound of the actual excited-state extinction coefficient, since it ignores any triplet absorption that may occur at the monitoring wavelength of the ground-state absorption. We obtained a value for ϵ_{350} of 3100 M^{-1} cm^{-1}, in reasonable agreement with the previously published value of 2500 M^{-1} cm^{-1} obtained by using the method of energy transfer with retinol as the acceptor.[32].

Table IV compares the calculated and observed triplet-state spectrum. Observed oscillator strengths (f) assume ϵ_{350} = 3100
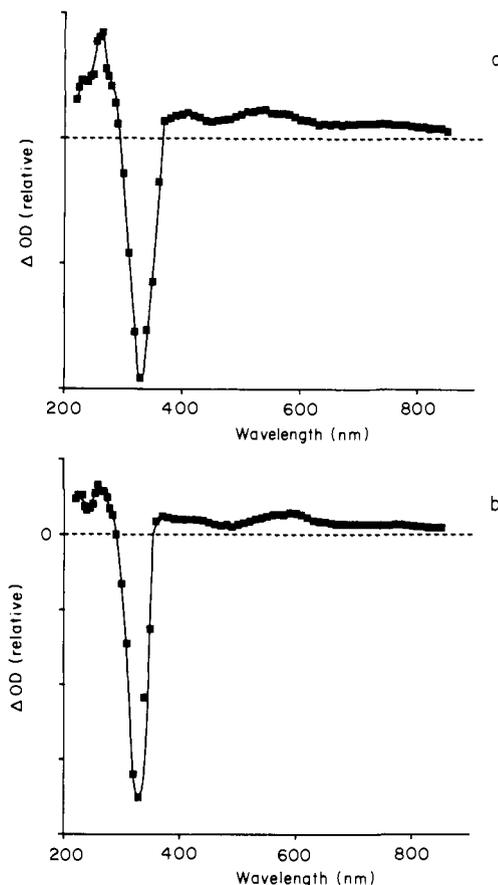
Figure 3. Transient absorption spectra of Ar-saturated H_2O solutions of 4t-Urd (a) and DMTU (b) taken directly after photolysis with a 7-ns, 355-nm laser pulse. Note the large signal at 330 nm due to ground-state bleaching relative to the triplet-state absorption in the visible region.

M^{-1} cm^{-1}. Since the bands in the triplet spectrum are weak and overlapping, the experimental oscillator strengths are only approximate and may be off by as much as a factor of 2. As predicted, weak bands are observed in the red, and other, stronger absorptions are observed in the visible and near-UV. The transitions predicted at 36 200 cm^{-1} and above are masked by the bleaching of the ground-state absorption. Table IV also indicates the transitions where a single electron promotion connects the largest components of the CI matrices. In most cases, these are transitions predicted to have moderate to strong transition strengths. Transitions which are primarily single electron $n\pi^*$ excitations are weak as expected. In one case, $T_{19} \leftarrow T_1$, there is a large transition strength predicted because single electron excitation contributions from minor components of the states are significant. Generally, Table IV shows that the INDO/S calculations accurately predict the observed triplet transitions in U.

Previous reports have shown that at room temperature in water, 4t-Urd does not fluoresce. It does, however, phosphoresce with a quantum yield of approximately 3×10^{-4} and a lifetime of approximately 250 ns.[4,9] Some photophysical results for 4t-Urd have been interpreted to indicate that an $n\pi^*$ state is the lowest triplet.[33] It is clear from ODMR studies, however, that the lowest, emissive triplet has $\pi\pi^*$ character.[34] Figure 1 shows the state ordering of the singlet and triplet states of 1Me-4-tU calculated by the INDO/S method. Even though the lowest singlet is $n\pi^*$ (see above), the exchange energy of the lowest $\pi\pi^*$ state is large so that, as in U, the lowest triplet is a $\pi\pi^*$ state.

Figure 3 shows the triplet absorption spectrum of 4t-Urd and DMTU measured from 220 to 850 nm in Ar-saturated H_2O at room temperature. This shows the relative optical density of the

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Table IV. Absorption Spectrum of the Lowest Triplet State of Uracil

triplet state[a]	single electron excitations[b]	$10^3 E$, cm^{-1} $(f)^c$	
		calcd	obsd[d]
T_2	$n\pi^*$	$n\pi$	6.4 (0.000)	
T_3	$\pi\pi^*$	$\pi^*\pi^*$	11.2 (0.003)	14.3 (<0.01)
T_4	$n\pi^*$		13.4 (0.000)	
T_5	$\pi\pi^*$		15.3 (0.003)	16.6 (<0.01)
T_6	$\pi\pi^*$	$\pi\pi$	23.6 (0.046)	28.6 (0.15)
T_7	$\pi\pi^*$	$\pi^*\pi^*$	24.9 (0.019)	
T_8	$n\pi^*$		29.9 (0.000)	
T_9	$n\pi^*$	$n\pi^*$	31.7 (0.001)	
T_{10}	$\pi\pi^*$	$\pi^*\pi^*$	32.0 (0.075)	33.9 (0.02)[e]
T_{11}	$\pi\sigma^*$	$\pi^*\sigma^*$	32.2 (0.001)	
T_{12}	$n\sigma^*$		33.8 (0.001)	
T_{13}	$\pi\sigma^*$		35.6 (0.001)	
T_{14}	$\pi\pi^*$	$\pi\pi$	36.2 (0.099)	
T_{15}	$n\sigma^*$		37.4 (0.002)	
T_{16}	$\pi\sigma^*$	$\pi^*\sigma^*$	37.5 (0.018)	
T_{17}	$n\pi^*$		41.2 (0.000)	
T_{18}	$\pi\pi^*$	$\pi\pi$	41.4 (0.093)	
T_{19}	$\pi\pi^*$		44.9 (0.026)	
T_{20}			45.1 (0.002)	

