Long-Lived Charge-Separated Species Observed on Flash Photolysis of Peptide Conjugates. Interplay of Local and Radical Ion Pair Triplet States[1]

Guilford Jones II* and Lily N. Lu

Department of Chemistry, and the Center for Photonics, Boston University, Boston Massachusetts 02215

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Peptides composed of alanine (Ala) and tryptophan (Trp), modified with the (nitro)pyrenesulfonyl chromophore (Pyr and NPyr) at the N-terminus have been examined by nanosecond laser flash photolysis. A common phototransient for Pyr-AlaOEt and Pyr-Ala-TrpOEt was observed that exhibited broad absorption at 410−550 nm and decay time constants in the range, $\tau_{1/2} = 20-40\ \mu$s. This species was assigned to the triplet excited state that is local to the pyrene chromophore (^{3}Pyr). For the conjugates having a stronger electron acceptor group at the N-terminus, NPyr-Ala-TrpOEt and NPyr-Ala-Ala-TrpOEt, the local triplet was replaced with a phototransient whose principal feature is a sharp band at 440 nm (assigned to the $NPyr^{-\bullet}$ radical anion). The radical ion transients for the NPyr peptide derivatives were assigned to intermediates that result from the intramolecular electron transfer quenching of NP excited species by pendant groups (i.e., the indole ring of tryptophan). The lifetimes observed for the radical ion transients associated with the NPyr series were relatively long (extending to ca. 400 ns) and depended in an interesting way on the structure of the peptide linkage. A mechanism of electron transfer in the singlet manifold and recombination yielding a local Pyr triplet state is important for the Pyr series.

Introduction

A variety of systems that invoke the peptide link for connecting electron donors and acceptors have been reported in recent years.[2−7] A principal finding in much of this work is that a low-lying excited state or redox intermediate associated with a metal center or an organic chromophore is involved in a forward electron transfer step. For the organic chromophores, singlet quenching (and a presumed formation of singlet radical ion pairs) has been the most common mechanism. In several peptide systems, particularly those involving transition metal centers, the lifetimes of electron-transfer intermediates have been investigated, and dependences on donor−acceptor distance, thermodynamic driving force, temperature, and other experimental variables have been obtained.[4] The issue of spin multiplicity for either precursor or radical(ion) pair states, however, is not readily assessed for the donor−acceptor systems having heavy metal atoms, either because radical pair states are not involved or because of the complication that results from strong mixing of spin states for such systems.

In recent communications, we have reported on the synthesis and photochemical properties of a series of modified peptides composed of alanine (Ala) and tryptophan (Trp) amino acid residues.[6−8] This series was constructed for the purpose of investigating photoinduced intramolecular electron transfer that occurs between an electron acceptor group (and light-absorbing unit) that is attached to the N-terminus of selected peptides having 1−3 amino acid links and pendant electron donors. In several of these examples, the tryptophan residue played a direct role as electron donor due to the electroactivity of its side-chain indole ring [$E°$ ($Trp^{+\bullet} \rightarrow$ Trp) = 1.0 V vs saturated calomel electrode (SCE)]. Particular attention was paid to the rates of back electron transfer for the series:

$$Pyr^{-\bullet} - \text{peptide} - donor^{+\bullet} \rightarrow Pyr - \text{peptide} - donor \quad (1)$$

Reported separately[7,8] are results having to do with intramolecular electron transfer that occurs between pyrenesulfonyl (electron acceptor) chromophores (e.g., Pyr) and donor groups for a family of peptides, including Trp residues, investigated using flash photolysis methods and conducted on the picosecond time scale. The general trends are consistent with a moderate falloff in intramolecular electron-transfer rate with (through-bond) distance along the peptide backbone, a dependence on driving force, and a modest dependence on the presence of Ala vs Trp in the peptide sequence.[7,8] Decay times for

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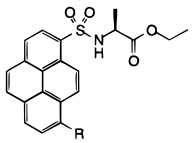
radical ion pair states varied for this series of measurements in the range of 10 ps to 1 ns. Data of this type provide reference points regarding the formation and decay of radicals derived from tryptophan, intermediates that been identified in studies of native proteins and other model systems containing Trp residues.[9]

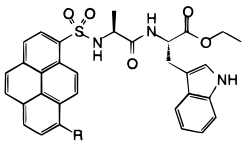
We report here phototransient data for a peptide series that is engaged in long-range electron transfer and for which the effects of spin for the precursor and radical ion pair states can be readily distinguished. Laser flash photolysis on the nanosecond time scale has confirmed that electron transfer takes place for a series of simple di- and tripeptides containing the Trp residue and bearing the pyrenesulfonyl chromophore. The appearance of long-lived intermediates depends critically on the relative energies of the local triplets [for substituted pyrenes, E_T = 46−48 kcal/mol (ca. 2.1 eV)] and the radical ion pair species (e.g., $Pyr^{-\bullet}$ − link- $Trp^{+\bullet}$). For the derivatives bearing the 8-nitro-1-pyrenesulfonamide (NP) moiety, we find that a larger driving force for electron transfer results in a positioning of the radical pair state below the local triplet. For peptides in which donor and acceptor groups are separated nominally by 1.0−1.3 nm (average edge-to-edge distances associated with randomly distributed conformations[7,8]), the decay times for radical pair intermediates are relatively long, reaching the microsecond domain. The present series has special value, because the singlet and triplet routes for electron transfer and the consequent influence of spin multiplicity on the lifetime of radical-pair intermediates are delineated.

Results and Discussion

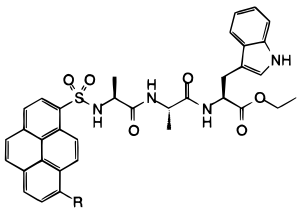
Pyrenesulfonamide Conjugates: Absorption and Fluorescence Properties. The selected peptides, as C-terminal ethyl esters, were prepared from commercially available N- and C-terminal protected L-amino acids, using standard peptide coupling methodology and 1-pyrenesulfonyl chloride as the agent for conjugation of the pyrene chromophore at N-termini.[10] The nitropyrenesulfonyl series was prepared starting with mononitration of Pyr-AlaOEt which introduced (preferentially)



R = H, Pyr-AlaOEt
R = NO_2, NPyr-AlaOEt



R = H, Pyr-Ala-TrpOEt
R = NO_2, NPyr-Ala-TrpOEt



R = H, Pyr-Ala-Ala-TrpOEt
R = NO_2, NPyr-Ala-Ala-TrpOEt

a nitro group at the 8-position of the pyrene chromophore (see Experimental Section for details). The conjugates of 1-pyrenesulfonic acid, Pyr-AlaOEt and Pyr-Ala-Trp,

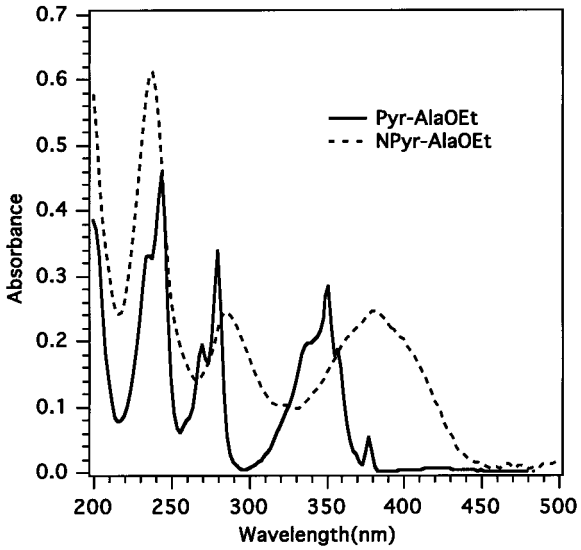


Figure 1. Absorption spectra for 10 μM Pyr-AlaOEt and 20 μM NPyr-AlaOEt in acetonitrile.

