Nucleic Acid Oxidation Mediated by Naphthalene and Benzophenone Imide and Diimide Derivatives: Consequences for DNA Redox Chemistry

Joy E. Rogers and Lisa A. Kelly*

Contribution from the Department of Chemistry and Biochemistry, University of Maryland, Baltimore County, 1000 Hilltop Circle, Baltimore, Maryland 21250

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Abstract: The rate constants for electron transfer from guanosine 5′-monophosphate (GMP), adenosine 5′-monophosphate (AMP), cytidine 5′-monophosphate (CMP), and thymidine 5′-monophosphate (TMP) to the triplet excited states of *N*-(3-propanol)-1,8-naphthalimide (NI), *N*,*N*′-(3-propanol)-1,4,5,8-naphthaldiimide (NDI), and *N*,*N*′-(3-propanol)-3,3′,4,4′-benzophenonediimide (BPDI) have been determined in 1:1 H_2O/CH_3CN solution. Upon 355-nm (8 ns) laser flash excitation of each of the imide or diimides in solution, the triplet states decayed by first-order kinetics under conditions of low excitation energy. Photoinduced electron transfer to the lowest electronically excited triplet state of *N*-(3-propanol)-1,8-naphthalimide from GMP occurred with a rate constant of 2.0×10^7 M^{-1} s^{-1}. Electron-transfer quenching by the other nucleotides was almost 2 orders of magnitude slower. In the case of BPDI, photooxidation rate constants ranged from 2.3×10^8 M^{-1} s^{-1} for quenching by CMP to 1.1×10^9 M^{-1} s^{-1} by GMP. In all cases, the imide radical anion was observed by laser flash photolysis, and the yields were quantified. From these investigations, nucleotide oxidation by the triplet state of a series of redox-active photosensitizers has been demonstrated. The results represent a systematic study of nucleotide oxidation by the triplet states of a series of structurally related organic photosensitizers in which the reduction potential can be tuned by ca. 800 mV. The greater than 100-fold variation in bimolecular rate constants for oxidation of base monophosphates by these photosensitizers offers the prospect of kinetic "selectivity" of oxidative damage in random-sequence DNA.

Introduction

The synthetic design of chemical systems useful as structural probes and therapeutic agents in biological systems has spawned enormous scientific advancements in molecular biology and chemotherapy. Specifically, the ability to deliver chemical reactivity on demand via photoexcitation offers the prospect of selectively activating chromophores that are associated with the target of interest. Such strategies are employed in photodynamic therapy, where the localized interaction of a chromophore and light results in tumor destruction. The development of synthetic chemical systems capable of initiating specific or nonspecific cleavage of DNA, RNA, or protein polymers has led to a growing interest in developing molecular chromophores for use as structural probes or sequencing agents. The goals of designing both specific and nonspecific cleavage agents are of interest for the purposes of developing both sequencing and "photo-footprinting" agents. A review of photoinitiated cleavage in nucleic acid systems has recently appeared.[1]

Of the chromophores employed, hydroxyl radical generators, singlet oxygen generators, and electron-transfer agents have been most studied. The naphthalene-derived imide and diimide chromophores have been shown to be a versatile class of chromophores capable of generating a multitude of reactive intermediates.[2−5] Specifically, it has been demonstrated that L-lysine-substituted naphthalene imides, when photolyzed in the

presence of DNA, initiate oxidative damage preferentially at the 5′-side of GG steps in duplex DNA.[4] Using 350-nm light, the quantum yield for this process was determined to be $<10^{-3}$. In this investigation, laser flash photolysis of a solution of the naphthalimide derivative and a duplex hexamer revealed production of the imide radical anion concomitant with the decay of the imide triplet. The bimolecular rate constant for this reaction was 5.3×10^7 M^{-1} s^{-1}. This observation, along with the decay of the imide radical anion via second-order kinetics, suggests that bimolecular interactions between imide triplet states and nucleic acids can initiate oxidative damage to DNA. A further demonstration of the versatility of naphthalimide derivatives was demonstrated by Saito et al., in which thymine-selective cleavage was initiated by nitro-substituted 1,8-naphthalimide chromophores.[5] Site-specific cleavage was attributed to hydrogen atom abstraction from the thymine methyl group by the photoexcited nitro group, not via abstraction from the DNA sugar backbone or electron transfer.

Utilization of these reactive intermediates to produce site-specific damage in nucleic acid polymers requires them to be associated with specific sites on the target. Cationic naphthalene imide derivatives have been reported to bind intercalatively to DNA and homopolymer derivatives ($K = 5 \times 10^4$ to 4×10^5 M^{-1}),[6] exhibiting preference for GC-rich DNA.[7] Thus, motiva-

* To whom correspondence should be addressed.
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tion to pursue and tune the specific reactivities of imide-based chromophores is driven by the observation of the versatile reactivity of imide and diimide excited states, along with precedented noncovalent interactions with nucleic acid polymers.

Although alkali labile sites in duplex DNA were shown to be produced upon irradiation of the L-lysine-substituted 1,8-naphthalimide chromophores, it is curious why cleavage products from analogous 1,4,5,8-naphthalenediimide systems were not observed.[3] The production of damaged sites, initiated by single-electron oxidation of the individual bases, should be correlated with the chromophore reduction potential. The ground-state of naphthalene diimide is a more powerful oxidant by almost 800 mV, suggesting that it should be a better redox-active cleavage agent.[8] To address this question and further understand the factors responsible for redox-initiated damage in DNA, we have embarked on a systematic investigation of the bimolecular reactivity of excited states in a series of structurally related imide and diimide chromophores with the individual nucleotides. Specifically, in naphthalene-derived imide chromophores that possess near unity ISC yields, it is of fundamental interest to characterize the kinetics of photoinduced electron transfer from nucleic acids to imide triplet excited states. Although previous studies have demonstrated nucleotide, nucleoside, and nucleobase oxidation using singlet excited states of organic chromophores,[9–11] base-specific oxidation by organic triplet states has not been demonstrated. Since the energy-wasting charge-recombination in triplet-derived radical ion pairs is slowed in comparison with that in singlet-derived products, the use of triplet states offers the prospects of increased charge-separation efficiency. Reports of nucleotide quenching of the ^{3}MLCT state of ruthenium(II) complexes have appeared.[12] However, spin−orbit coupling, induced by ruthenium, may facilitate charge recombination and result in diminished charge-separation efficiency.