[a] The orbital designation of the triplet state, T_n, as a single electron excitation from S_0. T_1 is $\pi\pi^*$. [b] Excitations are indicated for those transitions where the largest components of the CI matrices represent a single electron excitation for $T_n \leftarrow T_1$. [c] Transition energies from T_1. [d] From spectrum of U in CH_3CN. [e] Part of the intensity of this transitions is probably masked by ground-state bleaching.
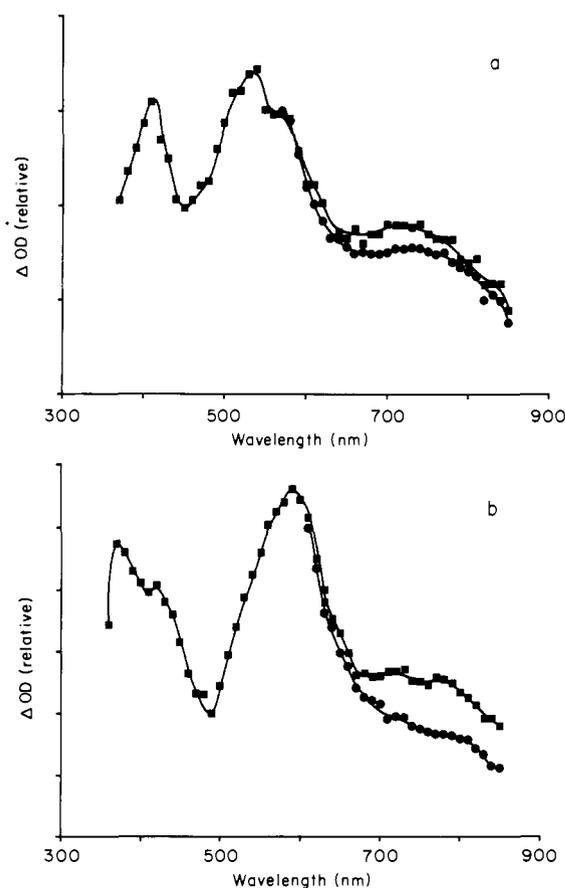

Figure 4. Triplet-state absorptions in the visible region of 4t-Urd (a) and DMTU (b) taken directly after photolysis with a 7-ns, 355-nm laser pulse. Spectra of both Ar-saturated (■) and N_2O-saturated (●) water solutions are shown. Note that N_2O decreases the transient absorptions in the red without affecting the blue portions of the spectra.

ground-state bleaching and the excited-state absorption. Spectra of solutions saturated with Ar and N_2O are shown in Figure 4 for the region 360–850nm. In spectra of both molecules, there is a small but significant decrease in absorption in the red when

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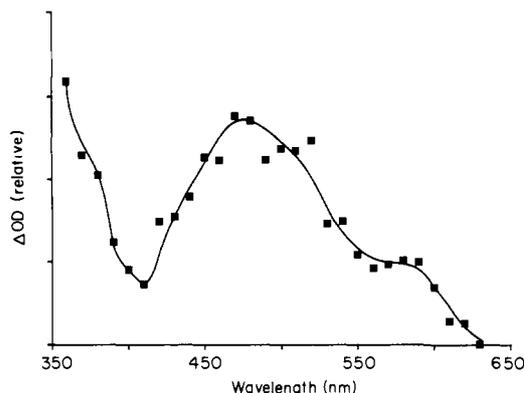*J. Am. Chem. Soc., Vol. 107, No. 25, 1985* 7369

Figure 5. Spectrum obtained 2 μs after photolysis of an N_2O-saturated solution of DMTU in H_2O. All DMTU triplets will have decayed by this time, and the spectrum is assigned to the absorption of $DMTU^+$. Absorption is weak, with ΔOD at 480 nm about 0.02.
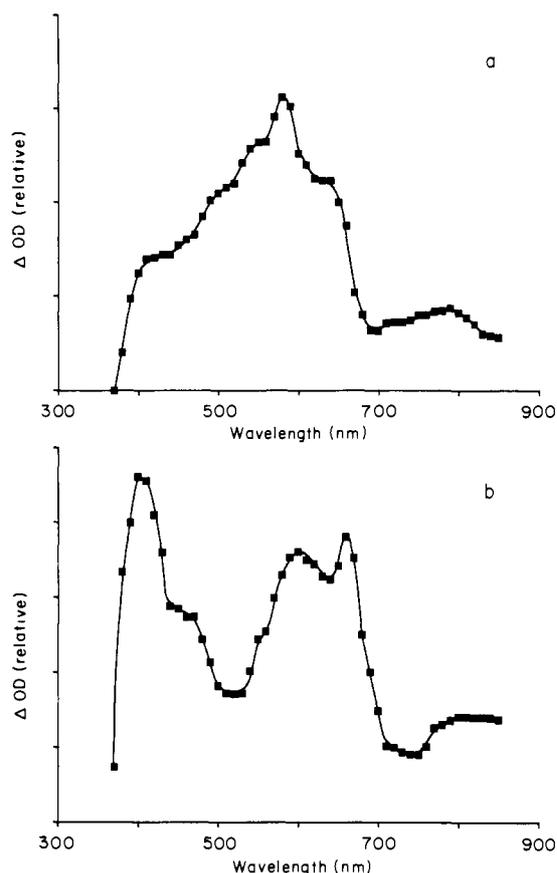