Table 1. Fluorescence and Phototransient Data for (Nitro)Pyrenesulfonamide Conjugates

compound	Φ_f[a]	transient (nm)[b]	τ ($\tau_{1/2}$), μs	k_{-et} (10^6 s^{-1})
Pyr-AlaOEt	0.31	430 (^{3}Pyr)	(38)	
Pyr-Ala-TrpOEt	0.014	430 (^{3}Pyr)	(20)	
NPyr-AlaOEt	0.0040	450 (^{3}NPyr)	12.2 (6.5)	
NPyr-Ala-TrpOEt	0.0006	440 ($NPyr^{-\bullet}$)	0.40	2.5
NPyr-Ala-Ala-TrpOEt	0.0009	440 ($NPyr^{-\bullet}$)	0.16	6.2
		515 ($Trp^{+\bullet}$)	0.20	5.0

[a] Aerated samples (10−20 μM in CH_3CN, 20 °C); λ_{exc} = 330−350 nm. [b] Nd/YAG laser (355 nm) irradiation of 10−20 μM conjugates in Ar-purged CH_3CN.

displayed UV spectra that showed a small perturbation arising from introduction of the Trp moiety (ca. 280 nm) and prominent bands associated with Pyr moieties (Figure 1, for Pyr-AlaOEt, ϵ_{350} = 16,000 M^{-1} cm^{-1}, acetonitrile solvent). The fluorescence observed for the Pyr conjugates (λ_{max} = 382 and 400 nm) revealed an emission quantum efficiency for acetonitrile solutions that depended on the tethering of Trp residues (Table 1). The emission data are consistent with the quenching of the singlet state for those peptides containing an electroactive substituent (the indole ring of tryptophan, Trp).[6−8] The introduction of a nitro group in the 8-position of the 1-pyrenesulfonamide chromophore led to substantial alteration in photophysical properties, including red-shifts in the principal absorption maxima (Figure 1). Fluorescence appears with λ_{max} = 430 nm for the NPyr series, with quantum efficiencies that are diminished (Table), consistent with the established effects of a nitro substituent on the parent pyrene chromophore and related hydrocarbons (more efficient intersystem crossing, vide infra).[11,12]

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(10) Hirashima, Y. et al. *Biochim Biophys. Acta* **1990**, *1047*, 35.

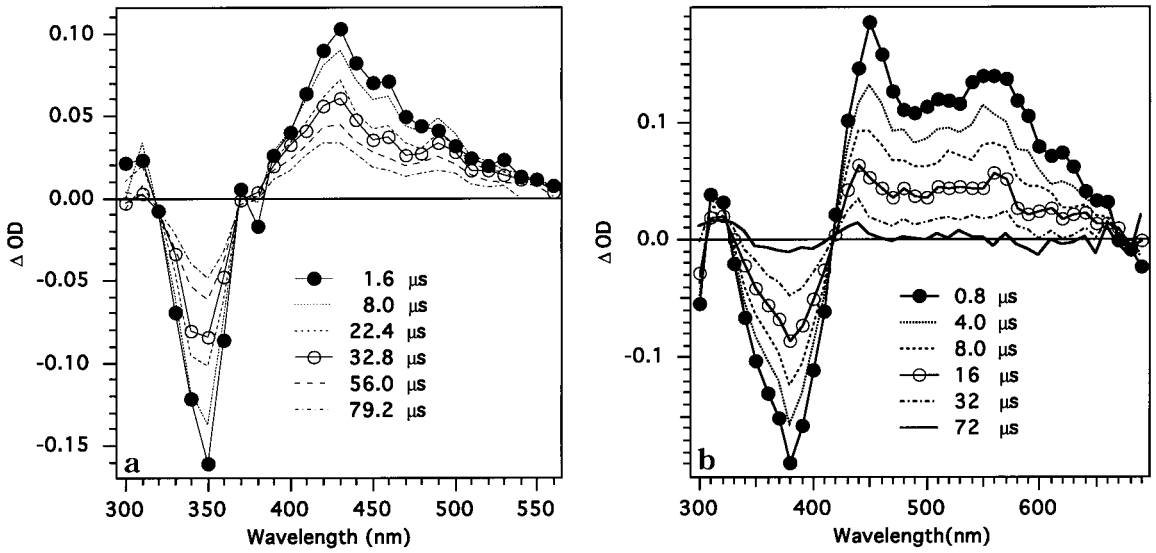


Figure 2. Transient absorption spectra for (a) 10 μM Pyr-AlaOEt and (b) 20 μM NPyr-AlaOEt in Ar-purged acetonitrile (λ_{ex} = 355 nm).

Flash Photolysis of Pyrenesulfonamide Conjugates. Pulsed laser excitation of Pyr-AlaOEt at 355 nm (Nd:YAG laser, 80 mJ/7-ns pulses) resulted in the spectra shown in Figure 2a. The decay half-life for this transient observed at 430 nm for Ar-purged solutions was about 40 μs; aeration of the acetonitrile samples resulted in a diminution of the decay time to the submicrosecond regime (a first-order fit of the decay data for aerated solutions yielded $\tau = 0.23$ μs). The data for Pyr-AlaOEt are consistent with the assignment of the phototransient observed in the 100 ns−100 μs regime to the local triplet excited state associated with the pyrene chromophore (λ_{max} = 420 nm[11−13]). Notably absent in the transient spectra for Pyr-AlaOEt is an absorption that has been identified with the radical anion species, $Pyr^{-\bullet}$; this transient, a narrow band centered at 490 nm,[7,8] in turn is similar to the radical anion of the parent hydrocarbon, pyrene (λ_{max} = 490; ϵ = 49,000 M^{-1} cm^{-1}) and several simple pyrene derivatives.[13]

Spectra obtained on flash photolysis of Pyr-Ala-TrpOEt contrasted with those for Pyr-AlaOEt; an inspection of the earliest possible time domain that could be interrogated with nanosecond resolution was informative (Figure 3). The transient behavior observed in the first 200-ns time interval after the laser pulse showed (1) a bleach of the absorption due to the ground state of Pyr-Ala-TrpOEt (at 350 nm); (2) sharp bands associated with residual (local pyrene) fluorescence at 380 nm that dissipate completely within 50 ns; (3) a small feature at 490 nm in the early time frame that is likely to be due to absorption by $Pyr^{-\bullet}$; and (4) growth of a broader transient absorption (λ_{max} = 425 nm) and other features that appear similar to those observed for the longer time domains for Pyr-AlaOEt (those assigned to the local triplet, Figure 2a).