The triplet excited states of three novel imide and diimide electron acceptors have been characterized and the bimolecular rate constants for forward electron transfer with a series of base monophosphates measured. To facilitate solubility in aqueous acetonitrile solvent systems, we have derivatized these sensitizers with *N*-(3-propanol) functional groups. Transient absorption spectra of the triplet excited states of these chromophores are reported. Bimolecular rate constants for electron transfer to the individual triplet states from the base monophosphates have been measured, and yields of charge-separated products resulting from triplet-state quenchings are reported.

Experimental Section

Chemicals. Guanosine 5′-monophosphate (GMP), adenosine 5′-monophosphate (AMP), cytidine 5′-monophosphate (CMP), and thymidine 5′-monophosphate (TMP) were obtained from Sigma in their disodium salt form (99%). Thioxanthene-9-one (thioxanthone) (98%), benzophenone (99%), methyl viologen dichloride hydrate (98%), and

1,4 diazobicyclo[2.2.2]-octane (DABCO) (98%) were used as received from Aldrich Chemicals. 1,8-Naphthalic anhydride, 3,3′,4,4′-benzophenone tetracarboxylic anhydride and 1,4,5,8-naphthalenetetracarboxylic acid dianhydride were obtained from Aldrich. 1,8-Naphthalic anhydride was recrystallized 2 × from *N,N*-dimethylacetimide (DMA) and dried in vacuo prior to use. 3-Aminopropanol was obtained from Acros and used as received. Tetraethylammonium perchlorate (TEAP) was obtained from Fluka (>99%) and dried in vacuo at 100° C prior to use. Acetonitrile (B&J spectrophotometric grade) was used as received for electrochemical and kinetic measurements. Water was deionized and freshly passed through an ion-pure reverse osmosis system. The system utilizes a point of use cartridge system, followed by UV irradiation to provide >18 MΩ ultrapure bacteria free water. Other materials were obtained from commercial sources.

N-(3-propanol)-1,8-naphthalimide (NI, **1**), *N,N′*-(3-propanol)-1,4,5,8-naphthalimide (NDI, **2**), and *N,N′*-(3-propanol)-3,3′,4,4′-benzophenonediimide (BPDI, **3**) were prepared by the reaction of 3-aminopropanol with the acid anhydride precursor. Synthetic, spectral and elemental analysis details are described elsewhere.[13]

General Techniques. Laser flash photolysis kinetic investigations were carried out at 22.0 ± 0.2° C using a water-jacketed sample holder connected to a Lauda RM6-B circulating water bath. Ground-state UV/vis absorption spectra were measured using a JASCO V-570 double-beam spectrophotometer. Proton NMR spectra were obtained using a GE QE-300 spectrometer. Fluorescence spectra were measured using a SLM 48000 fluorescence spectrometer. Phosphorescence spectra were obtained in frozen ethanol glasses at 77 K using an Aminico-Bowman 8202 fluorescence spectrometer with a rotating excitation shutter assembly. If necessary, 10% methyl iodide was added to the samples to induce phosphorescence. Excitation wavelengths of 350 nm (compounds **1** and **2**) and 264 nm (compound **3**) were employed for fluorescence and phosphorescence measurements.

One-electron reduction potentials of the imide and diimides were measured in anhydrous acetonitrile containing ca. 1 mM imide and 0.10 M TEAP as a supporting electrolyte. Solutions were bubbled with nitrogen prior to measurement. The cyclic voltammograms were obtained using a BAS CV-1B CV controller that was interfaced to a pentium PC for data acquisition. For these measurements, platinum working and counter-electrodes were employed, with a silver/silver chloride (approximately 3 M KCl) reference electrode. Ferrocene was employed as a standard in these measurements. The electrochemical half-wave potentials of the imides, measured with respect to the Ag/AgCl reference electrode, were converted to a NHE reference using $E^o_{1/2} = 0.69$ V vs NHE for the Fc$^+$/Fc couple in acetonitrile.[14] A scan rate of 100 mV/sec was typically employed.

Laser Flash Photolysis. Nanosecond transient absorption measurements employed the technique of laser flash photolysis. The third-harmonic (355 nm) of a Q-switched Nd:YAG laser (Continuum Surelight II, pulse width ca. 8 ns) was used for laser flash excitation. Pulse energies of up to 25 mJ cm^{-2} pulse^{-1} were typically employed at the excitation wavelength. Laser-induced transmittance changes were monitored using white light from a 75-W xenon source (Oriel) focused through the sample and re-imaged on the entrance slit of an Acton SP-150 monochromator. The monochromated light was detected with a Hamamatsu R-928 PMT, and the current was routed through a back-off circuit which stored and compensated for I_o.[15] The real-time current was recorded across 50 ohms on a Tektronix TDS 380 digital oscilloscope. The timing pulses to trigger the laser flashlamps and Q-switch, Uniblitz (Vincent Associates) probe shutter, back-off trigger, and scope trigger were generated using a National Instruments PC-TIO I/O timing card. Data collection and analysis routines were written using the National Instruments LabView 4.0 for Windows 95.

Triplet Sensitization. Triplet sensitization experiments were employed to obtain the molar absorption coefficients of the imide and diimide triplet states. Thioxanthene-9-one was employed as the triplet energy donor due to its unity triplet yield and long triplet lifetime (τ_T

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= 73 μs in acetonitrile).[16] Photosensitization experiments were carried out using 355- or 266-nm pulsed excitation of the thioxanthone triplet energy donor. Triplet sensitization of **1** and **3** employed 355-nm pulsed excitation of the thioxanthone. Sensitization of the triplet state of **2** required 266-nm excitation (8 ns pulse, up to 20 mJ cm^{-2} pulse^{-1}) owing to the sizable ground-state extinction coefficient of NDI at 355 nm. Pulsed excitation of thioxanthene-9-one, followed by energy transfer to the imide or diimide energy acceptor, resulted exclusively in sensitized production of the imide triplet.[17] Under the conditions employed in these experiments, >90% of the excitation light was absorbed by the thioxanthene-9-one. The sensitized imide or diimide $T_1 - T_n$ absorption ($\Delta A^{\circ}_{T(I)}$) extrapolated to time = 0 was measured as a function of imide or diimide concentration. The extinction coefficients for the $T_1 - T_n$ transition ($\Delta\epsilon_{T(I)}$) were determined from eq 1

$$\Delta A^{\circ}_{T(I)} = \Delta A^{\circ}_{T(Tz)} \frac{\Delta\epsilon_{T(I)}}{\Delta\epsilon_{T(Tz)}} f_q \qquad (1)$$