Figure 6. Absorption spectra of the triplet states of 4t-Urd (a) and DMTU (b) taken at 77 K in EPA.

the solution is saturated with N_2O. This implies that there is a small amount of solvated electron produced upon photolysis. Figure 5 shows the transient spectrum of DMTU in H_2O saturated with N_2O taken 2 μs after photolysis. By this time, the triplet absorption has completely decayed. This absorption is very weak, and we tentatively assign it to the $DMTU^+$ radical. Production of this species may be the cause of the slow photodegradation of thiouracils in pure solvents and may explain previously observed solvent photoaddition in alcohols.[35,36]

Figures 6 and 7 show the triplet spectra of 4t-Urd and DMTU obtained at 77 K in EPA and 2Me-THF. Relative to the room-temperature water spectra, the transitions in the EPA spectra are
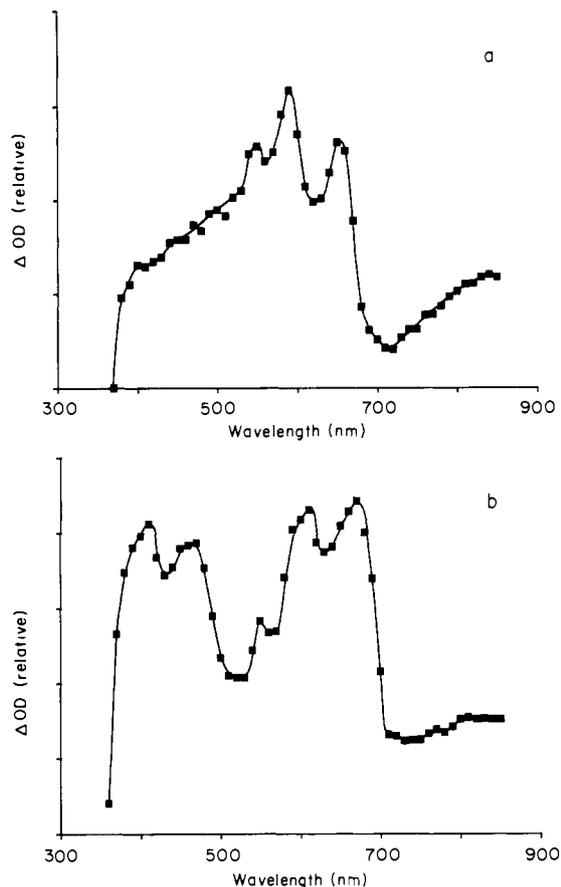

Figure 7. Absorption spectra of the triplet states of 4t-Urd (a) and DMTU (b) taken at 77 K in 2Me-THF.

shifted in energy and show sharpening and some structure in the visible. This is even clearer in the 2Me-THF spectra which show a vibrational progression in the large visible absorption band. The vibrational spacing the two triplet spectra at 77 K in 2Me-THF are the same within our resolution and represent a vibrational progression somewhere between 1400 and 1650 cm^{-1}. Both C=C and coupled S=C—N vibrations are found in this frequency range.[10,37,38]

As shown in Figure 3, in water at room temperature the triplet spectra of 4t-Urd and DMTU were obtained as far into the UV as 220 nm. Bleaching of the ground-state absorption was observed around 330 nm, and two triplet absorption bands were seen between 220 and 300 nm. By comparing the relative size of the bleaching signals at 330 nm with the transient absorption signals, triplet-state extinction coefficients for 4t-Urd and DMTU in water at room temperature were found to be $\epsilon_{540nm} = 2400$ M^{-1} cm^{-1} and $\epsilon_{590nm} = 2500$ M^{-1} cm^{-1} for 4t-Urd and DMTU, respectively. These values are much smaller than the value of 40000 M^{-1} cm^{-1} previously reported for $\epsilon_{540nm}(4t-Urd)^{33}$ which was obtained by using the energy-transfer method with retinol as the acceptor. We will discuss this discrepancy in detail below.

The calculated and observed triplet absorption spectra of the thiouracils are summarized in Table V. The experimental oscillator strengths were calculated by using the extinction coefficients cited above and are, as with other oscillator strengths determined in this study, only approximate due to overlapping transitions. All the observed transitions are easily correlated with calculated transitions. A few transitions predicted to occur near 30000 cm^{-1} are not seen. They are probably obscured by the transient bleaching of the ground state. As in the U triplet spectrum, most of the strong transitions occur between states where

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Table V. Absorption Spectrum of the Lowest Triplet State of 4-Thiouracil Derivatives

triplet state[a]		single electron excitations[b]	$10^3 E$, cm^{-1} $(f)^c$		
			calculated 1Me-4-TU	observed[d]	
				4t-Urd	DMTU
T_2	$n\pi^*$	$n\pi$	3.7 (0.000)		
T_3	$\pi\pi^*$	$\pi\pi$	9.5 (0.019)	13.7 (0.02)	12.8 (0.01)
T_4	$\pi\pi^*$		16.7 (0.030)	18.5 (0.06)	16.9 (0.05)
T_5	$n\pi^*$		18.4 (0.000)		
T_6	$n\sigma^*$		22.4 (0.022)	24.4 (0.05)	23.8 (0.04)
T_7	$\pi\pi^*$	$\pi^*\pi^*$	22.7 (0.049)		
T_8	$\pi\sigma^*$	$\pi^*\sigma^*$	23.6 (0.009)		
T_9	$n\pi^*$		27.1 (0.001)		
T_{10}	$\pi\sigma^*$	$\pi^*\sigma^*$	28.5 (0.012)		27.0 (0.04)
T_{11}	$\pi\pi^*$	$\pi^*\pi^*$	30.1 (0.079)		
T_{12}	$n\pi^*$		30.3 (0.035)		
T_{13}	$n\pi^*$	nn	32.3 (0.055)		
T_{14}	$\pi\sigma^*$	$\pi^*\sigma^*$	35.4 (0.009)		
T_{15}	$\pi\pi^*$		37.8 (0.163)	37.7 (0.15)	38.5 (0.1)
T_{16}	$n\pi^*$		38.1 (0.000)		
T_{17}	$n\sigma^*$		39.2 (0.003)		
T_{18}	$n\pi^*$	$n\pi^*$	39.4 (0.014)		
T_{19}	$n\sigma^*$		41.3 (0.008)		
T_{20}			42.2 (0.118)	43.5 (0.1)	43.5 (0.1)