The transient behavior for the Pyr series can be understood in terms of a mechanism of electron transfer and charge recombination suggested by the relative

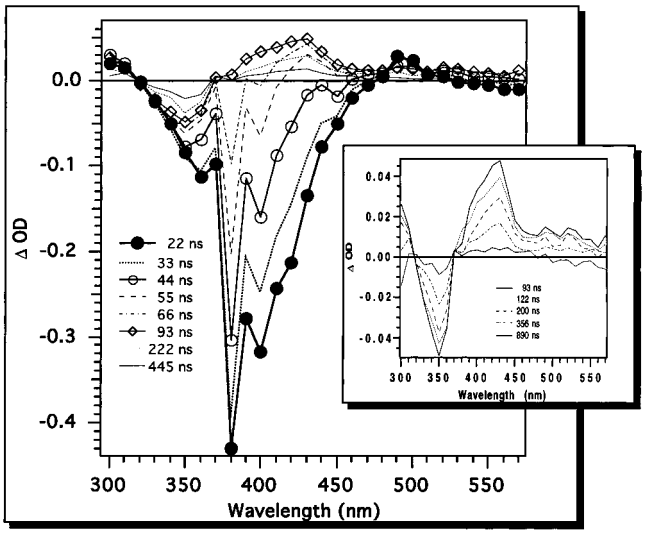


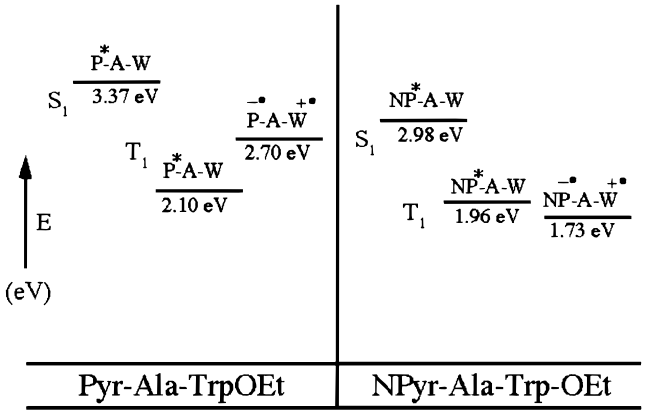
Figure 3. Transient absorption spectra for 10 μM Pyr-Ala-TrpOEt in air-saturated acetonitrile. Negative ΔOD corresponds to fluorescence decay at 380 nm for shorter time domain; the longer time domain featuring triplet absorption at 430 nm appears in the inset (λ_{ex} = 355 nm).

positioning of electronic state energies, as shown in Scheme 1. Unlike the simply substituted model compound, Pyr-AlaOEt, which gives rise to triplets via direct intersystem crossing, Pyr-Ala-TrpOEt participates in electron transfer in the singlet manifold leading to a decrease in fluorescence yield and lifetime [for Pyr-AlaOEt and Pyr-Ala-TrpOEt (acetonitrile), lifetimes of 11.5 and 0.88 ns have been obtained[8,14]]. The radical ion pairs thus formed (accounting for >90% of nonradiative singlet decay) lie in energy above the locally excited

(11) Scheere, R.; Henglein, A. *Ber. Bunseng.-Ges. Phys. Chem.* **1977**, *12*, 1234.
(12) Borg, R. A. J. *J. Phys. Chem.* **1994**, *98*, 11439.
(13) Gratzel, M.; Kalyanasundaram, K.; Thomas, J. K. *J. Am. Chem. Soc.* **1974**, *96*, 7869.

(14) The fluorescence lifetime observed for Pyr-Ala-TrpOEt only partially reflects the degree to which singlet quenching by the remote Trp leads to the radical ion pair state. According to pulse-probe measurements with subpicosecond resolution,[7,8] the rise times for the radical pair state via the singlet manifold can be shorter than 10 ps. The variations are due either to the distribution of conformations for the peptides, some of which undergo more rapid electron transfer,[5] or to direct excitation of a weak CT absorption observed at longer wavelengths around 400 nm corresponding to direct population of the radical ion pair state.[8]

Scheme 1



triplet species, ^{3}Pyr*. Intersystem crossing for singlet radical ion pairs is possible either via direct conversion to the local triplet or via (an equilibrium with) the triplet radical ion pair state. For the latter path, decay to the local triplet occurs by way of a spin-allowed charge recombination.

This type of electron-transfer-induced intersystem crossing has been reported previously for linked electron donor−acceptor systems.[15] The relative energies required for the mechanism shown in Scheme 1 are derived from the following parameters: (1) the singlet energy associated with the pyrenesulfonamide chromophore (^{1}Pyr*) (3.37 eV) obtained from the frequency at the intersection of absorption and emission spectra; (2) the reduction potential for Pyr-AlaOEt, obtained from cyclic voltammetry of the alanine conjugate ($E_{1/2} = -1.69$ V vs SCE[16]); and (3) the tryptophan reduction potential (for $Trp^{+\bullet} \rightarrow$ Trp, $E° = 1.0$ V vs SCE) derived from data for radical(ion) equilibria measured by pulse radiolysis.[17]

Flash Photolysis of Nitropyrenesulfonamide Conjugates. On flash excitation NPyr-AlaOEt provided a broadly absorbing transient (Figure 2b), the yield of which was higher than that observed for Pyr-AlaOEt, in accord with a higher yield of intersystem crossing for the nitro compound.[11] The half-life for this transient was 6.5 μs for Ar-purged acetonitrile solutions; oxygen again was effective in quenching this species ($\tau = 0.27$ μs for air-saturated acetonitrile solutions). The broadly absorbing transient observed on flash photolysis of NPyr-AlaOEt was compared with that reported for irradiation of 1-nitropyrene (a discernible peak at 450 nm and a broad absorption feature at 520−620 nm with $\epsilon_{530} = 10{,}000$ M^{-1} cm^{-1})[11]; the strong similarities confirmed the assignment of the triplet ^{3}NPyr* intermediate.

Spectral data for the NPyr conjugates with the electroactive amino acid substituent (Trp) led again to significantly different results. Laser irradiation of acetonitrile solutions at 355 nm of NPyr-Ala-TrpOEt and NPyr-Ala-Ala-TrpOEt resulted in strongly absorbing phototransients having well-defined maxima [$\lambda_{max} = 440$ nm (Figure 4a)] and (first-order) decay times in the submicrosecond regime ($\tau = 0.40$ and 0.16 μs, respectively; vide infra). These bands appeared to be virtually identical with that observed for the radical anion of nitropyrene ($\epsilon_{440} = 24{,}000$ M^{-1} cm^{-1}).[11] Less well resolved were weaker bands that appeared at 510−580 nm for NPyr-Ala-TrpOEt and for NPyr-Ala-Ala-TrpOEt. A summary regarding the identification of transients and decay data for the various peptide derivatives is presented in the Table.

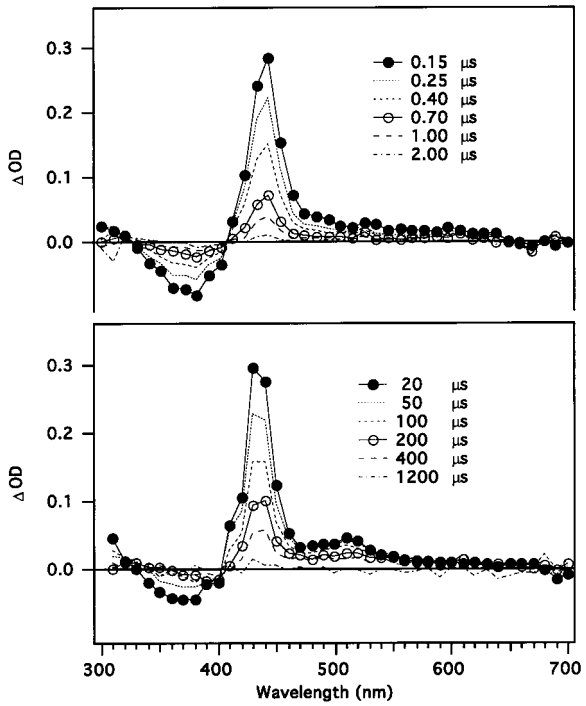


Figure 4. Transient absorption spectra for (a) 20 μM NPyr-Ala-TrpOEt and (b) 20 μM NPyr-AlaOEt with 10 mM Cbz-TrpOEt (Ar-purged acetonitrile; $\lambda_{ex} = 355$ nm).