In eq 1, $\Delta A^{\circ}_{T(I)}$ is the measured thioxanthene-9-one $T_1 - T_n$ absorbance change at 590 nm, immediately following the laser pulse, $\Delta\epsilon_{T(Tz)}$ is the extinction coefficient for this transition, and f_q is the fraction of thioxanthene-9-one triplet states that are quenched by the imide/diimide employed (eq 2)

$$f_q = \frac{k_{obs}}{k_{obs} - k_d} \qquad (2)$$

The fraction of triplet states quenched by the imide energy acceptor was calculated using k_d and k_{obs}, the rate constants for thioxanthene-9-one triplet decay in the absence and presence of the energy acceptor, respectively. A plot of $\Delta A^{\circ}_{T(I)}$ vs. f_q, along with measured values of $\Delta A^{\circ}_{T(Tz)}$ and $\Delta\epsilon_{T(Tz)}$, yielded the imide or diimide $T_1 - T_n$ extinction coefficients ($\Delta\epsilon_{T(I)}$). The thioxanthene-9-one $T_1 - T_n$ extinction coefficient ($\Delta\epsilon_{T(Tz)}$) in 1:1 CH$_3$CN/H$_2$O was determined by the method of comparative actinometry using a solution of benzophenone. For the thioxanthene-9-one and benzophenone solutions of matched optical density, plots of the absorbance at time zero at 590 nm ($\Delta A^{\circ}_{T(Tz)}$) and 520 nm ($\Delta A^{\circ}_{T(Bp)}$), respectively, vs relative laser energy were constructed. From the ratio of the slopes, along with the known $\Delta\epsilon_{T(Bp)}$ (6500 M^{-1} cm^{-1} at 520 nm)[18] in acetonitrile, the thioxanthene-9-one triplet−triplet extinction coefficient at 590 nm was determined to be 10 500 M^{-1} cm^{-1} in 1:1 CH$_3$CN/H$_2$O.

Results

Photophysical and Redox Properties. The structures of the imide and diimides **1** (NI), **2** (NDI), and **3** (BPDI) are shown in Figure 1. The *N*-(3-propanol) derivatives were synthesized to facilitate solubility in polar solvents. Specifically, *N,N′*-(alkyl)- and *N,N′*-(H)- derivatives of 1,2,4,5-naphthaldiimide and 3,3′,4,4′-benzophenone diimide were not sufficiently soluble in aqueous or acetonitrile solvents.

In the case of the naphthalene-derived imides, NI and NDI, the $S_o - S_1$ vibronic progression is observed at wavelengths longer than 300 nm. Higher energy $\pi-\pi^*$ transitions are observed at wavelengths shorter than 250 nm. The absorption and fluorescence spectra of these compounds are nearly identical to those previously reported for other 1,8-naphthalimide derivatives.[2,19] In both cases, the fluorescence is observed as a mirror image of the absorption spectra. Singlet-state energies, obtained
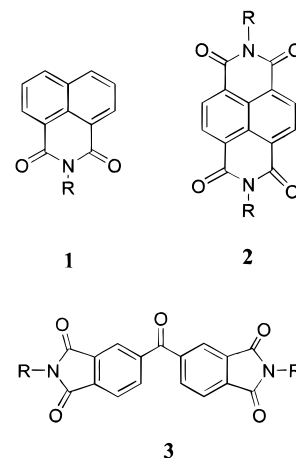
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Figure 1. Structures of NI, NDI, and BPDI (**1**, **2**, and **3**, respectively; R = −CH$_2$CH$_2$CH$_2$OH).

from the intersection points of the absorption and fluorescence spectra, were determined to be 3.43 and 3.21 eV for NI and NDI, respectively. The absorption spectra of a series of bis-(*N,N′*-aryl and -alkyl)-substituted benzophenone-derived diimides have been reported.[20] Consistent with the absorption spectrum shown in Figure 2c, the UV spectra reveal a transition of moderate intensity ($\epsilon \approx 8000$ M^{-1} cm^{-1}) that is superimposed on weak n−π^* transition in parent benzophenone. Fluorescence from **3** was not observed at room temperature or in frozen ethanol glasses. The long-lived and intense luminescence that was observed and is shown in Figure 2c was assigned as phosphorescence from the lowest triplet excited state of the chromophore. The phosphorescence spectrum is nearly identical to that reported by Green and Fox for *N,N′*-didodecyl-1,4,5,8-naphthalenediimide in frozen MTHF glasses.[21] In frozen ethanol glasses, phosphorescence from **1** or **2** was not observed. Upon addition of 10% methyl iodide, phosphorescence from NI and NDI was induced. The energies of the T_1 state, obtained from the phosphorescence spectra, are summarized in Table 1.

The one-electron reduction potentials, measured in acetonitrile for each of the imides, were determined using cyclic voltammetry. In all cases, the reduction peak was found to be completely reversible, and in the case of **2** and **3**, a second and third reduction waves were observed at more negative potentials (results not given). The half-wave potentials are given in Table 1. They are found to be in good agreement with the literature.[8]

Upon 355-nm pulsed excitation of a 1:1 CH$_3$CN:H$_2$O solution of compounds **1**, **2** or **3**, ground-state bleaching is observed, concomitant with the formation of transients absorbing at longer wavelengths (Figure 3). In all cases, the transients are quenched by oxygen. For all three of the imides, the spectra observed were identical to the $T_1 - T_n$ spectra produced upon triplet sensitization with thioxanthene as a triplet energy donor. With use of the methods described above, the extinction coefficients for the imide $T_1 - T_n$ transition were determined and are shown in Figure 3. The transient absorption spectrum of **1** shown in Figure 3 is identical to the $T_1 - T_n$ spectrum of 1,8-naphthalimide in deaerated CH$_3$CN that has been previously published.[2] At low excitation energies, the triplet decay kinetics are first-order. At higher excitation energies, triplet−triplet annihilation is observed. In addition, at high NI or NDI concentrations, efficient electron transfer was observed, following quenching of the triplet photosensitizer by ground states.

