VOLUME 119, NUMBER 23
JUNE 11, 1997

JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

Electric Field Effects on Electron Transfer Rates in Dichromophoric Peptides: The Effect of Helix Unfolding

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Received September 17, 1996[⊗]

Abstract: The effect of helix unfolding on the rates of photoinduced electron transfer in model dichromophoric peptides was investigated. Two α-helical peptides, **1** and **2**, having an alternating Ala-Aib backbone and differing only in the position of an appended electron donor (*N*,*N*-dimethylaniline) and an appended photoexcited electron acceptor (pyrene) relative to the electric field generated by the helix, had shown a difference in photoinduced electron transfer rates which had been ascribed to a helix dipole effect. Upon denaturation by protic solvents (EtOH, MeOH, H_2O, CF_3CH_2OH) or guanidinium, the observed electron transfer rates in **1** and **2** became identical. The helix unfolding was studied by circular dichroism (CD) analysis. A second pair of model oligopeptides, **3** and **4**, analogous to **1** and **2** but having L-proline (Pro) instead of α-methylalanine (Aib) incorporated into the backbone, were prepared in order to study unfolded peptides in low dielectric constant solvents. The CD, NMR, and steady-state fluorescence spectra in a variety of solvents establish that one of the chromophores experiences a different local environment in **3** than in **4** and that the two peptides have different average conformations.

Introduction

In an α-helical oligopeptide, the dipole moments of the amino acid residues align in the same direction, nearly parallel to the helix axis. The resulting macroscopic dipole generates an electrostatic potential, directed from the N-terminus to the carboxy-terminus, Figure 1.[1] This electrostatic field, estimated to be up to 10^9 V/m in an α-helix,[1b,2] plays an important role in the structure and functions of proteins, and probably also influences the primary electron transfer event in photosynthesis. It has been proposed, for example, that the permanent field of the first layer of proteins that surrounds the photosynthetic reaction center promotes a rapid electron transfer over a relatively long distance, producing a charge-separated pair oriented antiparallel to the helices' electric field.[1a] This effect has been the object of both theoretical and experimental investigations.[1a,3]

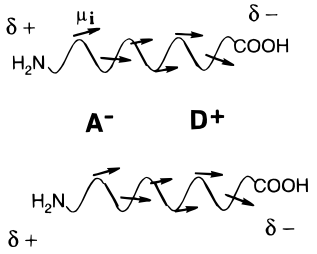


Figure 1. Schematic drawing of the postulate orientation of the charge-separated pair in a photosynthetic center embedded in parallel helices.

[⊗] Abstract published in *Advance ACS Abstracts,* May 15, 1997.

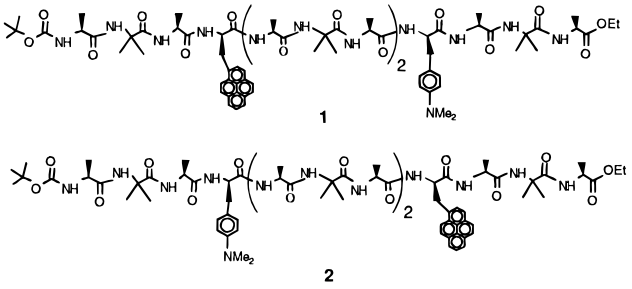
(1) For a review on the role of an α-helix dipole on protein function and structure see: (a) Hol, W. G. J. *Prog. Biophys. Mol. Biol.* **1985**, *45*, 149. (b) Hol, W. G. J.; van Duijnen, P. T.; Berendsen, H. J. C. *Nature* **1978**, *273*, 443. (c) Wada, A. *Adv. Biophys.* **1976**, *9*, 1. (d) Abraham, R. J.; Hudson, B. D.; Thomas, W. A.; Krohn, A. *J. Mol. Graph.* **1986**, *4*, 28.

(2) The reported value of 10^9 V/m is calculated from vacuum electrostatics.

(3) (a) Gosztola, D.; Yamada, H.; Wasielewski, M. R. *J. Am. Chem. Soc.* **1995**, *117*, 2041. (b) Brothers, H. M., III; Zhou, J. S.; Kostic, N. M. *J. Inorg. Organomet. Polym.* **1993**, *3*, 59. (c) Lockhart, D. J.; Kim, P. S. *Science* **1992**, *257*, 947. (d) Franzen, S.; Lao, K.; Boxer, S. G.; *Chem. Phys.*

S0002-7863(96)03269-6 CCC: $14.00

In previous work, we reported the photophysics of α-helical model peptides **1** and **2**, showing that the rates of electron transfer between a donor−acceptor pair are indeed significantly influenced by the orientation of the charge-separated ion pair with or against the helix dipole.[4,5] These two peptides differ



only in the positions of the *N*,*N*-dimethyl-*p*-anilino (the electron donor) and 2-pyrenyl (the electron acceptor) groups with respect to the helix ends. The calculated dipole moment for each peptide is approximately 40 D.[4b] From analysis of measurements of time-resolved pyrene fluorescence, the rates of electron transfer were shown to be 5−27 times faster in **1** (k_1) than in **2** (k_2), with smaller ratios being observed in solvents of higher dielectric constant.[4a] This result is in agreement with the postulated effect of the helix dipole, because faster rates were observed in **1**, where the radical ion pair is antiparallel to the helix field.

However, we consistently observed biexponential decays in **1** and **2**, a clear indication of multiple conformations of the oligopeptides in the ground state, probably caused by changes in the torsional angle of the methylenic unit that links each chromophore to the backbone.[6] Extended MM2−force field model calculations and semiempirical single-point calculations suggested that, although the peptide backbone is rigid, the side chains do experience sufficient conformational mobility[4b] to restrict the precision of the interpretation of the observed difference in electron transfer rates.

To understand the relative importance of different conformations and the helix electric field, we have studied the effect of denaturation of **1** and **2** on the observed electron transfer rates. If the observed difference in rates is caused mainly by the helix dipole, the difference in electron transfer rates in denatured **1** and **2** should disappear, as the net dipole of a random coil peptide is zero.

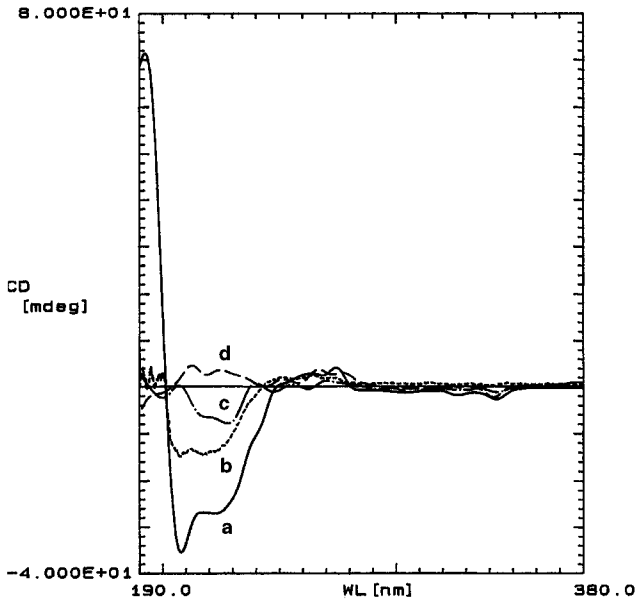


Figure 2. Circular dichroism spectra of **2** (1×10^{-4} M solutions in CH_3CN (a); MeOH (b); CH_3CN/H_2O (c); CF_3CH_2OH (d)).