Several control experiments further verified that the transients that appeared in the microsecond time domain were correctly ascribed to electron transfer intermediates. Flash photolysis of NPyr-AlaOEt in the presence of a tryptophan derivative, benzyloxycarbonyltryptophan ethyl ester (Cbz-TrpOEt), gave rise to the spectra shown in Figure 4b. The small feature that is discernible at 515 nm is most readily associated with the radical species that results from one-electron oxidation of tryptophan (Trp).[17,18] The data are consistent with the formation of nitropyrenesulfonamide triplet for NPA in relatively high yield (a quantum yield of intersystem crossing, $\Phi_{isc} = 0.6$, has been reported for 1-nitropyrene[11]) and effective bimolecular electron-transfer quenching by Cbz-TrpOEt. For the tryptophan moiety, the absorption properties of the radical-cation ($\lambda_{max} = 560$ nm; $\epsilon = 3{,}000$ M^{-1} cm^{-1}) have been distinguished from the deprotonated radical species ($\lambda_{max} = 510$ nm; $\epsilon = 2{,}000$ M^{-1} cm^{-1}) in pulse radiolysis and other transient studies of aqueous solutions, for which a pK_a of the $Trp^{+\bullet}$ species in water (4.3) has been determined.[17,18] The present data regarding the weaker absorbing transients for NPyr peptide derivatives in the 500−600 nm range are less compelling for precise

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(18) Merenyi, G.; Lind, J.; Shen, X. *J. Phys. Chem.* **1988**, *92*, 134. Bryant, F. D.; Santus, R.; Grossweiner, L. I. *J. Phys. Chem.* **1975**, *79*, 2711

assignment with regard to state of protonation. Contributions from both radical and radical cation forms in the time domain near 1 μs are possible; we are unaware of an independent assignment for the Trp radical(ion) species for dry acetonitrile solvent.[20]

The feasibility of the electron-transfer step involving NPyr excited states and Trp can be assessed from parameters that include the triplet state energy for the nitropyrenesulfonyl chromophore [after the value for nitropyrene, E_T = 46 kcal/mol (2.0 eV)],[16] and the reduction potential recorded for NPyr-AlaOEt ($E_{1/2}$ = −0.73 V vs SCE)[16] and for Trp (vide supra). The resulting energetics data (Scheme 1) show that the driving force for electron transfer is clearly more favorable for the combination, NPyr-AlaOEt and Trp vs Pyr-AlaOEt and Trp. The effectiveness of excited *singlet state* quenching of NPyr-AlaOEt by Cbz-TrpOEt (in a bimolecular process) was assessed from fluorescence quenching data. The quenching of the emission of NPyr-AlaOEt at 430 nm on addition of Cbz-TrpOEt (1.8−20 mM, acetonitrile solvent) yielded as Stern−Volmer plot with slope = 0.062 M^{-1}. Using a lifetime for the singlet of NPyr-AlaOEt, τ = 8.0 ps,[8] a bimolecular quenching constant was determined, $k = 7.7 \times 10^9$ M^{-1} s^{-1}, a value near that expected for a diffusion-limited rate.

The fate of radical(ion) species observed on nanosecond flash photolysis of NPyr-Ala-TrpOEt and NPyr-Ala-Ala-TrpOEt can be understood most readily in terms of return to ground state species via back electron transfer. The peptide conjugates were relatively resilient to photobleaching on repetitious pulsed photolysis. Within a 3-fold concentration range, the decay times were not dependent on peptide concentration. The depletion of the 440-nm transient for the peptides was examined in detail using trial fits of the decay data to first and second order and multicomponent decay parameters. The most satisfactory fits, those associated with the simple first-order rate law, yielded lifetimes and rate constants (in the range of 10^6 s^{-1}) as shown in the Table. The decay of transients at 440 and 515 nm appeared to be simultaneous, again consistent with a mechanism of depletion for the transients involving charge recombination. Notably, the decay of the radical ion pair intermediate *was somewhat faster for the longer peptide.* Also, for solutions of NPyr-Ala-TrpOEt, the 440-nm transient decayed more readily when aerated (τ = 0.12 μs); for undegassed samples the ratio of the absorbances at 440/(510−560) decreased also, consistent with the interception of the radical ion pair state by molecular oxygen (and electron transfer from $NPyr^{-\bullet}$ to O_2).[21]

Peptide Conformational Analysis. The peptide, Pyr-Ala-TrpOEt, was selected for molecular modeling, particularly with regard to the discovery of any conformational preferences shown by the peptide series. Geometries and distance relationships with respect to the Pyr chromophore and the potential electron donor group, the indole ring of tryptophan residues, were established. The distribution of conformations for Pyr-Ala-TrpOEt obtained from the simulations shown in Figure 5 includes

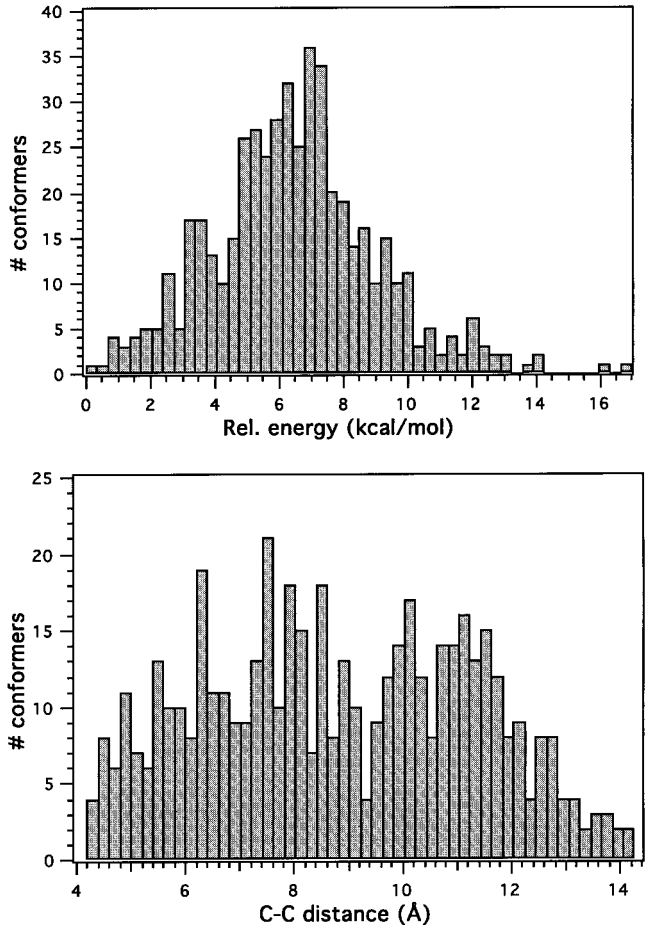

Figure 5. Histogram displaying distributions of relative energies (top) and center-to-center distances (bottom) of Pyr-Ala-TrpOEt conformations from computer simulations. (a) Aerated samples (10−20 μM in CH_3CN, 20 °C); λ_{exc} = 330−350 nm. (b) Nd:YAG laser (355 nm) irradiation of 10−20 μM conjugates in Ar-purged CH_3CN.

arrays of energies for individual conformations, or groupings in histogram form according to energy and critical distances. The important distances between putative electron donor and acceptor groups, defined in terms of spans through space between points "center-to-center" (c−c) for aromatic rings and distances through bonds between these same groups, "edge-to-edge" (e−e) (C1 of pyrene and C3 of the indole ring) were computed as an average to be 0.88 and 1.2 nm, respectively. A similar set of population/energy/distance data was obtained for the homologous peptide, Pyr-Ala-Ala-TrpOEt, which also served as a model for the nitro derivative; the simulations provided for the homologous peptide c−c and e−e distances of 1.1 and 1.5 nm, respectively.