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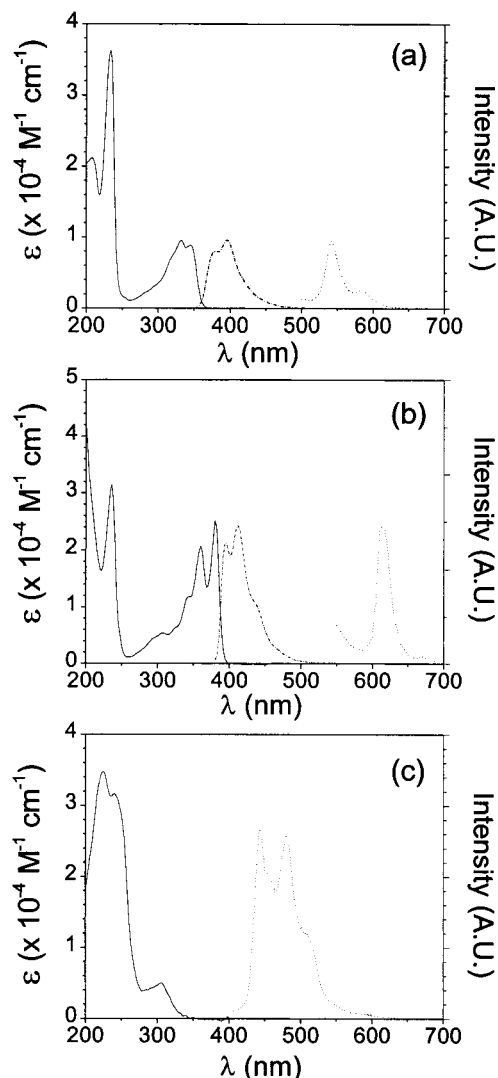(21) Green, S. and Fox, M. A. *J. Phys. Chem.* **1995**, *99*, 14752.

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Figure 2. Ground-state absorption (solid line), fluorescence (dashed line), and phosphorescence (dotted line) of (a) NI, (b) NDI, and (c) BPDI. Absorption and fluorescence spectra were recorded at room temperature in 1:1 CH_3CN/H_2O. Phosphorescence spectra were measured in frozen ethanol glass (77 K) (10% MeI was added to (a) and (b) to induce phosphorescence).

Table 1. Summary of Ground-State Reduction Potentials ($E_{1/2}$(I/I$^{•-}$)), Triplet-State Energies (E_T), and Triplet-State Lifetimes (τ_T) of Compounds **1**, **2**, and **3**

	$E_{1/2}$(I/I$^{•-}$) V vs NHE	E_T (eV)	$E_{1/2}$(^{3}I*/I$^{•-}$) V vs NHE	$\tau_T{}^d$
NI, **1**	−1.01	2.29^a	1.28	65 μs
NDI, **2**	−0.22	2.03^b	1.81	62 μs
BPDI, **3**	−0.76	2.80^c	2.04	84 μs

^{a} From 77 K phosphorescence in EtOH (10% methyl iodide). Value reported is identical to that reported by Wintgens et al. for *N*-methyl-1,8-naphthalimide in CH_3CN.[34] ^{b} From 77 K phosphorescence in EtOH (10% methyl iodide) glass. ^{c} From 77 K phosphorescence in EtOH. ^{d} 1:1 CH_3CN/H_2O at room temperature. Ground-state concentrations of NI, NDI, and BPDI were 21, 11, and 840 μM, respectively.

A detailed investigation of this with related naphthalene-derived imides has appeared.[2] Self-quenching was not observed when BPDI was used (up to a ground-state concentration of 1 mM). The triplet lifetimes, measured at low excitation intensity and ground-state concentration, are reported in Table 1. The T$_1$ − T$_n$ absorption spectrum of **3** (Figure 3c) is identical to that reported by Scaiano et al.[22] Specifically, the 360 nm absorption maximum, along with the long-wavelength tail out to 700 nm,
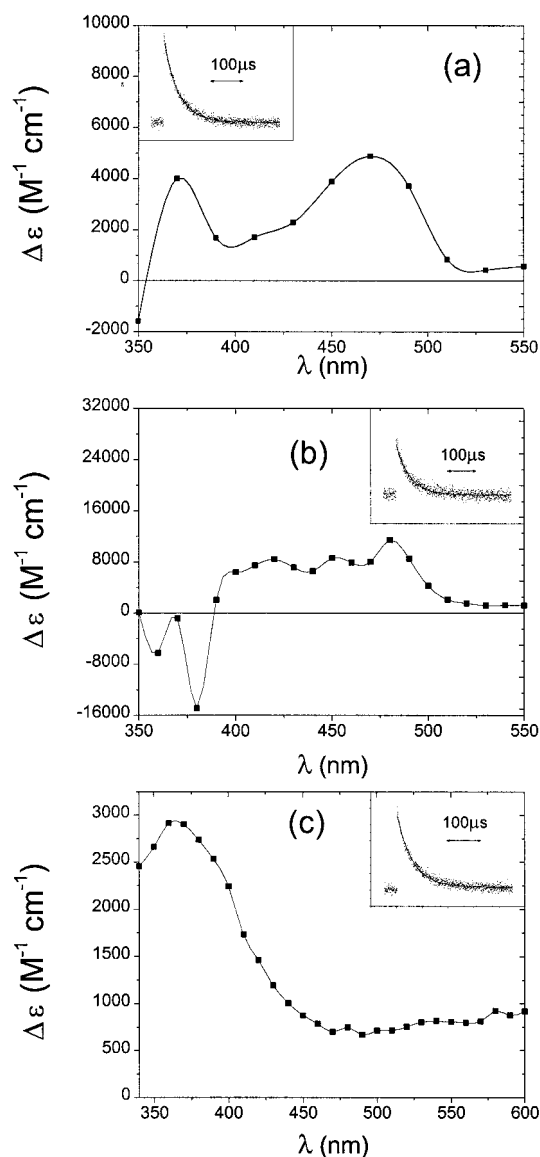

Figure 3. T$_1$ − T$_n$ absorption spectra observed after pulsed 355-nm excitation of (a) NI, [NI] = 21 μM; (b) NDI, [NDI] = 11 μM; and (c) BPDI [BPDI] = 820 μM in 1:1 CH_3CN/H_2O under argon-saturated conditions. Extinction coefficients were obtained by triplet sensitization methods as reported in the text.

was observed by Scaiano in the T$_1$ − T$_n$ absorption spectrum of *N,N*′-(bis(2-isopropylphenyl))-3,3′4,4′-benzophenonediimide.