We report here the effect of denaturation on photoinduced electron transfer between appended donor- and acceptor-substituted peptides, achieved by changing the external environment, such as solvents or salts, and by incorporation of proline into the oligopeptide sequence.[7] Thermal denaturation was not employed in order to avoid ambiguities caused by partial chemical decomposition.[8]

Results and Discussion

Denaturation of Peptides 1 and 2. Protic solvents and guanidinium salts often unfold peptides that exist as helices in organic solvents by promoting the formation of intermolecular hydrogen bonding, thus disrupting the intramolecular hydrogen bonding which leads to the helix. This effect is shown by the circular dichroism (CD) spectra of **2** in protic solvents, Figure 2. Whereas the CD spectrum of **2** in acetonitrile shows a strong positive band at 190 nm and two negative bands near 210 and 220 nm, which are characteristic of a right-handed helical conformation,[7,9] the spectra in MeOH, CH_3CN/H_2O (70/30 (v/v)), and CF_3CH_2OH provide evidence that the helical structure is partially retained in methanol and that water and 2,2,2-trifluoroethanol disrupt the helix. An analogous observation was made for peptide **1**. The CD spectrum of **1** in the presence of aqueous guanidinium carbonate, Figure 3, indicates a strong perturbation of the continuity of the helical structure, with possibly local helical subunits but not overall helicity (cf. Figure 6). However, it is unclear whether the major effect is exerted by guanidinium or by water.

Time-Resolved Fluorescence Studies of 1 and 2 in Protic Solvents. The electron transfer rates of unfolded **1** and **2** were studied by single photon counting. Pyrene fluorescence lifetimes in the denatured peptides, Table 1, were compared with the values obtained in solvents in which **1** and **2** are helical.

When guanidinum carbonate was added to THF solutions of **1** and **2**, the ratio between the electron transfer rates of **1** and **2**,

Lett. **1992**, *197*, 380. (e) Alegria, G.; Dutton, P. L. *Biochim. Biophys. Acta* **1991**, *234*, 1057 and 258. (f) Warshel, A.; Åqvist, J. *Annu. Rev. Biophys. Chem.* **1991**, *20*, 267. (g) Boxer, S. G.; Lockhart, D. J.; Franzen, S. In *Photochemical Energy Conversion*; Norris, J. R., Meisel, D., Eds.; Elsevier: Amsterdam, 1989; p 196. (h) Boxer, S. G.; Goldstein, R. A.; Franzen, S. In *Photoinduced Electron Transfer Part B. Experimental Techniques and Medium Effect*; Fox, M. A., Chanon, M., Eds.; Elsevier: Amsterdam, 1988; p 163. (i) Abraham, R. J.; Hudson, B. D.; Thomas, W. A.; Krohn, A. *J. Mol. Graph.* **1986**, *4*, 28.

(4) (a) Galoppini, E.; Fox, M. A. *J. Am. Chem. Soc.* **1996**, *118*, 2299. This reference describes the synthesis of **1** and **2** in Supporting Information. (b) Knorr, A.; Galoppini, E.; Fox, M. A. *J. Phys. Org. Chem.*, in press. The calculated value of 40 D is probably overstimated, since the calculation does not take into account a saturation effect that occurs with increasing number of residues.[1]

(5) Evidence of the formation of a radical ion pair was provided by observation of the pyrene radical anion in the transient absorption spectra of **1** and **2**.[4b]

(6) Short (5−15 amino acids) peptides can adopt rapidly interconverting conformations in solution, and biexponential decays were often observed in studies of electron transfer in peptides: (a) Pispisa, B.; Venanzi, M.; Palleschi, A.; Zanotti, G. *J. Phys. Chem.* **1996**, *100*, 6835. (b) Pispisa, B.; Venanzi, M.; Palleschi, A.; Zanotti, G. *Macromolecules* **1994**, *27*, 7800. (c) Inai, Y.; Sisido, M.; Imanishi, Y. *J. Phys. Chem.* **1991**, *95*, 3847. (d) Inai, Y.; Sisido, M.; Imanishi, Y. *J. Phys. Chem.* **1990**, *94*, 6237. (e) Basu, G.; Kubasik, M.; Anglos, D.; Secor, B.; Kuki, A. *J. Am. Chem. Soc.* **1990**, *112*, 9410.

(7) For a review about helix conformations, see: Goodman, M.; Verdini, A.; Choi, N. S; Masuda, Y. *Top. Stereochem.* **1970**, *5*, 69. Methods for denaturation of helical peptides are used or mentioned by Lockhart and Kim.[3c]

(8) We observed that **1** and **2** rather easily undergo chemical decomposition when heated or exposed to light and air for a few weeks.

(9) THF, the solvent used in some of the time-resolved fluorescence measurements, could not be used as the solvent for CD spectra because the UV cutoff of THF is 210 nm.

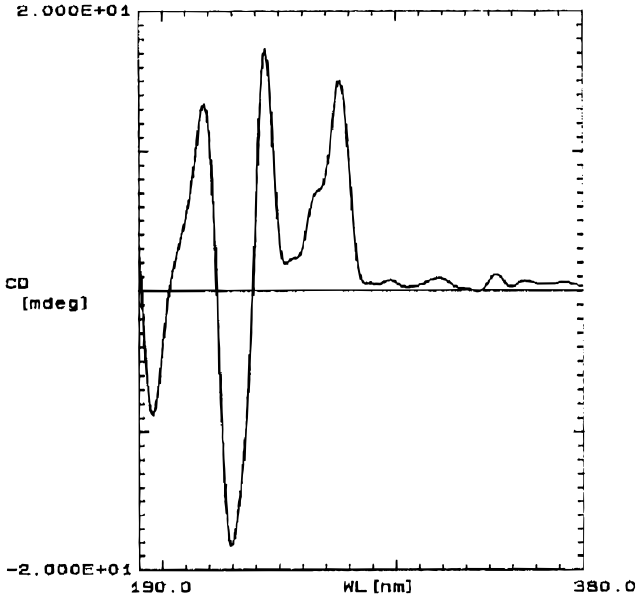
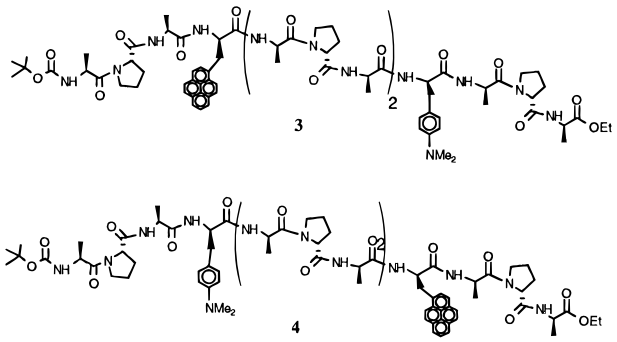


Figure 3. Circular dichroism spectrum of a 1×10^{-4} M solution of **1** in CH_3CN/H_2O/guanidinium carbonate.

k_1/k_2, decreased from 27 to 11 (Table 1, entries 1 and 2). Although guanidinium carbonate is not soluble in THF, it is likely that a small amount of salt slowly interacts by intramolecular hydrogen bonding with the helix, probably partially unfolding the peptides. When water was added to THF solutions of **1** and **2**, the ratio k_1/k_2 decreased to 1.8 (Table 1, entry 3). With and without guanidinium, k_1/k_2 approaches unity for aqueous solutions where helix−coil transition has taken place.[10] Finally, the electron transfer rates of **1** and **2** were identical in 2,2,2-trifluoroethanol, the solvent in which the peptides exist as random coils (Table 1, entry 6, and Figure 2).