[a] The orbital designation of the triplet state, T_n, as a single electron excitation from S_0. T_1 is $\pi\pi^*$. [b] Transitions where the largest components of the CI matrices represent a single electron excitation for $T_n \leftarrow T_1$. [c] Transition energies from T_1. [d] From spectra of 4t-Urd and DMTU in H_2O.

Table VI. Observed Lifetimes of Transient Absorption for 2×10^{-5} M Solutions of 4t-Urd and DMTU at 295 K

solvent	τ_{obsd}, ns	
	4t-Urd	DMTU
H_2O	320	220
CH_3CN	290	150
THF	50	190
DMSO	15	30
DMF	110	230
ETOH	40	90

the largest components of the CI matrices are connected by a single electron excitation. Again, when there is a strong transition where this is not the case, the intensity comes from minor configurations contributing to the two states.

In addition to the above spectra, we also obtained the triplet spectra of 4t-Urd and DMTU in a number of other solvents. The general features of the triplet spectra do not depend on the solvent. With identical photolysis conditions in all solvents, the size of the signal at the visible absorption maximum was the same to within 25%. This indicates that the product of the intersystem crossing yield and the triplet maximum extinction coefficient does not change significantly from solvent to solvent. Table VI reports the lifetimes of the triplet absorption in the solvents studied.

Excited-State Extinction Coefficients. As noted above, the triplet extinction coefficient obtained here by the ground-state bleaching method and the one previously reported for the triplet absorption of 4t-Urd differ by more than an order of magnitude. This was surprising since the triplet extinction coefficient we obtained for U by the ground-state bleaching method is in reasonable agreement with the previously published value.[32] Since the ground-state bleaching method gave triplet extinction coefficients for both U and 4t-Urd which lead to experimental oscillator strengths close to the values predicted by the INDO/S calculations, it seemed as if this method gave reasonable results. To understand the discrepancies in the triplet extinction coefficient of 4t-Urd, we thus measured the extinction coefficient of 4t-Urd and DMTU by using other methods.

There are four methods commonly used for obtaining extinction coefficients of excited-state absorptions. In the first, the transient signal is observed as a function of laser power, and the signal size is extrapolated to infinite power. The extrapolated signal represents the signal at complete saturation of the laser absorption.

Table VII. Triplet–Triplet Extinction Coefficients Obtained by Different Methods

method	ϵ_{540}(4t-Urd)	ϵ_{590}(DMTU)
energy transfer to		
retinol/CH_3CN	25 000	17 000
trans-stilbene/CH_3CN	29 000	18 000
naphthalene/CH_3CN	36 000	6 400
bleaching of ground state/H_2O	2 400	2 500

Here $\epsilon_t = \Delta A_{max}/lc\phi_{isc}$, where ΔA_{max} is the triplet absorption signal at ground-state saturation. In the second method, a species with known triplet absorption and intersystem crossing yield is used. A sample of this reference molecule is prepared in which the ground-state absorption at the laser excitation wavelength is identical with that of the sample whose transient absorption is being measured. The transient absorption of the reference (ΔA_r) and transient being studied (ΔA_t) are taken under identical photolysis conditions using low laser powers to avoid saturation of the ground-state absorption. Here $\epsilon_t = \epsilon_r(\Delta A_t \phi_r/\Delta A_r \phi_t)$. To use either of these two methods in this work, the intersystem crossing yields for 4t-Urd and DMTU would be needed. We tried to obtain these triplet yields by using Lamola and Eisinger's[39] method of triplet energy transfer to Eu^{3+}. However, the lifetime of the triplets of 4t-Urd and DMTU are so short that there was insufficient quenching to obtain a reliable extrapolation to 100% quenching. We thus had to rely on the two other methods of obtaining triplet extinction coefficients.

In the third method for obtaining extinction coefficients, the size of the transient absorption in the sample under study is compared to the size of the transient bleaching at a wavelength where the ground-state extinction coefficient is known. Here $\epsilon_t = \epsilon_{gs}(-\Delta A_t/\Delta A_{gs})$. It is assumed in this method that the excited state does not absorb at the bleaching wavelength. If this assumption is not correct, the excited-state extinction coefficient obtained will be an overestimate. In the fourth method, the triplet extinction coefficient can be determined when the triplet is quenched by a triplet energy acceptor whose triplet spectrum and extinction coefficients are known. Assuming complete quenching, $\epsilon_t = \epsilon_r(\Delta A_t/\Delta A_r)$, where ΔA_t is the absorbance of the triplet under study in the absence of the quencher. This method assumes that the amount of triplet quenching is known and that every quenching event leads to energy transfer to the acceptor triplet state. If other quenching pathways occur, the calculated extinction coefficient will be too high.