Triplet-State Reactivity with Base Monophosphates. After the characterization of the ground- and excited-state properties of compounds **1**, **2**, and **3**, the bimolecular reactivity of each of the triplet states with four nucleic acid bases was investigated. Upon 355-nm excitation of a solution of each of the imides in the presence of varying concentrations of the base monophosphates, the triplet state was found to be quenched by one or more of the bases. Shown in Figure 4(a) is the transient spectrum observed upon flash excitation of a solution of compound **1** containing 11 mM GMP. Concomitant with the decay of the triplet state at 480 nm, a transient at 410 nm was observed to grow in. The inset of Figure 4a shows the concomitant growth of this transient with triplet-state decay. The transient was identical to that observed following quenching of ^{3}NI* by a known electron donor, DABCO (1,4-diazobicylco[2.2.2]octane)
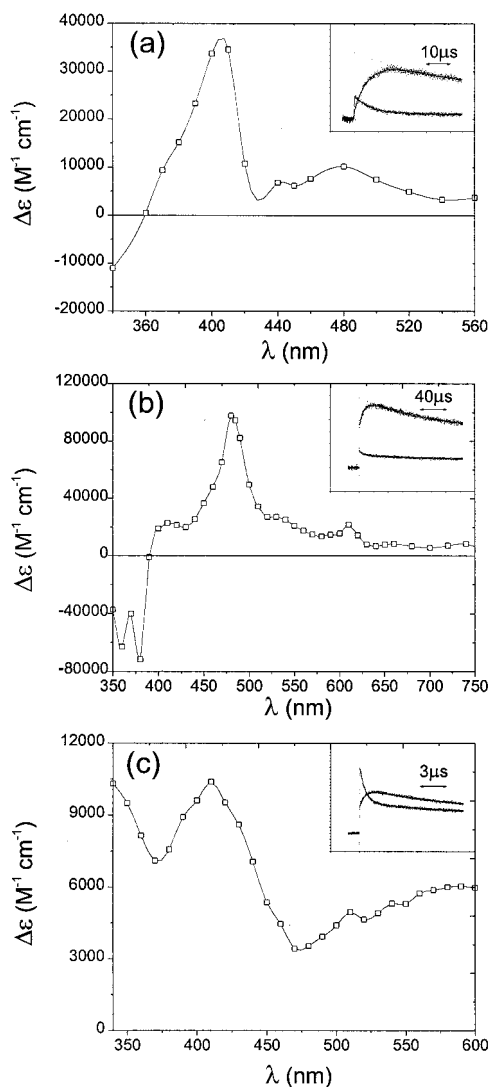
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Figure 4. Transient absorption spectra of imide radical anion observed under argon-saturated conditions upon 355-nm excitation of a 1:1 CH$_3$-CN/H$_2$O solution of (a) 9 μM NI containing 0.011 M GMP; (b) 12 μM NDI containing 830 μM GMP; and (c) 710 μM BPDI containing 770 μM GMP. Insets: Triplet decay observed at (a) 470, (b) 370, and (c) 400 nm with concomitant growth of quenching product at (a) 410, (b) 430, and (c) 480 nm. Fits shown are to double exponential decay and growth & decay, respectively.

and was thus assigned as NI$^{\bullet-}$, the one-electron reduced form of NI. The observed pseudo-first-order growth of the product or concomitant decay of the triplet was linear with GMP concentration, consistent with competitive Stern−Volmer kinetics (eq 3).

$$^3I^* \rightarrow I \qquad k_d \qquad (3a)$$

$$^3I^* + XMP \rightarrow I^{\bullet-} + XMP^{\bullet+} \qquad k_q \qquad (3b)$$

The bimolecular quenching plot for the reaction of ^{3}NI* with GMP is shown in Figure 5(a). The rate constant (k_q) for forward electron transfer from the base monophosphate to the imide triplet state, obtained from the slope of this plot, is given in Table 2. Quenching by AMP, CMP, and TMP was substantially slower. In these cases, only an upper limit for the quenching constant was estimated from a determination of the observed rate constant for triplet decay in the presence of 0.10 M nucleotide (limited by nucleotide solubility). On longer time
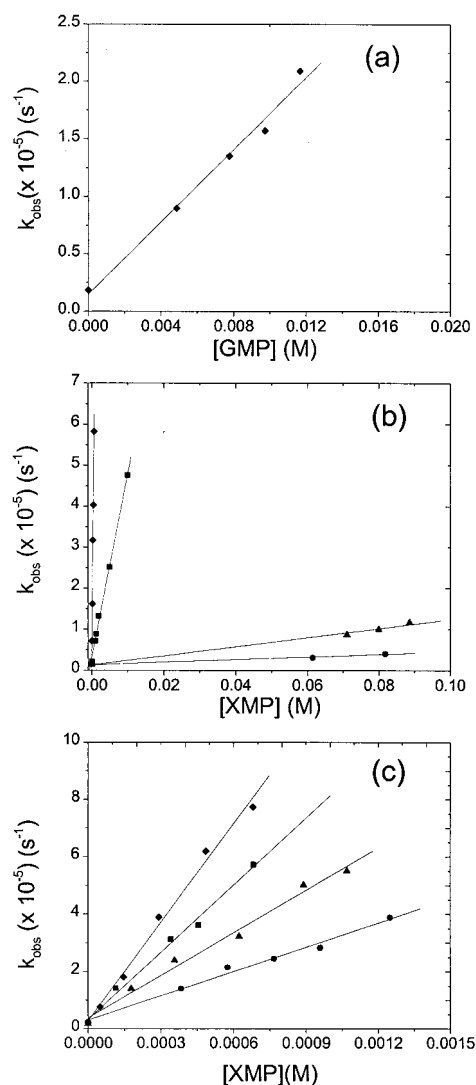

Figure 5. Bimolecular quenching plot for the reaction of the triplet state of (a) NI (b) NDI, and (c) BPDI with various nucleotides in 1:1 CH$_3$CN/H$_2$O. GMP (◆), AMP (■), TMP (▲), and CMP (●). XMP refers to the different base nucleotides.

scales, the reduced imide radical decayed via second-order kinetics, consistent with the bimolecular recombination of NI$^{\bullet-}$ and GMP.