From the data reported in Table 1 it appears that a decrease of k_1/k_2 is consistently observed when peptides are unfolded (protic solvents) from that observed when the same compounds exist as helices (nonprotic solvents). However, an electric field is smaller in high dielectric constant (ϵ) solvents, and the decrease of the effective k_1/k_2 upon addition of water to THF could be the result not only of the helix disruption but also of the increase in dielectric constant of the medium.[11] This effect cannot be responsible for the results observed in 2,2,2-trifluoroethanol: k_1/k_2 in acetonitrile ($\epsilon = 37$) is 7, and in 2,2,2-trifluoroethanol ($\epsilon = 27$) it is 1.2 (Table 1, entries 7 and 6). Since the peptides exist as α-helices in acetonitrile and as random coils in 2,2,2-trifluoroethanol, Figure 2, the identical electron transfer rates of **1** and **2** in 2,2,2-trifluoroethanol probably result from the cancellation of the helix electric field upon unfolding of the helix. Finally, it is important to emphasize that the observed complex multiexponential decays yield only approximations to microscopic rate constants.

Proline-Substituted Peptides. To study electron transfer rates in the absence of helical structure, but otherwise identical conditions, and possibly without using high dielectric constant solvents, we prepared oligopeptides **3** and **4**, which are



analogous to **1** and **2** except that L-proline has replaced Aib in the backbone.[12] In a peptide formed upon coupling proline with another amino acid, there are no amide protons on the proline residue which are available for intramolecular hydrogen bonding. Although oligoproline $(L\text{-Pro})_n$ may have a helical structure induced by the five-membered ring constraint,[7] the helical structure and the consequent electric field along the helix are perturbed in proline-containing peptides, because the intramolecular hydrogen bonding pattern is disrupted. Lockhart and Kim, for example, employed proline as the helix breaking residue to prepare nonhelical oligopeptides as controls in their studies of the helix electric field.[3c] Furthermore, the absorption spectra of these proline-containing oligopeptides did not exhibit the characteristic shift caused by the helical peptide electric field.

However, the prediction of precise secondary structure of proline-containing peptides is not simple because they can adopt a variety of conformations. The rotational restriction about the C−N−C bonds in the proline five-membered ring limits the rotation about the peptide bond. As a result, proline can adopt *cis* and *trans* conformations, which can be interconverted, Figure 4, with an energy barrier of about 16 kcal/mol.[13e] In $(L\text{-Pro})_n$ the *cis*−*trans* isomerization equilibrium is affected by the polarity of the solvent, pH, and ionic strength of the medium, the *trans* conformation being favored in hydrogen-bonding solvents, at low pH, or in the presence of salts such as $CaCl_2$. On the basis of CD[7,12c] and ^1H and ^{13}C NMR[13] spectra of the two conformers of oligoproline in water, MeOH, and DMSO, it is thought to be generally true that the *all-cis* form of $(L\text{-Pro})^n$ is a right-handed helix and the *all-trans* form of $(L\text{-Pro})^n$ is a left-handed, more extended helix.[7,12,13]

(10) The k_1/k_2 ratio did not change appreciably when the measurements were repeated on the same samples on sequential days (Table 1, entries 4 and 5), implying that the unfolding process had reached equilibrium by the time the measurements were made.

(11) Lockhart and Kim found that the effective value of the dielectric constant at the boundary of an oligopeptide was lower than the dielectric constant of the solvent, and the experimentally measured field was 1 order of magnitude stronger than expected on the basis of the ϵ of the bulk solvent.[3c] For calculations of ϵ in proteins, see also: (a) King, G; Lee, F. S.; Warshel, A. *J. Chem. Phys.* **1991**, *95*, 4366. (b) Wipff, G.; Dearing, A.; Weiner, P. K.; Blaney, J. M.; Kollman, P. A. *J. Am. Chem. Soc.* **1983**, *105*, 997. (c) Blaney, J. M.; Weiner, P. K.; Dearing, A.; Kollman, P. A.; Jorgensen, E. C.; Oatley, S. J.; Burridge, J. M.; Blake, C. C. F. *J. Am. Chem. Soc.* **1982**, *104*, 6424.

(12) Because of the conformational restraint of the five-membered ring, *all-cis*- or *all-trans*-$(L\text{-Pro})_n$ peptides are more rigid than an α-helical peptide, and are valuable rigid spacers. Photoinduced electron transfer across oligoproline peptides ($0 < n < 4$) has been extensively studied by Isied and co-workers. Their models are structurally very different from **3** and **4**, as the donor and acceptor (inorganic complexes) are linked at the two ends of the peptide backbones, which contain only proline units (nonalternating), and the experimental conditions (low pH, water) were selected to favor the *trans* conformation. Therefore, a direct comparison of the conformation with **3** and **4** with elegant earlier work by the Isied group is not possible. (a) Vassilian, A.; Wishart, J. F.; van Hemelryck, B.; Schwarz, H.; Isied, S. S. *J. Am. Chem. Soc.* **1990**, *112*, 7278. (b) Isied, S. S.; Vassilian, A.; Magnuson, R. H.; Schwarz, H. *J. Am. Chem. Soc.* **1985**, *107*, 7432. (c) Isied, S. S.; Vassilian, A. *J. Am. Chem. Soc.* **1984**, *106*, 1732 and references therein for energy-transfer studies across oligoproline.

(13) For ^1H and ^{13}C NMR studies of the *cis*−*trans* equilibrium in oligoproline- and proline-substituted peptides, see: (a) Chao, Y.-Y. H.; Bersohn, R. *Biopolymers* **1978**, *17*, 2761. (b) *Idem. Biopolymers* **1977**, *16*, 277. (c) Grathwohl, C.; Wüthrich, K. *Biopolymers* **1976**, *15*, 2025. (d) *Ibid.* p 2043. For molecular mechanics calculations of preferred conformations of $(L\text{-Pro-X})_n$ peptides, see: (a) McDonald, D. Q.; Still, W. C. *J. Org. Chem.* **1996**, *61*, 1385. (b) Oka, M.; Nakajima, A. *Polym. Bull.* **1994**, *33*, 693.