Table VII presents the values obtained for the triplet absorption extinction coefficient at the visible absorption maximum for 4t-Urd and DMTU. The results obtained by the energy-transfer method depend considerably on the acceptor used. In addition, the value of ϵ_{max} obtained with this method for 4t-Urd and DMTU differs by over a factor of 5 for some acceptors. Since both the energy transfer and ground-state bleaching methods give upper limits to ϵ_{max}, it appears that retinol, *trans*-stilbene, and naphthalene must all quench the thiouracil triplets by a second mechanism which kinetically competes with the observed energy-transfer mechanism. It is interesting that there was only a small discrepancy between the triplet extinction coefficients obtained for U using the relative bleaching/absorption method in the present study and the energy-transfer method (with retinol as the acceptor) obtained in a previously reported study. Thus, there is little or no competition to energy transfer in the quenching mechanism from U to retinol.

Electron-Transfer Photochemistry. The above results show that the quenching of the triplets of 4t-Urd and DMTU proceeds predominantly by a pathway other than energy transfer. As most excited-state quenching occurs by either energy transfer or electron transfer, the implication is that the triplets of the thiouracils are quenched by electron transfer. However, we did not observe absorption of any long-lived intermediate other than that of the acceptor's triplet after the quenching of 4t-Urd or DMTU with either retinol or naphthalene. Since no electron-transfer products

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Table VIII. Observed Quenching Rate Constants

quencher/solvent	$k_Q(\text{4t-Urd})^a$	$k_Q(\text{DMTU})^a$
Cl^-/H_2O	0.3^b	0.05
Br^-/H_2O	6^b	1
diethylaniline/CH_3CN	2	0.2
triethylamine/CH_3CN	6	0.2
naphthalene/CH_3CN	3	0.4
naphthalene/cyclohexane	c	0.6
methylviologen/CH_3CN	7	14

$^a k_Q$ in units of 10^9 M^{-1} s^{-1}. ^{b} From ref 33. ^{c} 4t-Urd is not soluble in cyclohexane.
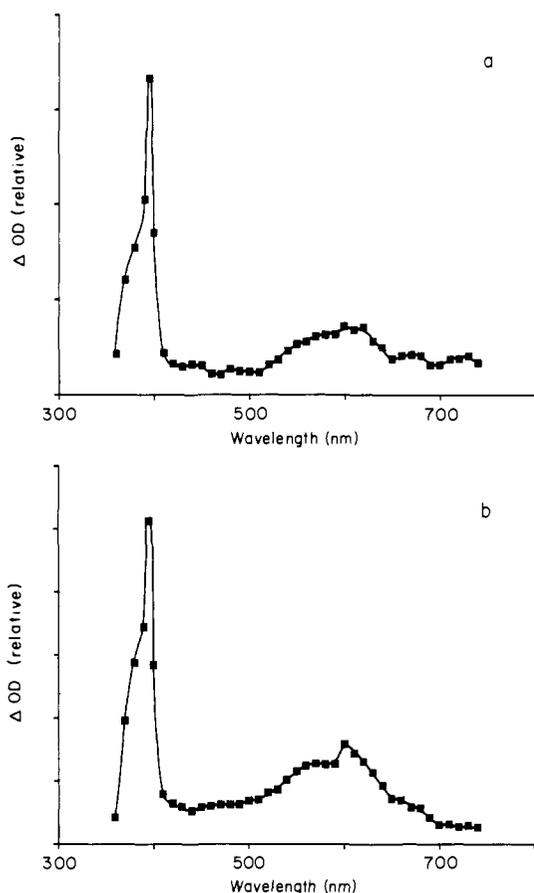

Figure 8. Spectra taken directly after photolysis with a 7-ns, 355-nm laser pulse of Ar-saturated CH_3CN solutions of 4t-Urd (a) and DMTU (b) that contained 0.05 M $MV(BF_4)_2$. Near-complete excited-state quenching and electron transfer are both observed. The spectra readily show the absorption of MV^+ ($\lambda = 395$ and 600 nm) and suggest the appearance of weak absorption from 400 to 500 nm due to the thiouracil cation radicals.

were observed, any electron-transfer products formed must have undergone back electron transfer within the solvent cage in which they were created more quickly than they diffused apart. To test for the presence of electron-transfer photochemistry, we investigated the triplet quenching of 4t-Urd and DMTU with other triplet quenchers. Table VIII shows the observed quenching rates of a number of species which, except for naphthalene, do not have triplet states near to or lower in energy than the thiouracils. They all quench at rates faster than 10^8 M^{-1} s^{-1}, and some quench at the diffusion-controlled rate. The quenching was followed by observing the decrease in the lifetime of the thiouracil triplet transient absorption upon adding quencher. In all cases, Stern–Volmer kinetics were observed. Furthermore, in all cases where the quenching occurs by reduction of the triplet, the quenching rate is faster for 4t-Urd than for DMTU, implying that the triplet of 4t-Urd has a more positive reduction potential than the triplet of DMTU.