For NDI and BPDI, pulsed excitation of a solution of the imide in the presence of nucleotide produced a transient whose spectra are shown in Figure 4b and c, respectively. As in the case of NI, the spectra were identical to that produced by DABCO quenching. Thus, the product of NDI and BPDI quenching by the nucleotides was identified as the one-electron reduced form of the diimide. In the case of BPDI, significant triplet quenching was observed with all four of the bases employed (Figure 5c). The one-electron reduced forms of NDI and BPDI were independently generated via quenching of the triplet state by the electron donor DABCO. In all cases, the transient produced from this process was identical to that observed following the reaction of the diimide triplet state with the base monophosphates. A summary of the bimolecular rate constants is included in Table 2. For **1**, **2**, and **3**, the bimolecular rate constants for the reaction of the imide triplet state with DABCO were found to be approximately diffusion-controlled ($k_q = 1.1 \times 10^9$, 1.5×10^9, and 2.7×10^9 M^{-1} s^{-1} for NI, NDI, and BPDI, respectively). The transient spectra produced following quenching of the imide triplet by either the base

Table 2. Bimolecular Rate Constants (k_q) and Radical Yields (Φ_{CE}) for Reactions of Imide and Diimide Triplet States with Nucleic Acid Base Monophosphates (1:1 H_2O/CH_3CN, $T = 22$ °C)

	NI		NDI		BPDI	
	$k_q \times 10^{-7}$ (M^{-1} s^{-1})	$\Phi_{CE}{}^a$	$k_q \times 10^{-8}$ (M^{-1} s^{-1})	$\Phi_{CE}{}^b$	$k_q \times 10^{-9}$ (M^{-1} s^{-1})	$\Phi_{CE}{}^c$
GMP	2.0 ± 0.1	0.37 ± 0.03	9.2 ± 0.5	0.19 ± 0.01	1.1 ± 0.1	0.26 ± 0.01
AMP	<0.06		0.45 ± 0.02	0.23 ± 0.01	0.59 ± 0.11	0.20 ± 0.03
CMP	<0.004		$.003 \pm 0.001$		0.27 ± 0.03	0.20 ± 0.01
TMP	<0.02		0.011 ± 0.001		0.47 ± 0.04	0.17 ± 0.01

^{a} [GMP] = 0.020 M. ^{b} [GMP] = 2.9×10^{-4} M; [AMP] = 6.0×10^{-3} M. ^{c} [GMP] = 1.9×10^{-4} M; [AMP] = 5.7×10^{-4} M; [CMP] = 1.9×10^{-3} M; [TMP] = 6.2×10^{-4} M.

monophosphates or DABCO contain contributions from both the imide radical anion and the oxidized base or DABCO electron donor. The absorption spectra of oxidized purine and pyrimidine derivatives have been generated by pulse radiolysis, and the extinction coefficients above 360 nm, for all four of the bases employed, were determined to be less than 1000 M^{-1} cm^{-1}.[23] Likewise, the visible absorption spectrum of DABCO radical cation exhibits a peak at 470 nm with an extinction coefficient of only 1500 M^{-1} cm^{-1}.[24] Since the extinction coefficients of these latter products are small compared to those of the reduced imide moieties (see below), it is assumed that the observed spectra are due exclusively to the imide radical anion.

Transient Absorption Spectra of Imide Radical Anion. After the characterization of the kinetics of triplet-state reactivity with the individual base monophosphates, the yields of charge-separated products produced, in competition with cage recombination, were quantified. Determination of the yield of charge-separated products produced from each quenching event necessitated a determination of the visible or UV extinction coefficients for each reduced imide. To determine these absorption coefficients, imide solutions containing the reductant DABCO were irradiated with pulsed 355-nm light. The experiment was carried out under conditions where the triplet excited state of the imide was 99% quenched by DABCO to rapidly form the imide radical anion. The concentration of reduced imide that was formed was evaluated by subsequent reactivity with methyl viologen as the secondary electron acceptor (Figure 6).[25] Slow triplet-state quenching by methyl viologen was observed ($k_q < 4 \times 10^7$ M^{-1} s^{-1} for all three imides) but was not a competitive triplet decay pathway under the conditions employed. The extinction coefficients of the imide radical anions are given in Figure 4. The bimolecular quenching constants for the electron transfer from the imide radical anions to methyl viologen were diffusion-controlled for **1** and **3**, but somewhat slower for **2**, owing to its more positive reduction potential. Bimolecular rate constants for eq 5 were determined to be 5.8 × 10⁹, 2.4 × 10⁸, and 3.4 × 10⁹ M^{-1} s^{-1} for NI, NDI, and BPDI, respectively. From a knowledge of the concentration of viologen produced, extrapolated to time zero and the imide radical anion absorbance change, the extinction coefficients of the imide radical anions were determined and are shown in Figure 4.

Radical Yields from Base Monophosphate Quenching. After the quantification of the absorption coefficients for the triplet states and radical anions of the three imides, the yields of imide radical anions, produced following base monophosphate quenching of the triplet state and escape from the solvent cage, were determined. Laser flash photolyses (355 nm) of solutions of **1**, **2**, and **3**, in the presence of each of the four base monophosphates were carried out. The quencher concentration
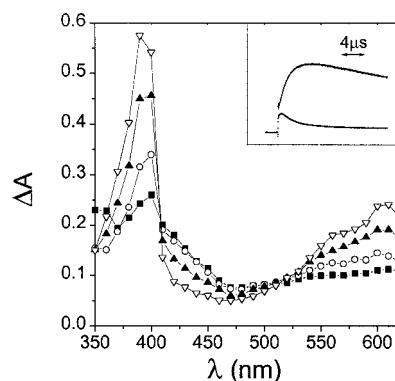

Figure 6. Transient absorption spectrum observed upon 355-nm excitation of a solution of BPDI (830 μM) and DABCO (990 μM) in the presence of 68 μM methyl viologen (1:1 CH_3CN/H_2O). Under the conditions employed, BPDI$^{•-}$ is produced in less than 0.5 μs. Spectra show the growth of MV$^{•+}$ at 395 and 606 nm, concomitant with the decay of the BPDI$^{•-}$ absorption band at 430 nm. Spectra are shown at 0.38 μs (■), 0.84 μs (○), 1.74 μs (▲), and 3.66 μs (▽) after the laser pulse. Inset: Growth of the quenching product (MV$^{•+}$) at 400 nm concomitant with radical anion decay (430 nm).

was adjusted to ensure >98% quenching takes place in competition with radiative and nonradiative decay of the triplet excited state. The radical yields (Φ_{CE} or cage escape yields) from each quenching event (eq 3) were determined from eq 4.

$$\Delta A_{I^{•-}} = \Delta\epsilon_{I^{•-}}\Phi_{CE}[^3I^*]_o b \qquad (4)$$

In eq 4, $[^3I^*]_o$ is the excited triplet-state concentration immediately after the laser pulse, $\Delta\epsilon_{I^{•-}}$ is the extinction coefficient of the radical anion of the imide, and Φ_{CE} is the cage escape yield. The charge separation yields from the triplet-state quenching by the base monophosphates are listed in Table 2.