The modeling results tended to confirm that long-range ordering of the chains (e.g., as with α-helices) or favor for particular conformations (i.e., folded vs extended) is not important for the short peptide chains under investigation, in accord with 2D-NMR (nuclear Overhauser effect) findings that are reported separately.[8] This determination is important because folded forms would introduce a mechanism of electron transfer that could be dominated by through-space interaction if distances between electroactive pendants for highly favored conformations were as close as 5 Å.[22] Mechanisms that use through-bond interaction have been shown to be quite effective in providing pathways for electron transfer via

(19) Creed, D. *Photochem. Photobiol.* **1984**, *39*, 537.
(20) A detailed examination of spectra at higher resolution for the model system, NPyr-AlaOEt and Cbz-TrpOEt in dry acetonitrile, showed only the single distinct (although weak) feature at 515 nm at 20 μs and longer times after a 355-nm laser pulse; Lu, N., Dissertation, Boston University, 1995, chap 4.
(21) See, for example, Jones, II, G.; Malba, V.; Bergmark, W. R. *J. Am. Chem. Soc.* **1986**, *108*, 4214.

long-range orbital overlaps or tunneling paths.[22,23] In terms of previous studies on peptides, rate data for electron transfer between terminal groups held via oligopeptide spacers (e.g., prolines) have been consistent with a mechanism of through-bond interaction via peptide backbone linkages extending over distances of 1.0−4.0 nm.[2,4] The most important finding for the present peptide models is that a very large number of favorable conformations lie in energy within a range of 2−3 kcal/mol and that the terminal and side-chain groups (as well as chain segments) are oriented in largely random fashion (with a probable time constant of a few nanosecond for conformational averaging[24]).

Summary. The more important general features of the electron-transfer mechanisms for the pyrenesulfonamide derivatives are illustrated with the aid of Scheme 1. For the linkage of tryptophan (Trp) and pyrenesulfonamide (Pyr), electron transfer is a dominant path for the singlet manifold; significant fluorescence quenching is observed. Charge recombination, either with or after radical pair intersystem crossing leads to the local pyrene triplet (^{3}Pyr*) and ultimate nonradiative decay. The alternative involves the nitropyrene chromophore that enters the triplet manifold of states, either by an accelerated rate of intersystem crossing that is inherent to the nitroaromatic stage or via fast singlet electron transfer and intersystem crossing at the radical pair stage.[25] In either event the lower lying species is the triplet radical ion pair for the NPyr series. The lifetimes for these species (abbreviated $NP^{-\cdot}A(A)W^{+\cdot}$, Scheme 1) are impressively long for charge-separated intermediates having relatively short links and through-bond distances of separation of 12−15 Å. A direct comparison can be made with picosecond flash photolysis data for the Pyr-Ala (Ala)TrpOEt series for which return electron transfer from radical ion pair states *in the singlet manifold* has been associated with shorter decay times, 0.76 and 3.1 ns for the peptides with one and two alanine spacers, respectively.[7,25] Thus, the slow rate of charge recombination for the NPyr series may be controlled by the rate of intersystem crossing (ISC) for the linked radical ion pair

$$^3[NPyr^{-\bullet} - Link - Trp^{+\bullet}] \rightarrow {}^1[NPyr^{-\bullet} - Link - Trp^{+\bullet}] \quad (2)$$

a rate that will be influenced by several factors that include spin−orbit and hyperfine interaction.[26] The spacer length (number of intervening residues) and the distribution of conformations for the peptide backbone and side chains are no doubt important factors in controlling this ISC step. There have been several reports of molecules in which donor and acceptor subunits are held together by a tether of significant length (commonly 5−15 σ bond separations), for which radical ion pair states that remain spin-correlated evolve on photoexcitation.[27] A distinction between singlet and triplet paths for charge separation across peptide bonds could be made for only a few cases in which amino acid derivatives provide short links between donor and acceptor groups (e.g., glycine spacers or a diketopiperazine bridge,[5] or a thiourea or thiohydantoin-linked N-terminal group[6]), coupled with the observation of radical pair intermediates that live in the microsecond time domain.[6]

The interesting finding that the longer peptide link, NPyr-Ala-Ala-TrpOEt, is as effective (or slightly more effective) in producing a shorter lived radical ion pair may be the result of a requirement that through-space interaction between remote groups contribute at least moderately to the ISC mechanism. Models show that the longer peptide, Pyr-Ala-Ala-TrpOEt (and presumeably the nitro derivative), provides a similarly large number of low-energy conformations that have a moderate distance of separation for through-space interaction (<0.8 nm, analogous to data shown in Figure 5), even though the average center-to-center distance between electron-transfer sites is somewhat larger than that for Pyr-AlaTrpOEt.

Recent refinements regarding the mechanism of long-range electronic coupling that leads to ISC for radical pairs focus on the role of random motion of spacer and substituent groups which modulates the through-space exchange interaction that is assumed to have an exponential falloff with distance.[28] Another recent view[29] of long-distance exchange electronic coupling envisions a "through excluded volume" mechanism to which there can be through-bond, through-vacuum, and through-solvent contributions, with evidence of some favor for the through-bond component.

Experimental Section

Materials and General Procedures. The benzyloxycarbonyl-protected amino acids were obtained from Bachem Bioscience Inc. The amino acid ethyl esters were obtained from Sigma. The deuterated NMR solvents were purchased from Cambridge Isotope Laboratories. The high-performance liquid chromatography (HPLC) grade acetonitrile was obtained from Fisher Scientific. Palladium on activated carbon (palladium content, 10%), along with other reagents and solvents, was obtained from Aldrich. The Silica Gel 60 (230−400 mesh, E. Merck Inc.) was used as the stationary phase for flash column chromatography (typically, a 2.5 cm ID × 32 cm column for a 0.5−1.0-g sample of crude product);[30] solvent composed of 5−50% ethyl acetate in petroleum ether, which gave an R_f for product of 0.2−0.3 on thin-layer chromatography

(22) Beratan, D. N.; Onuchic, J. N.; Betts, J. N.; Bowler, B. J.; Gray, H. B. *J. Am. Chem. Soc.*, **1990**, *112*, 7915.

(23) For a review, see Jordan, K. D.; Paddon-Row, M. N. *Chem. Rev.* **1992**, *92*, 395.

(24) Fasman, G. D. *Prediction of Protein Structure and the Principles of Protein Conformation*; Plenum Press: New York, 1989.

(25) The values for radical pair decay time for the Pyr series correspond to faster components from transient decay data from double exponential fits on fs laser flash photolysis − acetonitrile solvent).[7,8] An investigation of the fast processes that involve electron transfer quenching of the local singlet state of NPyr chromophores is presently underway (note fluorescence quantum yield trends in the table). The early data from flash experiments show formation and at least partial decay of radical ion pairs in the 1−50-ps time domain; Jones, G. II; Lu, L. N.; Gosztola, D. J.; Greenfield, S. R.; Wasielewski, M. R., unpublished results.