Table 1. Time-Resolved Fluorescence of **1** and **2**[a,b]

solvent	**1** τ (ns) (A)[c]	**2** τ (ns) (A)[c]	k_{et1}[d] ($\times 10^{-8}$ s^{-1})	k_{et2}[d] ($\times 10^{-8}$ s^{-1})	k_{et1}/k_{et2}
THF	0.7 (42), 2.5 (58)	33 (88), 81 (11)	6.2	0.2	27
THF[e]	1.5 (62), 3.7 (34), 59 (4)	39 (93), 117 (7)	2.2	0.2	11
THF/H_2O	2.5 (53), 8.6 (40), 67 (7)	19 (92), 56 (8)	1.0	0.5	1.8
THF/H_2O[e]	8 (38), 2.7 (54), 70 (6)	17 (85), 46 (15)	1.1	0.4	2.4
THF/H_2O[e,f]	3.7 (61), 14 (31), 97 (8)	19 (90), 80 (10)	0.6	0.3	1.8
CF_3CH_2OH	16 (83), 46 (9), 162 (8)	17 (85), 46 (15)	0.3	0.2	1.2
CH_3CN	0.9 (67), 27 (33)	9 (97), 96 (2)	7.1	0.9	8

[a] The time-resolved fluorescence experiments were performed on a single-photon counting apparatus, with a 20 ps time resolution, $\lambda_{exc} = 344$ nm, $\lambda_{obs} = 400$ nm. $T = 20-22$ °C. [b] Concentrations were low ($\cong$30 μM, od(355) < 0.1) in order to avoid self-aggregation. Samples were degassed by bubbling Ar for 20 min before and during the measurements. [c] The numbers in parentheses indicate the relative weighting, A = amplitude × 100, of the component. [d] Calculated from $k_i = 1/\langle\tau\rangle_i - 1/\tau_o$ where $\langle\tau\rangle_i = \tau'(A') + \tau''(A'')$, where A' and A'' are the amplitude of each component and $\tau_o = 300$ ns (time decay of a control oligopeptide substituted only with pyrene). [e] Guanidinium carbonate (about 4 g/mL) added. [f] Measurement repeated on sample of entry 4 after 1 day.

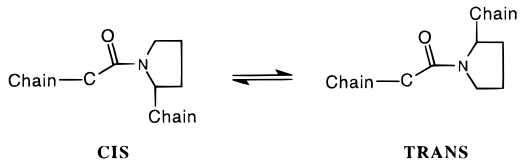


Figure 4. *cis*−*trans* isomerization of proline-substituted peptides.

The conformation of (L-Pro-X)$_n$ peptides, where X = Ala or a non-proline peptide, is more complicated because the equilibrium is dependent also on the nature of X and on the several possible hydrogen bonding patterns between the carbonyl group of Pro and the NH of X.[13e] Therefore, in addition to *cis* and *trans* isomerism, the internal hydrogen bonding pattern in these mixed polypeptides generates β and/or γ turns, and the number of possible conformations is higher than in the parent (L-Pro)$_n$ peptides. In most organic solvents, proline-substituted peptides exist as a mixture of conformers and are nonhelical.

Oligopeptides **3** and **4** were prepared following the same standard solution-phase coupling procedure employed for the synthesis of **1** and **2**.[4a,14] Both oligopeptides were much more polar on silica gel and less soluble in organic solvents than **1** and **2**, probably because proline-rich peptides are more rigid and extended than alternating Ala-Aib oligomers. The ^{1}H and ^{13}C NMR spectra in $CDCl_3$ of **3** and **4**, as well as the spectra of some of the intermediates, show the presence of more than one conformer.[15] The *cis*−*trans* distribution in **3** and **4** depends on the pH and solvent polarity, complicating the interpretation of the time-resolved fluorescence measurements. However, from literature data of (L-Pro-L-Ala)$_n$ peptides and by analysis of their CD spectra (*vide infra*), it appears that the *trans* conformation of the Pro-Ala bonds is the dominant conformation in the conditions used for the data reported in Table 2.[13c,d]

In the ^{1}H NMR spectrum of **4**, one of the aniline doublets is shifted downfield by 0.2 ppm from the corresponding doublet in **3**, Figure 5, indicating a different average environment for this group in $CDCl_3$.[16]

Circular Dichroism Spectra of 3 and 4. An analysis of the CD spectra of **3** and **4** in CH_3CN shows that **3** and **4** have a low helical content, as evidenced by the single minimum at approximately 230 nm, Figure 6. The CD spectra of **3** and **4** in EtOH and CH_3CN, with negative bands at $\lambda = 210$ nm and weak positive bands at 230 nm, are similar in shape to the previously reported CD spectra of *all-trans*-polyproline and are very different from the CD spectra of *all-cis*-polyproline, which has a strong negative band at $\lambda = 190$ nm and a strong positive band at $\lambda = 220$ nm.[12a]

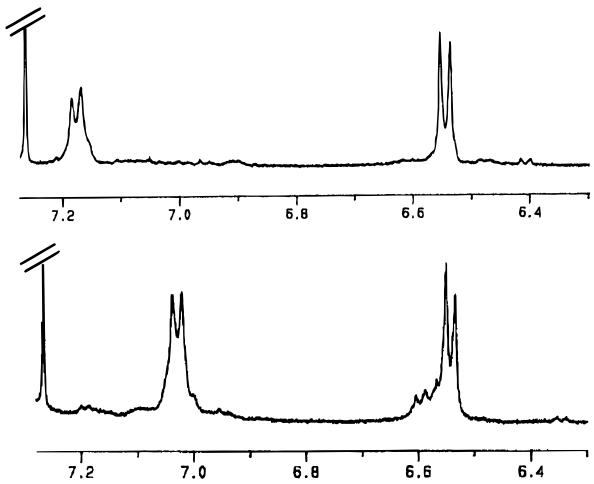


Figure 5. ^{1}H NMR spectra in $CDCl_3$ of **3** (top) and **4** (bottom) in the aromatic region.

Protic solvents also influence the secondary structure of **4**, as shown by the CD spectra in CH_3CN, EtOH, MeOH, CH_3CN/H_2O (70/30 (v/v)), and CF_3CH_2OH, Figure 7.[9] As in **1** and **2**, peptides **3** and **4** are completely unfolded in the presence of water or in CF_3CH_2OH. Although **1** and **2** are denatured in protic solvents because of disruption of intramolecular hydrogen bonding, the changes in the secondary structure established by the CD spectra in protic solvents of **3** and **4** may instead reflect changes in the *cis*−*trans* equilibrium and/or denaturation caused by interaction of the solvent with the intramolecular hydrogen bonds[13e] between the proline carbonyl and the alanine NH groups.

Steady-State Fluorescence Spectra of 3 and 4. The fluorescence spectra of **3** and **4** in THF, CH_2Cl_2, MeOH, EtOH, and CH_3CN exhibit dramatic solvent dependence, Figure 8. In nonpolar solvents, like THF and CH_2Cl_2, a broad structureless fluorescence emission is observed at $\lambda_{max} = 500$ nm for **4** and at $\lambda_{max} = 520$ nm for **3**. This emission has a lower intensity or is absent in polar or protic solvents such as CH_3CN, EtOH, and MeOH, Figure 5. In all solvents studied, the emission intensity of **4** was higher than that of **3**. In both **3** and **4**, substantial quenching of the pyrene singlet by the *N*,*N*-dimethylanilino group can be observed ($\Phi_{f3} = 0.13$, $\Phi_{f4} =$

(14) For general coupling procedures, see: Bodanszky, M. *The Practice of Peptide Synthesis*; Springer-Verlag: Berlin, 1994.