Upon adding enough methylviologen (MV^{2+}) to quench essentially all of the triplets of either 4t-Urd or DMTU in CH_3CN,
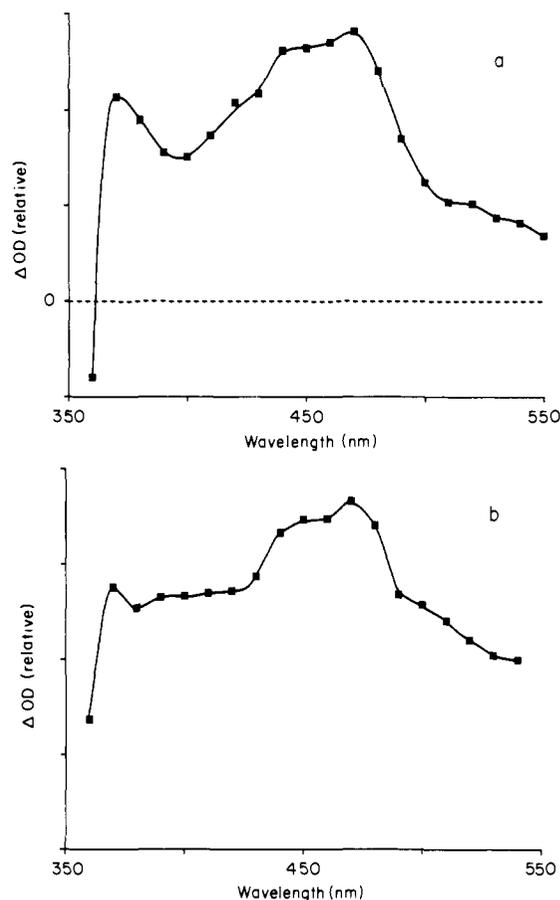

Figure 9. Spectra taken directly after photolysis with a 7-ns, 355-nm laser pulse of Ar-saturated CH_3CN solutions of 4t-Urd (a) and DMTU (b) that contained 0.3 M DEA. Near-complete excited-state quenching and electron transer are both observed. Absorption at 470 nm is due to DEA^+, while absorption at 370 nm is assigned to the thiouracil anion radicals.

we obtained the transient spectra displayed in Figure 8. The spectra represent the transient signals obtained directly after the laser pulse. There was no significant decay of the transients over a 5-μs time period, but the signals did decay by the time of the next laser pulse (100 ms). Very little permanent photochemistry was observed, and the samples could absorb hundreds of laser pulses before significant decomposition of the thiouracils was observed. The transient spectra shown in Figure 8 are very similar to that of the MV^+ radical reported previously in CH_3CN.[40] Thus, it is clear that MV^{2+} oxidizes the triplet states of 4t-Urd and DMTU. There does appear to be broad, weak absorption in the transient spectra between 400 and 500 nm, the same region as the weak absorption ascribed to the $DMTU^+$ radical which is shown in Figure 5. This again is consient with the hypothesis that the quenching leads to single electron transfer.

Reductive quenching would be expected from halide ions, and Cl^- and Br^- were observed to quench both 4t-Urd and DMTU. As expected, Br^- quenches faster than Cl^-. No absorptions due to the thiouracil anions or to radical dihalides, which are readily formed by $X + X^- \rightarrow X_2^-$,[41] were seen. Thus, as is often reported in the literature, halide quenching does not lead to separated electron-transfer products.[42] Reductive quenching with diethylaniline (DEA) did, however, give observable electron-transfer products. Figure 9 shows the spectra taken directly after the laser pulse for CH_3CN solutions of 4t-Urd and DMTU quenched with DEA. The absorption band with a maximum at 470 nm is similar to the absorption reported for the dimethylaniline radical cation[43]

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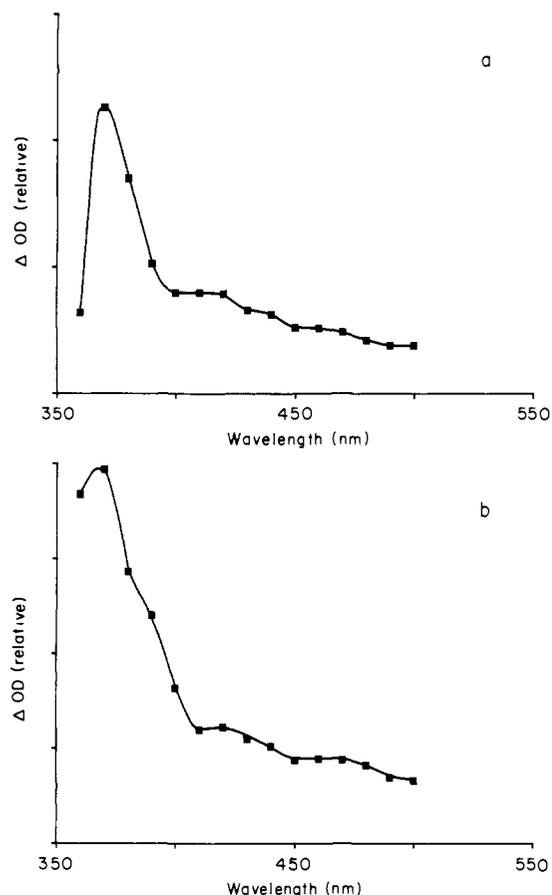(43) Shida, T.; Nosaka, Y.; Kato, T. *J. Phys. Chem.* **1978**, *82*, 695.

Figure 10. Spectra taken directly after photolysis with a 7-ns, 355-nm laser pulse of Ar-saturated CH_3CN solutions of 4t-Urd (a) and DMTU (b) that contained saturated (<0.1 M) TEA. Near-complete excited-state quenching and electron transfer are both observed. Absorption at 370 nm is due to the thiouracil anion radicals, while the weak, broad visible absorption is assigned to TEA^+.

and is thus assigned as absorption from DEA^+. The absorptions seen in both spectra with maxima at 370 nm are then assigned to the radical anions 4t-Urd⁻ and DMTU⁻. Assuming that the extinction coefficient of DEA^+ is similar to that of dimethylaniline cation radical[43] ($\simeq 4000$ M^{-1} cm^{-1}), we estimate the extinction coefficients of 4t-Urd⁻ and DMTU⁻ at 370 nm to be about 3000 M^{-1} cm^{-1}. The observed decay kinetics of the transient signals are quite complex. However, all transients decayed within the 100-ms period between laser pulses. The rate of photodegradation under our conditions was small, and hundreds of laser pulses hit the samples before significant photodecomposition occurred.