(26) Steiner, U. E.; Wolff, H. J. In *Photochemistry and Photophysics*; Rabek, J. F., Ed.; CRC Press: Boca Raton, 1991; Vol. 14; Jenks, W. S.; Turro, N. J. *Res. Chem. Intermed.* **1990**, *13*, 237.

(27) See, for example, Wasielewski, M. R. *Chem. Rev.* **1992**, *92*, 435; van Dijk, S. I.; Groen, C. P.; Hartl, F.; Brouwer, A. M.; Verhoeven, J. W. *J. Am. Chem. Soc.* **1996**, *118*, 8425; Hakamura, H.; Usui, S.; Matsuda, Y.; Matsuo, T.; Maeda, K.; Azumi, T. *J. Phys. Chem.* **1993**, *97*, 534; Staerk, H.; Kuhnle, W.; Treichel, R.; Weller, A. *Chem. Phys. Lett.* **1985**, *118*, 19.

(28) Staerk, H.; Busmann, H. G.; Kuhnle, W.; Treichel, R. *J. Phys. Chem.* **1991**, *95*, 1906; Shafirovich, V. Y.; Batova, E. E.; Levin, P. P. *J. Phys. Chem.* **1993**, *97*, 4877; Schulten, K.; Bittl, R. *J. Chem. Phys.* **1986**, *85*, 228.

(29) Forbes, M. D. E.; Ball, J. D.; Avdievich, N. I. *J. Am. Chem. Soc.*, **1996**, *118*, 4707.

(30) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

(TLC) (Silica Gel 60 F254), was used for elution of amino acid or peptide derivatives.

***N*-(1-Pyrenesulfonyl)-L-alanine Ethyl Ester (Pyr-AlaOEt).** 1-Pyrenesulfonyl chloride[10] (0.80 g, 2.8 mmol) in 15 mL tetrahydrofuran (THF) was added dropwise to a mixture of the hydrochloride salt of L-alanine ethyl ester (0.43 g, 2.8 mmol) in 10 mL of THF and 2 mL of triethylamine. The solution was stirred at room temperature for 5 h. The precipitate that formed was filtered and the solvent removed by rotary evaporation to obtain a yellow solid. The latter product was purified by flash column chromatography on silica gel and recrystallized from ethyl acetate/petroleum ether to yield 0.64 g (62%) of yellow cubic crystals (mp 104−105 °C): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.95 (d, J = 9.5 Hz, H-10), 8.66 (d, J = 8.2 Hz, H-2), 8.30 (d, J = 7.8 Hz, H-8), 8.28 (d, J = 9.5 Hz, H-9), 8.26 (d, J = 7.8 Hz, H-6), 8.17 (d, J = 8.8 Hz, H-5), 8.16 (d, J = 8.2 Hz, H-3), 8.08 (t, J = 7.8 Hz, H-7), 8.04 (d, J = 8.8 Hz, H-4), 5.63 (d, J = 8.3 Hz, A−N*H*), 3.98 (qd, J = 7.1, 8.3 Hz, A−C*H*), 3.6 (m, O$CH_2$$CH_3$), 1.27 (d, J = 7.1 Hz, A−CH_3), 0.78 (t, J = 7.2 Hz, OCH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5 (C═O), 134.0 (C1), 132.6 (C3a), 130.5 (C5a), 130.0 (C9), 129.7 (C8a), 129.3 (C5), 127.3 (C10c), 127.1 (C6, C8), 127.0 (C7), 126.8 (C4), 126.7 (C3), 124.2 (C10a), 124.0 (C2), 123.7 (C10), 123.1 (C10b), 60.3 (−O$CH_2$$CH_3$), 51.2 (A-*C*H), 18.3 (A-*C*H_3), 13.2 (−OCH_2*C*H_3). LRMS (EI, 70 eV) *m*/*z* (relative intensity) 381 (M^+, 33), 308 (11), 256 (38), 217 (30), 202 (38), 201 (100), 200 (34); HRMS (EI, 70 eV) *m*/*z* 381.1032 (M^+, calcd for $C_{21}H_{19}NO_4S$ 381.1035).

***N*-Benzyloxycarbonyl-L-alanine-*p*-nitrophenyl Ester.** A solution of *N*-benzyloxycarbonyl-L-alanine (5.0 g, 0.022 mol) and *p*-nitrophenol (3.3 g, 0.024 mol) in 200 mL ethyl acetate was cooled to 0 °C, and dicyclohexylcarbodiimide (DCC) (4.6 g, 0.022 mol) in 20 mL of ethyl acetate was added dropwise. The solution was stirred at 0 °C for 2 h and left in the refrigerator overnight. The precipitate that formed was filtered, and the filtrate was washed successively with solutions of 5% Na_2CO_3, 5% HCl, and 5% NaCl and dried over anhydrous sodium sulfate. The solution was concentrated by rotary evaporation until 10 mL remained. The concentrated solution was added dropwise to 200 mL of petroleum ether to form a white precipitate. The product was collected by filtration and dried under vacuum to yield 6.9 g (89%) of a white powder (mp 78−79 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, J = 8.5 Hz, ΦNO$_2$-2H), 7.35 (s, Φ-5H), 7.27 (d, J = 8.5 Hz, ΦNO$_2$-2H), 5.25 (d, J = 5.6 Hz, A−C*H*), 5.13 (s, Φ-CH_2), 4.61 (m, A−C*H*), 1.58 (d, J = 7.3 Hz, A−CH_3).

***N*-Benzyloxycarbonyl-L-alanyl-L-tryptophan ethyl ester (Cbz-Ala-TrpOEt).** A solution of *N*-benzyloxycarbonyl-L-alanine, *p*-nitrophenyl ester (0.90 g, 2.6 mmol) in 25 mL pyridine (freshly distilled) was cooled to 0 °C, and 0.8 mL of triethylamine was added. The hydrochloride salt of L-tryptophan ethyl ester (0.70 g, 2.6 mmol) in 5 mL pyridine was added dropwise. The mixture was stirred at 0 °C for an hour and at room temperature overnight. After evaporation of pyridine under a vacuum the resulting oil was dissolved in 80 mL of ethyl acetate. The ethyl acetate solution was washed successively with solutions of 5% Na_2CO_3, 5% HCl, and 5% NaCl, and dried over anhydrous sodium sulfate. After evaporation of most of the ethyl acetate under vacuum, the resulting liquid was added to 80 mL of petroleum ether which led to the formation of a white precipitate. The product was collected by filtration and dried under vacuum to yield 0.97 g (85%) of a white powder (mp 123−124 °C): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (s, W−H-3), 7.49 (d, J = 7.8 Hz, W−H-7), 7.30 (s, Φ-5H), 7.30 (d, W−H-4, overlap with Φ-5H), 7.14 (t, J = 8.2, 7.1 Hz, W−H-5), 7.07 (t, J = 7.8, 7.1 Hz, W−H-6), 6.93 (s, W−H-2), 6.49 (d, J = 6.8 Hz, A−N*H*), 5.25 (d, J = 6.8 Hz, W−N*H*), 5.03 (m, Φ-CH_2), 4.85 (m, W−C*H*), 4.18 (m, A−C*H*), 4.09 (m, OCH_2-CH_3), 3.28 (d, J = 5.4 Hz, W−CH_2), 1.29 (d, J = 6.8 Hz, A−CH_3), 1.19 (t, J = 7.1 Hz, OCH2CH_3).