(15) The ^{1}H and ^{13}C NMR spectra in $CDCl_3$ of peptides **3** and **4** and of their precursors are a mixture of at least two isomers, one present in a smaller amount (8−25%). In the Experimental Section, we report the resonances of the major component, but we were not able to unambiguously assign each peak to a *cis* or *trans* conformation. NMR spectra of *Z*-Ala-Pro-Ala-OMe in CD_3OD, D_2O, and DMSO showed that in these solvents the Pro-Ala bonds are mostly *trans*, with 11−13% of *cis*.[13c]

(16) No difference in the chemical shift of the chromophore protons was observed between the spectra of **1** and **2**.

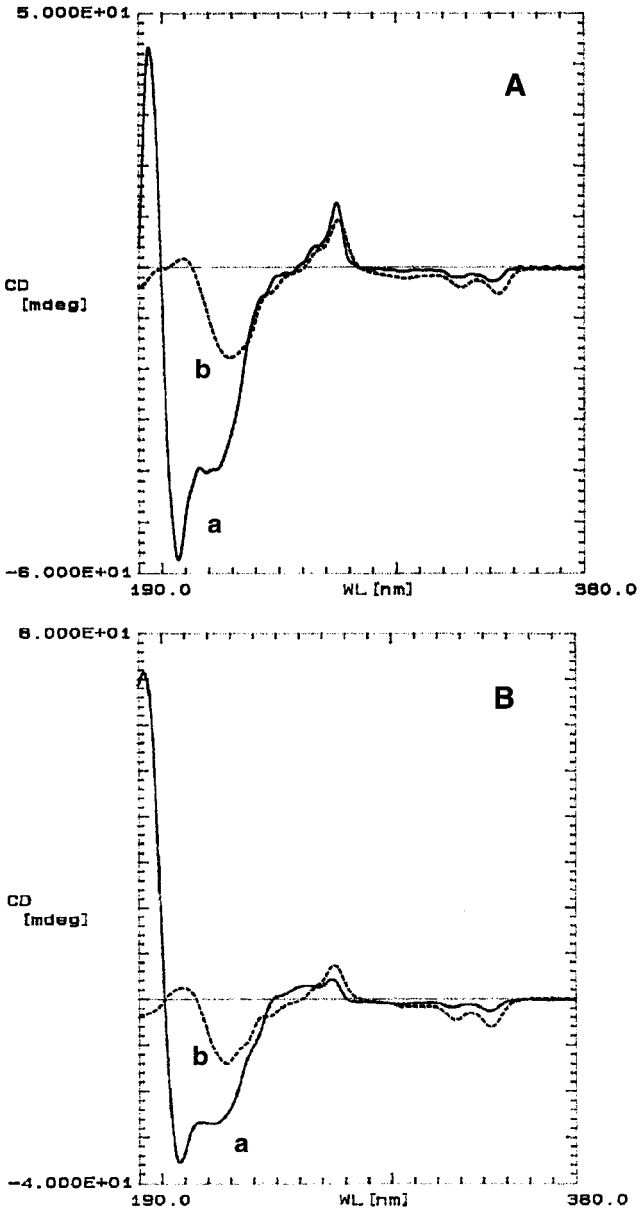


Figure 6. (A) Circular dichroism spectra of 1×10^{-4} M solutions of **1** (a) and **3** (b) in CH_3CN. (B) CD spectra of 1×10^{-4} M solutions of **2** (a) and **4** (b) in CH_3CN.

0.061, in CH_3CN, referenced to pyrene in ethanol, $\Phi_{fpyr} = 0.7$).[17] The fluorescence quantum yield of **3** was twice that of **4**, indicating more efficient quenching in **4**.

To establish whether the 500 nm band is an exciplex or excimer emission, and whether it is formed intramolecularly or intermolecularly (peptides **3** and **4** are sparingly soluble in nonpolar solvents and may aggregate), we studied the effect of dilution on the fluorescence spectra of **4** in THF and CH_2Cl_2, Figure 9. In THF, the emission intensity did not decrease with increasing dilution beyond that expected for Beer's law absorption changes. In CH_2Cl_2, the emission of a more dilute sample was stronger than that of a more concentrated sample, and then decreased upon further dilution. These observations suggest that the 500 nm emission is probably an intramolecular exciplex, and that the distance between the chromophores is regulated not only by the number of Ala-Pro residues that separate them

(17) The fluorescence quantum yields of **1** and **2** were calculated from the formula reported by Eaton, D. F. *Pure Appl. Chem.* **1988**, *60*, 1107. The pyrene fluorescence quantum yield is reported by Birks, J. B. *Photophysics of Aromatic Molecules*; Wiley & Sons: New York, 1970; p 252.

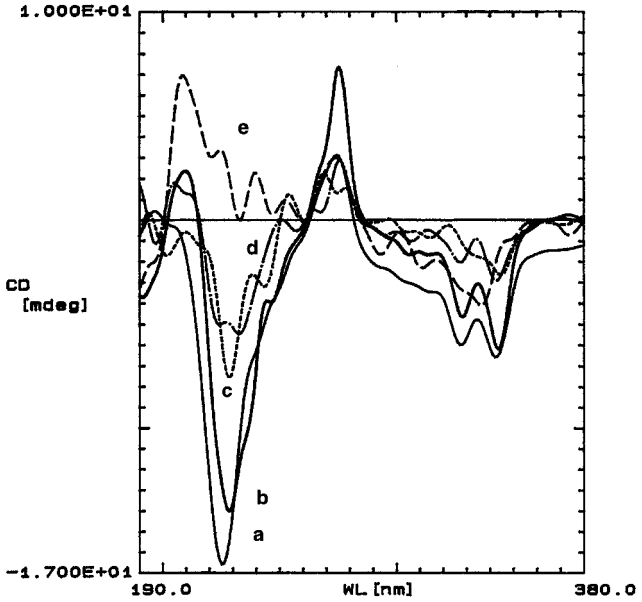


Figure 7. Circular dichroism spectra of 1×10^{-4} M solutions of **4** in EtOH (a), CH_3CN (b), MeOH (c), CH_3CN/H_2O (d), and CF_3CH_2OH (e).