Electron-transfer products were also seen from the photolysis of the thiouracils in the presence of triethylamine (TEA). Figure 10 shows the transient spectra taken in CH_3CN directly after laser excitation for 4t-Urd and DMTU solutions that were completely quenched by TEA. Both show the band with maximum at 370 nm due to the radical anion, either 4t-Urd⁻ or DMTU⁻. Both spectra also exhibit a weak absorption tail extending out to 530 nm. This is probably due to TEA^+, as previous work has shown that the TEA^+ has very weak absorption through the visible.[43] Again, the kinetic decay of the radicals is complex. This may be due in part to the abstraction of a hydrogen from TEA^+. Such a step has been proposed in the previously reported static photochemistry of DMTU with TEA.[44]

For either 4t-Urd or DMTU, others have reported the quenching by, or the photoreaction with, simple amines and amino acids such as n-butylamine and lysine.[33,45,46] From the current

work, it is likely that these reactions are due to an initial electron transfer. It has been reported that in water cytosine reacts with excited 4-thiouracil to give addition products.[47] We thus attempted to observe laser-induced electron transfer between 4t-Urd and cytosine in water. However, cytosine was not soluble enough to give a large amount of quenching, and no photoproduct transients were observed.

Though we were not able to directly observe electron transfer between 4t-Urd and cytosine under our conditions, it is clear that electron transfer is a reasonable first step in the photochemistry of tRNA. Reductive quenching of the triplet of the 4t-Urd in the 8-position by the cytidine in the 13-position cannot be totally efficient, as the triplet of 4t-Urd emits in tRNA.[9] This is consistent with the low quantum yield for photocrosslinking ($\simeq 5 \times 10^{-3}$) in tRNA.[4] The low quantum yield could also be due in part to the fact that the radicals may undergo rapid back electron transfer which would compete with chemical addition. Others have proposed a thietane intermediate in the photocrosslinking reaction.[47] It is possible that this intermediate follows from the initial electron-transfer products. However, our current results cannot discriminate between a thietane route or a different route subsequent to the electron transfer. Direct photochemical studies of tRNA will be needed to sort out the details of the mechanism.

Intersystem Crossing Yields. The intersystem crossing yield has been reported for 4t-Urd in water as 0.02.[33] However, this value depends on the assumption that the maximum triplet extinction coefficient is 40 000 M^{-1} cm^{-1}, a value shown above to be more than an order of magnitude too large. We thus determined ϕ_{isc} for 355-nm excitation for both 4t-Urd and DMTU. As energy transfer to Eu^{3+} did not give reliable results for reasons discussed above, a comparison method was used. Both 4t-Urd and DMTU in water were made the same optical density at 355 nm as a solution of anthracene in cyclohexane. Each sample was photolyzed at 355 nm at the same low laser power (<0.5 mJ/pulse) to avoid saturation effects, and the sizes of the transient absorptions at the absorption maxima were obtained. When a triplet extention coefficient of ϵ_a(422.5 nm) $= 64\,700$ M^{-1} cm^{-1} and intersystem crossing yield of $\phi_a = 0.7$ for anthracene in cyclohexane[33,48] were used, the intersystem crossing yield of the thiouracils was computed by $\phi_t = \phi_a(\Delta A_t \epsilon_a / \Delta A_a \epsilon_t)$. We obtained ϕ_{isc}(4t-Urd) $= 0.9 \pm 0.1$ and ϕ_{isc}(DMTU) $= 1.0 \pm 0.1$. For 4-tUrd, this value is much higher than the value reported previously. It is interesting to note that based on stuudies of singlet oxygen production upon 4t-Urd irradiation, the same authors have recently stated that ϕ_{isc} must be significantly greater than the previously reported value of 0.02.[49] The high intersystem crossing yields are consistent with the fast intersystem crossing expected from the lowest $n\pi^*$ singlet to the lowest energy $\pi\pi^*$ triplet. The internal heavy atom effect of the sulfur may also be a factor in these high triplet yields.

Conclusions. The ground-state and the excited triplet spectra of U, 4t-Urd, and DMTU are reported and are successfully modeled by using INDO/S molecular orbital calculations. The triplet spectra for 4t-Urd and DMTU are similar, showing that the excited state of 4t-Urd is predominately in the thione tautomer. For both 4t-Urd and DMTU, the lowest excited triplet state is $\pi\pi^*$.

In attempting to use the energy-transfer technique to measure the triplet–triplet extinction coefficients, we showed that for 4t-Urd and DMTU a major portion of the quenching by typical energy-transfer quenchers actually follows by an electron-transfer mechanism. The triplet–triplet extinction coefficient of 4t-Urd reported in a previous study was greatly overestimated as it was based on the assumption that all quenching occurred by energy transfer. The energy-transfer method is the most common means of determining excited-state extinction coefficients. Our results point out the care one must take in using this procedure. On determining accurate triplet–triplet extinction coefficients, we

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determined that the intersystem crossing yields are 0.9 ± 0.1 for 4t-Urd and 1.0 ± 0.1 for DMTU. This is a factor of 45 greater than reported previously for 4t-Urd.