***N*-(1-Pyrenesulfonyl)-L-alanyl-L-tryptophan Ethyl Ester (Pyr-Ala-TrpOEt).** A mixture of *N*-benzyloxycarbonyl-L-alanyl-L-tryptophan ethyl ester (1.0 g, 2.3 mmol) in 12 mL of methanol and 0.1 g of 10% Pd/C was stirred under hydrogen provided by a balloon for 4 h. The Pd/C was filtered and the methanol removed by rotary evaporation to yield 0.64 g of a light yellow solid (93%). The product, L-alanyl-L-tryptophan ethyl ester (0.64 g, 2.1 mmol), was used directly without further purification, starting with dissolution in 8 mL THF with 2 mL of triethylamine. 1-Pyrenesulfonylchloride (0.6 g, 2.1 mmol) in 12 mL THF was added dropwise, and the reaction was stirred at room temperature for 18 h. The precipitate was filtered and the solvent removed by rotary evaporation to yield a yellow solid. The product was purified by flash column chromatography and recrystallized from ethyl acetate/petroleum ether to yield 0.66 g (57%) of white crystals (mp 204−205 °C): ^{1}H NMR (400 MHz, $CDCl_3$, with one drop of DMSO-d_6) δ 8.93 (br, W−H-3), 8.85 (d, J = 9.4 Hz, H-10), 8.57 (d, J = 8.2 Hz, H-2), 8.21 (d, J = 7.6 Hz, H-8), 8.20 (d, J = 7.6 Hz, H-6), 8.16 (d, J = 9.4 Hz, H-9), 8.12 (d, J = 8.9 Hz, H-5), 8.06 (d, J = 8.2 Hz, H-3), 8.01 (t, J = 7.6 Hz, H-7), 7.99 (d, J = 8.9 Hz, H-4), 7.23 (d, J = 7.7 Hz, W−H-7), 7.23 (d, J = 8.1 Hz, W−H-7), 7.06 (td, J = 8.1, 7.0, 1.1 Hz, W−H-5), 7.00 (d, J = 7.4 Hz, A−N*H*), 6.95 (td, J = 7.6, 7.0, 1.0 Hz, W−H-6), 6.88 (d, J = 8.1 Hz, W−N*H*), 6.85 (d, J = 2.3 Hz, W−H-2), 4.51 (m, W−CH), 3.84 (m, O$CH_2$$CH_3$), 3.75 (m, A−C*H*), 3.07 (dd, J = 14.0, 5.5 Hz, W−CH_2-*Ha*), 3.02 (dd, J = 14.0, 6,3 Hz, W−CH_2-*Hb*), 1.00 (t, J = 7.1 Hz, OCH_2CH_3), 0.94 (d, J = 7.1 Hz, A−CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3 (W−C═O), 171.2 (A−C═O), 136.1 (W−C8), 133.9 (C1), 132.8 (C2a), 130.5 (C5a), 129.8 (C9), 129.7 (C8a), 129.2 (C5), 127.3 (C10c), 127.0 (C6, C7, C8), 126.9 (C4), 126.8 (W−C9, C2), 124.2 (C10a), 123.9 (C3), 123.8 (C2), 123.7 (C10), 123.2 (C10b), 121.0 (W−C7), 118.4 (W−C5), 117.9 (W−C6), 111.4 (W−C4), 109.0 (W−C1), 60.2 (O$CH_2$$CH_3$), 53.0 (W-*C*H), 51.4 (A-*C*H), 26.8 (W-*C*H_2), 19.0 (A-*C*H_3), 13.7 (OCH_2*C*H_3); LRMS (EI, 70 eV) *m*/*z* (relative intensity) 567 (M^+, 19), 352 (17), 217 (23), 216 (11), 215 (75), 207 (14), 202 (39), 201 (69), 200 (38). 143 (11), 131 (13), 130 (100); HRMS (EI, 70 eV) *m*/*z* 567.1826 (M^+, calcd for $C_{32}H_{29}N_3O_5S$ 567.1813).

***N*-[1-(8-Nitropyrenesulfonyl)]-L-alanine Ethyl Ester (NPyr-AlaOEt).** The conjugate, Pyr-AlaOEt (50 mg, 0.13 mmol) in 5 mL acetic anhydride was cooled to 0 °C, and nitric acid (8.4 μL, 69% in water) in 1 mL of acetic anhydride was added dropwise. The reaction was stirred at 0 °C for 2 h and at room temperature for 10 min. When the reaction mixture was added slowly to ice water, an oily yellow solid separated. The solid was washed with water. The product was purified by flash chromatography and recrystallized from ethyl acetate/petroleum ether to yield 20 mg (35%) of yellow crystals (mp 186−187 °C), R_f = 0.22 (ethyl acetate/petroleum ether, 1:3); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.24 (d, J = 10.0 Hz, H-9), 9.08 (d, J = 10.0 Hz, H-10), 8.78 (d, J = 8.0 Hz, H-7), 8.74 (d, J = 8.4 Hz, H-2), 8.35 (d, J = 8.0 Hz, H-6), 8.33 (d, J = 8.4 Hz, H-3),8.28 (s, H-4, H-5), 5.56 (d, J = 8.3 Hz, A−N*H*), 4.05 (m, A−C*H*), 3.68 (m, O$CH_2$$CH_3$), 1.30 (d, J = 7.2 Hz, A−CH_3), 0.88 (t, J = 7.1 Hz, OCH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7 (C═O), 144.1 (C8), 134.7 (C1), 134.6 (C5a), 133.6 (C3a), 130.3 (C4), 129.6 (C5), 128.3 (C6), 127.2 (C8a), 127.0 (C3), 126.4 (C9), 126.2(C10), 124.4 (C10a), 124.3 (C10c), 124.2 (C2), 123.5 (C10b), 123.4 (C7), 61.7 (O$CH_2$$CH_3$), 51.8 (A-CH), 19.8 (A-CH3), 13.6 (OCH_2*C*H_3); LRMS (EI, 70 eV) *m*/*z* (relative intensity) 426 (M^+, 71), 368 (93), 310 (92), 262 (46), 246 (100), 237 (83), 220 (38), 200 (50), 97 (29), 81 (31), 69 (58), 57 (40), 55 (38), 43 (36); HRMS (EI, 70 eV) *m*/*z* 426.0864 (M^+, calcd for $C_{21}H_{18}N_2O_6S$ 426.0885).

The major product (above) (R_f = 0.22) was accompanied by a significant minor product, R_f = 0.24 [TLC, ethyl acetate and petroleum ether (1:3) as eluant]. Formation of these monosubstituted products is consistent with nitration at positions 6 and 8.[31] A comparison of the ^{1}H spectra of the Pyr-AlaOEt with NPyr-AlaOEt showed that the major product is indicative of nitration at position 8 (see Supporting Information). Nuclear Overhauser effect observed between protons assigned to positions 9 and 10 further supported the identification of the 8-nitro isomer.

(31) Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. In *Vogel's Textbook of Practical Organic Chemistry*, 5th ed.; John Wiley & Sons: New York, 1989, chp 6.