Discussion

General Photophysical Properties and Redox Tunability. Imide and diimide systems offer attractive prospects for utilization as light-activated oxidizing agents. First, a variety of functional substituents can be readily attached at the imide nitrogen from a host of commercially available acid anhydrides and amines. This offers the prospect for developing the systems into ones that are capable of noncovalent interactions with, among others, biological substrates such as DNA. Second, besides the potential of the imide/diimide as efficient oxidizing agents in the electronically excited state, the carbonyl moiety introduces n–π* excited states that are well-known to abstract hydrogen atoms from appropriate donors. A fundamental understanding of the photophysical and redox properties of reactive excited states is crucial in elucidating the specific interactions of these states with potential target quencher

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(25) A detailed description of how the imide radical anion was quantified is provided in the Supporting Information.

molecules. In the case of simple N-substituted phthalimides, it has been shown that the lowest energy singlet excited state is primarily n−π* in nature, while the lowest triplet excited state behaves principally as π−π* state, and is unable to initiate hydrogen atom abstraction reactions.[26] The nature of the excited state of certain naphthalene and benzophenone-derived imide and diimides has been reported.[2,19−22] From solvent-induced spectral shifts, the lowest singlet excited state of naphthalimide chromophores is generally believed to be primarily π−π* in nature.[2] However, the presence of a nearly isoenergetic n−π* triplet state (T_2) results in efficient intersystem crossing into the triplet manifold ($\phi > 0.90$).[27] Efficient intersystem crossing has also been reported for naphthaldiimide derivatives.[21] However, for these systems, solvent-induced spectral shifts suggest that the lowest singlet possesses significant n−π* character, with the weak n−π* transition apparently lying beneath the intense π−π* transition observed at ca. 350 nm.[2] In both cases, the lowest electronically excited triplet states (T_1) of the naphthalene-derived chromophores are π−π* and are unreactive toward hydrogen atom donors.[2] For structurally similar naphthalimide, naphthaldiimide, and aromatic benzophenone diimide derivatives, the previously reported triplet-state energies agree extremely well with those reported in Table 1.[21,22,27] Scaiano et al. have reported that the lowest excited triplet state of *N,N′*-diaryl-substituted benzophenone diimide derivatives are quenched by hydrogen atom donors, thus behaving primarily as n−π* excited states.[22]

In several reports, Schuster has demonstrated the ability of organic triplet states to react with DNA and individual nucleotides via either an electron transfer or hydrogen atom abstraction (for n−π* states) pathway.[28] Quantum yields of $10^{-2}-10^{-3}$ for -GG- selective cleavage have been reported.[29] This observation, coupled with the knowledge that the lowest energy triplet excited state of **3** is likely to be principally n−π* in nature, suggests the possibility of triplet reactivity via both mechanisms. For the imide-derived systems investigated in this work (including **3**), the interactions of the electronically excited triplet states with the individual nucleotides result exclusively in electron-transfer products. The transient absorption spectrum that was observed following nucleotide quenching was identical to that produced following triplet-state quenching by the electron donor, DABCO. Proton-coupled electron transfer has been reported in other nucleobase- and nucleotide-quenching studies.[10,12] Since the experiments were carried out in aqueous acetonitrile solutions, this possibility was considered.[30] Shafirovich et al. observed a strong solvent isotope effect on the fluorescence quenching of benzo[*a*]pyrene derivatives by nucleotides,[9] specifically, the bimolecular rate constants that were a factor of 1.5−2.0 slower in D_2O than in H_2O. In this work, we measured the rate constant for triplet quenching of **1** by GMP in 1:1 CH_3CN/D_2O. Since the rate constant was within 5% of that measured in CH_3CN/H_2O, proton-coupled electron transfer does not appear to be rate-limiting.

The photosensitized cleavage of nucleic acid polymers must consider the possible mechanisms of the interaction of the chromophore excited state with individual nucleotides. These include (i) hydrogen atom abstraction from the deoxyribose groups of DNA, (ii) the generation of diffusible reactive intermediates such as singlet oxygen or hydroxyl radicals, and (iii) single-electron transfer to oxidize a nucleic acid base. Recent reports suggest that organic chromophores such as naphthalimide,[3−5] riboflavin,[31] and anthraquinone[28] derivatives initiate piperidine-requiring, selective cleavage at the 5′-side of -GG- steps, suggesting that the single-electron transfer mechanism is of significant importance in photocleavage processes. However, questions regarding the site-selectivity of these processes remain. Guanine-specific cleavage can be readily justified by a consideration of the one-electron oxidation potential of G, A, T, and C nucleosides. Steenken and Jovanovic have determined these potentials at pH 7 using pulse radiolytic techniques and shown that, in order of decreasing ease of oxidation, the reduction potentials of the nucleoside radicals are: 1.29 (G) < 1.42 (A) < 1.6 (C) < 1.7 V (T) (V vs NHE at pH 7).[32] Moreover, recent calculations have shown that -GG- steps are more easily oxidized than single guanine residues, with the HOMO localized almost exclusively on the 5′-side of the step.[33] Although these values have been determined for the individual nucleosides in aqueous solution, they remain undetermined in DNA polymers, where ion solvation is significantly reduced.