Both oxidative and reductive quenching of the triplet states of 4t-Urd and DMTU have been observed, and the spectra of the anion radicals of 4t-Urd and DMTU and the cation radical of DMTU are reported. These results lead to the conclusion that the first step in the photocrosslinking of 4t-Urd with cytidine in tRNA probably involves an electron transfer. There have been a number of recent reports of the photocrosslinking reactions of nucleic acid bases in DNA with amino acids, such as lysine, in proteins.[50] Our results suggest the possibility of electron-transfer

reactions in these systems as well.

Registry No. THF, 109-99-9; DMSO, 67-68-5; DMF, 68-12-2; Et$_2$NPh, 91-66-7; Et$_3$N, 121-44-8; H$_2$O, 7732-18-5; MeCN, 75-05-8; EtOH, 64-17-5; 4-thiouridine, 13957-31-8; 1,3-dimethyl-4-thiouracil, 49785-67-3; uracil, 66-22-8; naphthalene, 91-20-3; methylviologen, 1910-42-5; retinol, 68-26-8; (*trans*)-stilbene, 103-30-0.

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A Microelectrochemical Diode with Submicron Contact Spacing Based on the Connection of Two Microelectrodes Using Dissimilar Redox Polymers

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Abstract: Closely spaced, 0.2–1 μm, Au microelectrodes (50 μm long, 1–2 μm wide, and 0.1 μm thick) on Si$_3$N$_4$ can be functionalized with poly(vinylferrocene), PVFc$^{+/0}$, or with an *N*,*N'*-dibenzyl-4,4'-bipyridinium-based polymer, (BPQ$^{2+/+}$)$_n$, derived from hydrolysis of *N*,*N'*-bis[(*p*-trimethoxysilyl)benzyl]-4,4'-bipyridinium (I). Two- or eight-microelectrode arrays have been functionalized with PVFc$^{+/0}$ or (BPQ$^{2+/+}$)$_n$. Adjacent microelectrodes can be connected with either polymer in the sense that net current can pass from one microelectrode to another, through the polymer, when one electrode is held at a potential where the polymer is oxidized and the other electrode is held at a potential where the polymer is reduced. From such steady-state current an estimate of the diffusion coefficient for charge transport, D_{CT}, in the polymer can be made; values in the range 10^{-9}–10^{-10} cm^2/s are found and accord well with earlier measurements of D_{CT} for the polymers studied. A two-terminal diode can be fabricated by coating one electrode with (BPQ$^{2+/+}$)$_n$ and an adjacent electrode with PVFc$^{+/0}$ such that there is a connection between the microelectrodes via the (BPQ$^{2+/+}$)$_n$/PVFc$^{+/0}$ contact. Current passes when the applied potential is such that the negative lead is attached to the (BPQ$^{2+/+}$)$_n$-coated electrode and the positive lead is attached to the PVFc$^{+/0}$-coated electrode. When the applied potential approaches the difference in the $E°'$s of the two polymers, current flows with the crucial feature being a downhill (by ~0.9 V) cross redox reaction at the (BPQ$^{2+/+}$)$_n$/PVFc$^{+/0}$ interface, BPQ$^+$ + Fc$^+$ → BPQ^{2+} + Fc^0. Current does not flow between the microelectrodes when the applied potential is in the opposite sense, because the reaction BPQ^{2+} + Fc^0 → BPQ$^+$ + Fc$^+$ is uphill by ~0.9 V. The switching time of a microelectrochemical diode is controlled by the time required to oxidize and reduce the polymers.

In this article we wish to report procedures that yield the functionalization of two closely spaced (<1 μm) microelectrodes such that each electrode is coated with a different redox polymer in a manner as illustrated in Scheme I. The resulting assembly functions as a diode of the sort first demonstrated by Murray and co-workers.[1] The assembly behaves as a diode in the sense that steady-state current passes in only one direction upon application of a potential across the two electrodes. The principles of such a diode have been demonstrated with macroscopic electrodes derivatized first with one redox polymer followed by a second polymer having a different redox potential and then capped with a porous front contact forming a sandwich structure of metal/polymer/polymer/metal.[1] Our new results demonstrate a synthetic methodology for making an "open-faced" sandwich with a metal-to-metal separation of substantially less than one micron involving a combination of conventional microfabrication techniques and electrochemistry. We recently reported the derivatization

Scheme I. Cross-Sectional View of Redox Polymer Functionalized Microelectrodes^a
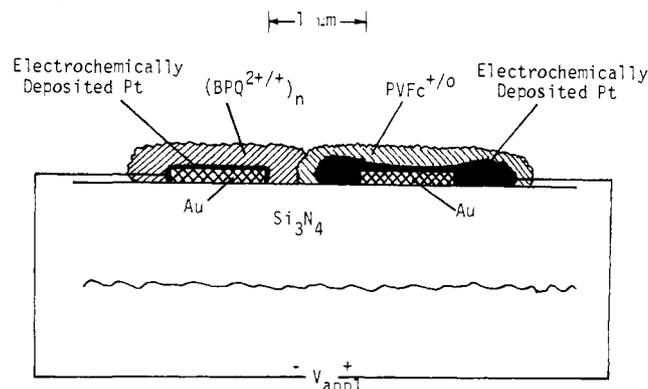
^{a} The two-terminal "device" can operate as a diode in the sense that current can pass in only one direction upon application of a potential across the two microelectrodes, since the two polymers have very different redox potentials.

of microelectrode arrays using a single polymer, polypyrrole,[2] poly(*N*-methylpyrrole),[3] polyaniline,[4] and poly(3-methyl-

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