***N*-[1-(8-Nitropyrenesulfonyl)]-L-alanyl-L-tryptophan Ethyl Ester (NPyr-Ala-TrpOEt).** A solution of *N*-[1-(8-nitropyrenesulfonyl)]-L-alanine ethyl ester (200 mg, 0.47 mmol) in 10 mL ethanol water (1:1) with 1 mL 2 N NaOH was stirred at 60−80 °C for 3 h. The solution was neutralized with concentrated HCl to pH 3 and extracted with ethyl acetate (3 × 10 mL). The ethyl acetate layer was dried over anhydrous sodium sulfate followed by rotary evaporation to yield a yellow solid. The dried solid was used directly in the following step. *N*-[1-(8-Nitropyrenesulfonyl)]-L-alanine (187 mg, 0.47 mmol) was dissolved in 20 mL THF and cooled to 0 °C. 1-Hydroxybenzotriazole (HOBt, 127 mg, 0.94 mmol), *N*-methylmorpholine (0.1 mL), and the hydrochloride salt of L-tryptophan ethyl ester (189 mg, 0.71 mmol) were added successively. DCC (97 mg, 0.47 mmol) in 3 mL of THF was added dropwise. The reaction was stirred at 0 °C for 2 h and then at room temperature overnight. The precipitate was filtered and the solvent removed by rotary evaporation to yield a yellow solid. The product was purified by flash column chromatography and recrystallized from ethyl acetate/petroleum ether to yield 104 mg (36%) of yellow crystals (mp 193−194 °C): ^{1}H NMR (400 MHz, CD_3COCD_3) δ 10.05 (s, W−H-3), 9.37 (d, J = 9.9 Hz, H-9), 8.92 (d, J = 9.9 Hz, H-10), 8.76 (d, J = 8.6 Hz, H-7), 8.69 (d, J = 8.2 Hz, H-2), 8.51 (d, J = 8.6 Hz, H-6), 8.38 (d, J = 8.8 Hz, H-5), 8.30 (d, J = 8.8 Hz, H-4), 8.38 (d, J = 8.2 Hz, H-3), 7.38 (d, J = 8.0 Hz, A−N*H*, W−N*H*), 7.35 (d, J = 8.1 Hz, W−H-7), 7.23 (d, J = 7.9 Hz, W−H-4), 7.08 (t, J = 7.9, 7.0 Hz, W−H-5), 7.01 (d, J = 2.4 Hz, W−H-2), 6.93 (t, J = 8.1, 7.0 Hz, W−H-6), 4.14 (m, A−C*H*, W−C_H), 3.83 (m, OCH_2CH_3), 2.82 (W−CH_2−Ha overlap with solvent), 2.68 (dd, J = 14.5, 6.7 Hz, W−CH_2−Hb), 1.15 (d, J = 7.1 Hz, A−CH_3), 0.96 (t, J = 7.1 Hz, OCH_2CH_3); LRMS (EI, 70 eV) *m*/*z* (relative intensity) 612 (M^+, 18), 582 (6), 310 (92), 269 (6), 216 (10), 215 (89), 131 (7), 130 (100); HRMS (EI, 70 eV) *m*/*z* 612.1677 (M^+, calcd for $C_{32}H_{28}N_4O_7S$ 612.1679).

***N*-[1-(8-Nitropyrenesulfonyl)]-L-alanyl-L-alanyl-L-tryptophan Ethyl Ester (NPyr-Ala-Ala-TrpOEt).** In a similar fashion, *N*-[1-(8-nitropyrenesulfonyl)]-L-alanine (187 mg, 0.47 mmol) was coupled to L-alanyl-L-tryptophan ethyl ester (213 mg, 0.71 mmol). The product was purified by flash chromatography and recrystallized from ethyl acetate/petroleum ether to yield 103 mg (32%) of yellow crystals (mp 273−274 °C): ^{1}H NMR (400 MHz, DMSO) δ 10.79 (s, W−H-3), 9.29 (d, J = 9.9 Hz, H-9), 8.84 (d, J = 9.9 Hz, H-10), 8.84 (d, J = 8.7 Hz, H-7), 8.70 (d, J = 8.4 Hz, H-2), 8.6 (m, overlap of H-6, H-3, A_1−N*H*), 8.5 (s, overlap of H-4, H-5), 8.01 (d, J = 7.2 Hz, W−N*H*), 7.86 (d, J = 7.5 Hz, A_2−N*H*), 7.38 (d, J = 8.0 Hz, W−H-7), 7.27 (d, J = 8.3 Hz, W−H-4), 7.03 (d, J = 2.3 Hz, W−H-2), 7.00 (t, J = 8.3, 7.4 Hz, W−H-5), 6.91 (t, J = 8.0, 7.4 Hz, W−H-6), 4.29 (m, W−C*H*), 3.99 (m, A_1−C*H*), 3.90 (m, OCH_2CH_3), 3.73 (m, A_2−C*H*), 2.98 (dd, J = 14.0, 5.7 Hz, W−CH_2−Ha), 2.94 (dd, J = 14.0, 8.3 Hz, W−CH_2−Hb), 0.97 (m, A_1−CH_3 overlap with OCH_2CH_3), 0.63 (d, J = 6.9 Hz, A_2−CH_3); LRMS (EI, 70 eV) *m*/*z* (relative intensity) 683 (M^+, 7), 217 (15), 215 (55), 130 (100), 111 (16), 97 (20), 85 (15), 84 (56), 83 (15), 70 (18), 44 (24), 43 (23); HRMS (EI, 70 eV) *m*/*z* 683.2073 (M^+, calcd for $C_{32}H_{28}N_4O_7S$ 683.2050).

Molecular Modeling.[32] The software used was based primarily on the QUANTA package from Molecular Simulations, Inc., including the conformational search subroutine that interfaces with the CHARMm molecular mechanics program. Additionally, a semiempirical quantum mechanical program, MOPAC (version 6.0; QCPE 455), was used in determining charge distributions. To arrive at an array of low-energy (most probable) conformations for a particular peptide, the sequence of steps began with a random selection of all the flexible dihedral angles. The resulting high-energy conformation was thoroughly energy-minimized by the adopted-basis Newton−Raphson algorithm. The energy-minimized conformation was used to initiate the next sequence in a cumulative manner. For the computations certain assumptions or constraints were made: (1) the solvent dielectric constant was assumed to have a value of 37 and a radial distance dependence; (2) the amide carbonyl and amide proton positions were assumed to be antiparallel and coplanar. Typical runs provided simulations of 500 low-energy conformations whose distribution, in terms of population and energy, was determined. The "average" parameters, such as average energy and average distance, were obtained by arithmetic averaging.

Laser Flash Photolysis. Nanosecond phototransient experiments were carried out using a Nd:YAG laser system and detection methods described previously,[33] with principal components consisting of a Quantel YG-581−10 Nd:YAG laser, a LeCroy 6880A 1.35 gigasamples/second waveform digitizer, a LeCroy 6010 MAGIC controller, a pulsed xenon monitoring lamp, a H-20 monochromator (version V grating) from Instruments SA, and a Hamamatsu R928 PMT. The samples for flash photolysis experiments were 1.0×10^{-5} M in concentration for the pyrene series (Pyr) and 2.0×10^{-5} M for the nitropyrene derivatives (NPyr) and prepared by injecting 5−10 μL of a 1.0×10^{-2} M stock solution of peptide in DMF into 5 mL of acetonitrile. All experiments were performed at room temperature with Ar-purged or air-saturated solutions as indicated. Excitation was carried out at 355 nm with 7-ns pulses using 2.2 cm × 1.0 cm rectangular Pyrex cells. Each measurement of decay time was obtained as an average of transient decay data for at least five pulse sequences and two independent samples. Decay times were shown not to be dependent on pulse energy (40−80 mJ/pulse) or peptide concentration (10−30 μM).

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Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra (5 pages). This material is contained in microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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