Driving Force Dependence on Forward and Back Electron Transfer. The rate constants for forward electron transfer that are reported in Table 2 span 2 orders of magnitude, depending upon the imide and base monophosphate employed. For a given nucleotide, the trend of increasing rate constants for forward electron transfer (Table 2) with increasing reduction potential of $^3NI^* < {}^3NDI^* < {}^3BPDI^*$ is consistent with forward electron transfer in the Marcus normal region. Since these rate constants are more than 2 orders of magnitude slower than one would predict for a diffusion-controlled process, the rate-limiting step is the electron-transfer process itself. Estimation of the thermodynamic driving force for electron transfer requires a knowledge of the base oxidation potentials. Using the values reported by Steenken and Jovanovich in aqueous solution at pH 7 (see above),[32] the driving force for forward electron transfer from the imide or diimide triplet state to the individual nucleotides (XMP) can be estimated from the Rehm−Weller equation (eq 5).

$$\Delta G_f = -\{E_{1/2}({}^3I^*/I^{\bullet-}) - E_{1/2}(XMP^{\bullet+}/XMP)\} +$$

$$\Delta(z_I z_{XMP})\frac{e^2}{4\pi\epsilon_o\epsilon_s r} \quad (5)$$

In eq 5, the first two terms are the one-electron reduction potentials of the imide triplet state and base monophosphate radical, respectively. The last term accounts for the electrostatic destabilization of the redox products ($I^{\bullet-}$ and $XMP^{\bullet+}$), relative to the reactants ($^3I^*$ and XMP). Treating the donor and acceptor as point charges, with the charges located at the center of the sphere, we estimate r, the distance of closest approach of the two spheres, to be of the order of 10 Å. Assuming that the

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Scheme 1. Energetics of Forward and Reverse Electron Transfer for the Oxidation of the Base Monophosphates by **1**, **2**, and **3**
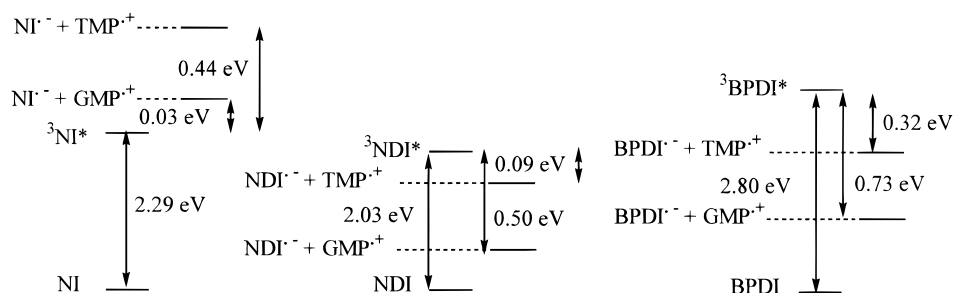
solvent dielectric constant in a binary solvent mixture is a weighted average of the individual dielectrics, we estimate the electrostatic interaction term to be +24 mV for forward electron transfer from the base monophosphate to the imide/diimide triplet state. By using this estimate, the redox potentials listed in Table 2, and the nucleoside oxidation potentials reported by Steenken, the driving force for forward electron transfer from the imide triplet states to the base monophosphates can be approximated. Although absolute values of nucleotide oxidation potentials in the solvent system employed remain unknown, an energy diagram such as that shown in Scheme 1 suggests that the driving force for the forward electron-transfer process can be "tuned" for desired base reactivity. Specifically, employing the excited triplet state of **1** results in electron transfer from only GMP, and this process is quite slow. GMP oxidation becomes more rapid when ^{3}NDI* is employed as an electron acceptor. Slow AMP oxidation is observed, but the excited state is essentially unreactive toward CMP and TMP. For the best oxidizing agent,^{3} BPDI*, the process becomes rapid for all of the nucleotides, with oxidation of TMP occurring less than 1 order of magnitude slower than that expected for a diffusion-controlled process.

The driving forces for charge recombination are all more negative than −1.5 eV. Thus, it is likely that back electron transfer from the reduced imide/diimides to oxidized base monophosphate is strongly in the Marcus inverted region. Moreover, the spin flip required for charge recombination processes within triplet-derived ion pairs is expected to slow this process down, thus favoring the competitive diffusion process of the ions from the solvent cage. From Table 2, ca. 17−37% of the triplet quenchings yield charge-separated radical ions in the systems employed. From the energy diagram depicted in Scheme 1, the free-energy change for recombination from NI$^{•−}$ to oxidized GMP would be significantly larger than that for the other recombination processes. The yield of charge-separated ions following the reaction of ^{3}NI* with GMP is largest, consistent with the process occurring in the Marcus inverted region. Although unity charge-separation yields are sometimes observed following electron-transfer quenching of organic triplet states, our data suggest that most of the triplet states that are quenched by electron-transfer undergo charge recombination within the water/acetonitrile solvent cage.

Conclusions

In summary, we have demonstrated the feasibility of utilizing functionalized imide or diimide chromophores as exclusively redox-active photonucleases. The laser flash photolysis data reported in this work suggest that the principle products resulting from interaction of organic imide and diimide triplet states with the individual nucleotides are exclusively those from redox chemistry. Significantly, the data suggest that the initial site of base oxidation can be tuned to include a specific subset of the bases, depending upon the thermodynamic properties of the imide or diimide employed. The kinetic selectivity that has been demonstrated has significant implications for doing photooxidative chemistry in a mixture of nucleotides, such as in DNA. Although it is certainly not the case that the base oxidation potentials in DNA are the same as in aqueous solution, the trend in ease of oxidation would be expected to be the same. Thus, the observed kinetic trend suggests that the series of structurally analogous imide and diimide electron acceptors may be employed to carry out selective and guanine-specific oxidative damage, or nonselective single-electron oxidation. Within the series, the redox potential of the lowest electronically excited triplet states can be readily tuned over ca. 800 mV. As a result, the kinetics for forward electron transfer to the electronically excited triplet state from a nucleic acid base can be tuned over more than 2 orders of magnitude. The strategy offers the prospect of kinetic control over the specific nucleotide(s) to be oxidized in mixed-sequence DNA. Specifically, within the series, it may be possible to develop chromophores in which it is kinetically feasible to initiate oxidative damage at only guanine residues (e.g., employing derivatives of compound **1**), nonspecifically at all residues (e.g., employing derivatives of compound **3**), or a subset of residues (e.g., employing derivatives of compound **2**).

Supporting Information Available: Experimental details for **1**, **2**, and **3** and determination of extinction coefficients (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.