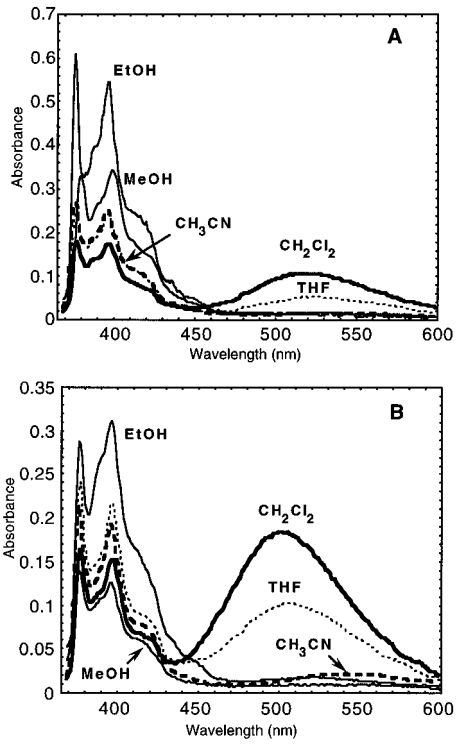


Figure 8. Steady-state fluorescence spectra of **3** (A) and **4** (B) in CH_2Cl_2, THF, EtOH, MeOH, and CH_3CN ($\lambda_{exc} = 355$ nm, 1×10^{-4} M). The λ_{max} of the exciplex emission is 520 nm in **3** and 500 nm in **4**.

but also by the backbone conformations. In nonprotic and nonpolar solvents, the Pro-Ala bonds adopt preferentially a *cis* conformation[12,13] and the intramolecular hydrogen bonds are not disrupted; hence, the peptide backbone is less extended and the chromophores are closer in space, permitting the formation of an intramolecular exciplex.[18]

Furthermore, the consistent observation of a more intense exciplex emission in **4** than in **3**, a 10 nm red shift of the

(18) From the data available, it is not possible to know whether both compounds have identical conformations in more polar solvents in which the peptide backbone is more extended and the exciplex emission is not observed.

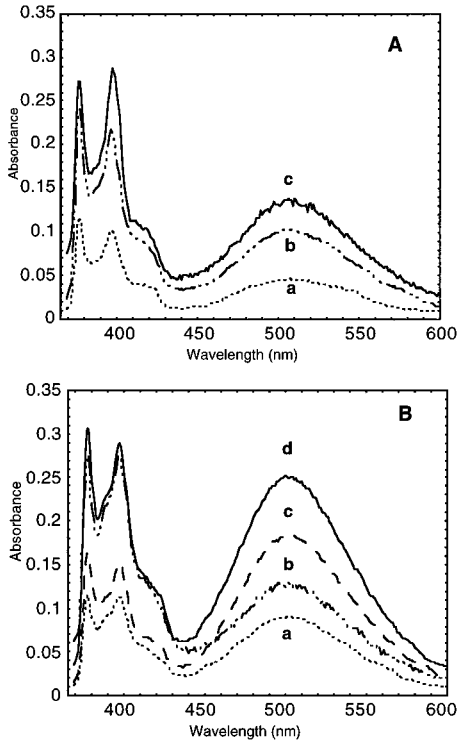


Figure 9. Steady-state fluorescence spectra of **4** in CH_2Cl_2 (λ_{exc} = 355 nm, 1×10^{-4} M) (od(355) = 0.061 (a); 0.014, gain = 10 (b); 0.099 (c); 0.177 (d)). In THF od(355) = 0.056 (a); 0.124 (b); 0.012, gain = 10 (c).

emission in **3** and the chemical shift differences between the ^{1}H NMR spectra of **3** and **4** all suggest that the two peptides populate different average conformations in solutions.

Time-Resolved Fluorescence Measurements. The electron transfer rates[19] of **3** and **4** were measured only in polar solvents in which the exciplex fluorescence is either not observed or has a low intensity: CH_3CN, EtOH, MeOH, THF/H_2O (70/30 (v/v)), and CF_3CH_2OH, Table 2.[20]

In both **3** and **4**, the observed fluorescence decays were biexponential and the electron transfer rates were about 1 order of magnitude slower than in **1** and **2**. The slower rates are probably the result of the more extended structure adopted by the proline-rich backbone of **3** and **4** in polar solvents, the distance between chromophores being greater than the distance between chromophores in α-helical **1** and **2** (about 10 Å). In THF/H_2O and CF_3CH_2OH, where the peptides exist as a random coil, the electron transfer rates of **3** and **4** were identical (Table 2, entries 5 and 6, cf. Figure 7).

In both MeOH and EtOH, the rates of electron transfer in **4** are faster than in **3** by about a factor of 2 (Table 2, entries 2−4). However, the CD spectra of **3** and **4** in ethanol show that both peptides have a similar residual secondary structure: in methanol the peptides are completely unfolded, implying that the small difference between k_3 and k_4 in that solvent probably has a conformational origin. In CH_3CN, the k_4/k_3 ratio is about 3, and the CD spectra show that in this solvent both peptides have a residual secondary structure.

The observed rate difference in CH_3CN is in the direction opposite to that anticipated from an intramolecular dipole effect. That is, faster rates are observed in **4**, in which charge separation takes place parallel to the helix field. However, the fluorescence, CD, and NMR spectra have provided evidence that the backbones in **3** and **4** are conformationally mobile and have a low helical content. Since the peptides populate different backbone conformations, it is likely that any residual difference in electron transfer rates between **3** and **4** can be attributed to a conformational effect rather than an electric field effect.

Experimental Section

Materials. Anhydrous *N*,*N*-dimethylformamide (DMF) and 1,4-dioxane were used as received (Aldrich). *N*-Methylmorpholine (NMM) was dried overnight over 4 Å molecular sieves, fractionally distilled over KOH under Ar, and stored in the dark under Ar. Ethanol was distilled from $Mg(OEt)_2$. Solvents for spectroscopic studies were either distilled or spectral grade and used as received. Commercially available 1-hydroxybenzotriazole trihydrate (HOBT), 1-(3-(dimethylamino)-propyl)-3-ethylcarbodiimide hydrochloride (EDCI), (*tert*-butoxycarbonyl)-L-alanine (Boc-Ala-OH), L-alanine ethyl ester hydrochloride, and L-proline methyl ester hydrochloride (HCl·H-Ala-OEt) were used without further purification. (L)-Boc-(p-(*N*,*N*-dimethylamino)phenyl)-alanine and (L)-pyrenyl-1-alanine methyl ester were prepared according to a previously reported procedure.[4a] Column chromatography was performed on Merck silica gel 60 (400/600 mesh). Thin-layer chromatography was carried out on 0.25 mm Polygram silica gel plates using 10% ethanolic phosphomolybdic acid, 1% ethanolic ninhydrin, and/or UV light and heat as developing agents. Protected unsubstituted oligopeptides were detected by using a previously described developing reagent.[22]

Instrumentation and Methods. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125 MHz, respectively, or at 300 and 75 MHz, respectively, on a General Electric GN-500 or on a Varian Unity Plus spectometer. The solvent was $CDCl_3$. Proton spectra were referenced to TMS, and carbon spectra were referenced to the solvent. Coupling constants are reported in hertz, with a precision of 0.1 Hz. MS and HRMS spectra were obtained on a VG2AB2-E mass spectrometer (VG analytical LDT).

Circular dichroism measurements (Figures 2, 3, 6, and 7) were performed on a Jasco J600 spectropolarimeter with a 1 cm quartz cell. The solutions had an optical density <0.1 at 355 nm, corresponding to 30 μM concentration. The steady-state fluorescence spectra reported in Figures 8 and 9 were recorded on a SLM-Aminco 500 C spectrofluorometer, λ_{exc} = 355 nm, with solutions of 0.1 AU (about 30 μM).

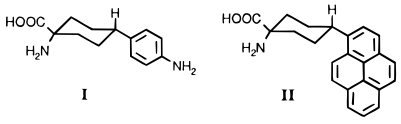
Single-photon counting measurements (Tables 1 and 2) were conducted at the Center for Fast Kinetics Research (CFKR) at the University of Texas at Austin on a single-photon counting apparatus.[23]

Peptide Syntheses. A. General Procedure for Peptide Coupling.[4a] All reactions were performed under Ar in dried glassware. In a typical procedure, NMM (2.8 mL) was added to a solution of the Boc-protected amino acid (15 mmol) in DMF (30 mL) cooled to −15 °C (ice/acetone bath). The methyl or ethyl ester of the neutral amino acid or the

(19) The transient absorption spectra of **3** and **4** show the presence of pyrene radical anion at 500 nm, in direct analogy to that observed in the transient absorption spectra of **1** and **2**.

(20) A possible way to simplify the operative conformational equilibrium was to use the experimental conditions in which $(Pro)_n$ peptides are in an *all-trans* conformation. However, these conditions (acidic aqueous solution) could not be employed, because at low pH the *N*,*N*-dimethylanilino group would be protonated and could no longer act as an electron donor.

(21) We attempted the synthesis of oligopeptides analogous to **1** and **2**, but substituted with the conformationally restricted amino acids **I** and **II**. (We thank Dr. Mark Minton for assistance in the preparation of these compounds.)



Unfortunately, we were unsuccessful in incorporating **I** or **II** into an Ala-Aib backbone, because of solubility problems and/or the difficulty of coupling bulky α,α-disubstituted amino acids. The latter is a problem which is well documented in the literature: Kaminski, Z.; Leplawy, M. T.; Olma, A.; Redlinski, A. In *Peptides 1980, Proceedings of the European Peptide Symposium*; Brunfeldt, K., Ed.; Scriptor: Copenhagen, 1981; Vol. 16, p 201.

(22) Von Arx, E.; Faupel, M.; Brugger, M. *J. Chromatogr.* **1976**, *120*, 224.

(23) O'Connor, D. V.; Phillips, D. *Time Correlated Single Photon Counting*; Academic Press: New York, 1984; Chapter 4.

Table 2. Time-Resolved Fluorescence of **3** and **4**[a]

solvent	**4** τ (ns) (weight)[c]	**3** τ (ns) (weight)[c]	k_{et4}[b] ($\times 10^{-7}$ s^{-1})	k_{et3}[b] ($\times 10^{-7}$ s^{-1})	k_{et4}/k_{et3}
CH_3CN[d]	21 (84), 140 (16)	53 (70), 220 (30)	2.1	0.6	3.4
MeOH[d]	30 (77), 110 (23)	34 (79), 230 (21)	1.7	1.0	1.8
MeOH[e]	29 (65), 96 (35)	35 (72), 200 (28)	1.6	0.9	1.8
EtOH[e]	28 (39), 100 (61)	26 (42), 170 (58)	1.0	0.6	1.8
H_2O/THF[d,f]	36 (45), 99 (55)	54 (80), 244 (20)	1.0	7.5	1.3
CF_3CH_2OH[d]	55 (38), 182 (62)	71 (59), 235 (41)	0.4	0.4	1.0

[a] The time-resolved fluorescence experiments were performed on a single-photon counting apparatus, with a 20 ps time resolution, $\lambda_{exc} = 344$ nm, $\lambda_{obs} = 400$ nm. $T = 22$ °C. Concentrations were low ($\cong$30 μM, od(355) < 0.1) in order to avoid self-aggregation. [b] Calculated from $k_i = 1/\langle\tau\rangle_i - 1/\tau_o$ where $\langle\tau\rangle_i = \tau'(A') + \tau''(A'')$, where A' and A'' are the amplitude of each component and $\tau_o = 300$ ns (time decay of a $(Ala\text{-}Aib)_3$ monosubstituted with pyrene). [c] The numbers in parentheses indicate the relative weighting, A = amplitude $\times$ 100, of the component. [d] Samples degassed by bubbling Ar for about 20 min before and during the measurements. [e] Samples degassed by six freeze−pump−thaw cycles. [f] Guanidinium carbonate (about 4 mg/mL) added.

hydrochloric salt (15 mmol) was added, followed by HOBT (17 mmol) and EDCI (17 mmol). The solution was stirred for 24 h, during which time it reached room temperature. It was then poured into ethyl acetate (100 mL) and water (50 mL). The organic layer was washed with 1 N HCl(aq) (3 × 50 mL), brine, 1 M $KHCO_3$(aq) (2 × 50 mL), and brine and then dried over Na_2SO_4 and concentrated *in vacuo*. The oil obtained was purified by column chromatography. All reactions and column chromatographies involving chromophore-substituted substrates were performed in the dark, protecting the flask and the column from light with aluminum foil. The peptides were stored at −10 °C in the dark, in vials purged with Ar.

B. General Procedure for Ester Hydrolysis. In a typical procedure, an aqueous solution of NaOH (8−10 mmol, in 4 mL of H_2O) was added to a solution of the peptide methyl or ethyl ester (7.3 mmol) in ethanol (80 mL). The solution was stirred for 6 h at room temperature, during which time the hydrolysis was complete (TLC). The solution was then acidified to pH 3−4 with 5% $NaHSO_4$(aq), the solvent was evaporated, and the residue was dissolved in ethyl acetate. The organic layer was washed with brine twice, and the solvent removed *in vacuo*. The white solid obtained was dried *in vacuo* overnight to afford the acid as a white solid (typically 90%), which was used in the following step without further purification, after checking the completion of reaction and purity by ^{1}H NMR. All reactions involving chromophore-substituted substrates were performed in the dark.

Boc-Ala-Pro-OMe was prepared following the general coupling procedure from Boc-Ala-OH (2.8 g, 15 mmol), HCl·H-Pro-OMe (2.5 g, 15 mmol), HOBT (2.3 g, 17 mmol), and EDCI (3.2 g, 17 mmol) in DMF (30 mL). Purification by column chromatography (40% hexane/60% ethyl acetate, v/v) afforded Boc-Aib-Ala-OMe as a colorless oil (3.8 g, 85%): ^{1}H NMR δ 5.35 (br d, 1H, NH, $J = 7.2$), 4.50−4.55 (dd, 1H), 4.40−4.50 (m, 1H), 3.72 (s, 1H, OCH_3), 3.65 (m, 1 H), 3.55−3.63 (m, 1 H), 1.8−2.3 (m, 1 H), 1.0−2.1 (m, 3 H), 1.42 (s, 9H, C(CH_3)$_3$), 1.35 (d, 3H, $J = 7.1$, CHCH_3) ppm; ^{13}C NMR δ 172.44, 171.77 ((CH$_3$)$_3$*C*(=O)−O−), 155.26 (*C*(=O)OMe), 79.57 (O−*C*(CH$_3$)$_3$), 58.72, 52.24, 47.75, 46.76, 28.93, 28.38 (3C, C(*C*H$_3$)$_3$), 24.92, 18.31 ppm; CI-MS *m*/*z* 302 (20), 301 (100), 287 (4), 245 (48), 227 (6), 201 (8), 175 (10); HRMS calcd for $C_{14}H_{25}N_2O_5$ 301.1763, found 301.1760.

Boc-Ala-Pro-OH was prepared following the general procedure for ester hydrolysis from NaOH (360 mg, in 4 mL of H_2O) and Boc-Ala-Pro-OMe (2.2 g, 7.3 mmol) in ethanol (80 mL). The solution was stirred for 6 h at room temperature. Boc-Ala-Pro-OH was obtained as a white solid which was used in the following step without further purification.

Boc-Ala-Pro-Ala-OEt was prepared following the general coupling procedure from HCl·H-Ala-OEt (2.7 g, 18 mmol), Boc-Ala-Pro-OH (15 mmol), HOBT (2.5 g, 18 mmol), EDCI (3.5 g, 18 mmol), and NMM (3.5 mL) in DMF (50 mL). Purification by column chromatography (80% ethyl acetate/20% hexane, v/v) and crystallization from ethyl acetate/hexane afforded Boc-Ala-Pro-Ala-OEt as white crystals (5.0 g, 86%): the ^{1}H NMR spectrum shows about 10% of another isomer (not reported); ^{1}H NMR δ 7.1 (br d, 1H, N*H*, $J = 7.2$), 6.6 (s, 1H, N*H*), 5.37 (br d, 1H, N*H*, $J = 8.0$), 4.6 (m, 1H), 4.4−4.55 (m, 2H), 4.19 (q, 2H, $J = 7.2$, OCH_2CH$_3$), 3.7 (m, 1H), 3.63−3.55 (m, 1 H), 3.38 (t, 1H, $J = 7.1$), 2.37 (t, 1H, $J = 5.0$), 2.3 (m, 1 H), 2.1 (m, 1 H), 2.05−1.90 (m, 1 H), 1.44 (s, 9H, C(CH_3)$_2$), 1.39 (d, 3H, $J = 7.3$, CHCH_3), 1.35 (d, 3H, $J = 7.3$, CHCH_3), 1.27 (t, 3H, $J = 7.2$, OCH$_2$CH_3) ppm; ^{13}C NMR δ 173.03, 172.74, 170.55 ((CH$_3$)$_3$*C*(=O)−O−), 155.20 (−*C*(=O)OEt), 79.66 (O−*C*(CH$_3$)$_3$), 61.39, 59.74, 49.43, 48.21, 47.76, 47.18, 28.29 (3C, C(*C*H$_3$)$_3$), 27.41, 25.08, 18.62, 14.12; CI-MS *m*/*z* 386 (50), 330 (20), 199 (70), 160 (7), 148 (7), 140 (14), 128 (17), 114 (17); HRMS calcd for $C_{18}H_{32}N_3O_6$ 386.2291, found 386.2282.

Boc-Ala-Pro-Ala-OH was prepared following the general procedure for ester hydrolysis from Boc-Ala-Pro-Ala-OEt (0.8 g, 2.2 mmol) and NaOH (90 mg in 15 mL of H_2O) in ethanol (20 mL). The solution was stirred for 4 h at room temperature. Boc-Ala-Pro-Ala-OH was a white solid which was used in the next reaction without further purification.

HCl·H-Ala-Pro-Ala-OEt. Boc-Ala-Pro-Ala-OEt (0.92 g, 2.3 mmol) was added to 100 mL of ice-cold dioxane in which HCl had been bubbled for 10−15 min. The ice bath was removed, and the solution was stirred for 3 h, during which time the reaction was complete (^{1}H NMR). The solvent was evaporated, and the white solid obtained was triturated with ether and dried *in vacuo* overnight to yield Boc-Ala-Pro-Ala-OEt as a white, hygroscopic solid (1.6 g, 100%).

Boc-dmaPhe-Ala-Pro-Ala-OEt was prepared following the general coupling procedure from HCl·H-Ala-Pro-Ala-OEt (2.3 mmol), Boc-dmaPhe-OH[24] (710 mg, 2.3 mmol), HOBT (320 mg, 2.5 mmol), EDCI (480 mg, 2.5 mmol), NMM (2 mL), and DMF (20 mL). Purification by column chromatography (ethyl acetate) and trituration with ether afforded **6** as an off-white solid (1.1 g, 86%): the ^{1}H NMR spectrum shows about 8% of another isomer (not reported); ^{1}H NMR δ 7.1−7.2 (2 br s, 3 H, N*H*), 7.11 (d, 2H, $J = 5.0$, phenyl), 6.68 (d, 2H, $J = 5.1$, phenyl), 4.95 (br s, 1H, N*H*), 4.73 (m, 1H), 4.50−4.55 (m, 1H), 4.47 (m, 1H), 4.4 (br s, 1H), 4.17 (q, 2H, $J = 7.1$, OCH_2CH$_3$), 3.61 (m, 1H), 3.55−3.60 (m, 1 H), 2.8−3.0 (m, 2H), 2.90 (s, 6H, −N(CH_3)$_2$), 2.25 (br m, 1H), 2.1 (br m, 1 H), 2.05−1.90 (br m, 1 H), 1.39 (s, 9H, C(CH_3)$_2$), 1.35 (d, 3H, $J = 2.5$, CHCH_3), 1.33 (d, 3H, $J = 2.1$, CHCH_3), 1.26 (t, 3H, $J = 7.1$, OCH$_2$CH_3) ppm; ^{13}C NMR δ 172.74, 171.91, 170.91, 170.62 ((CH$_3$)$_3$*C*(=O)−O−), 155.20 (−*C*(=O)−OEt), 149.71 (phenyl), 130.28, 130.01 (2C, phenyl), 124.11 (phenyl), 112.89 (2C, phenyl), 80.03 (O−*C*(CH$_3$)$_3$), 61.34, 59.79, 55.54, 48.10, 47.29, 46.71, 40.71 (2C, −N(*C*H$_3$)$_2$), 37.65, 28.30 (3C, C(*C*H$_3$)$_3$), 27.72, 25.07, 18.40, 18.13, 14.13 ppm; CI-MS *m*/*z* 576 (100), 562 (4), 520 (8), 503 (3), 330 (11), 286 (6); HRMS calcd for $C_{29}H_{46}N_5O_7$ 576.3397, found 576.3386.

H-dmaPhe-Ala-Pro-Ala-OEt. The deprotected tetrapeptide was prepared following a literature procedure for the removal of Boc group in aryl-substituted peptides.[25] Me_3SiI (0.3 mL, 2.1 mmol) was added at room temperature in a glovebag under nitrogen to a solution of Boc-dmaPhe-Ala-Pro-Ala-OEt (960 g, 1.6 mmol) in dry CH_3CN (10 mL). The reaction flask was protected from light. The pale yellow solution was stirred for 10 min, and dry MeOH (0.5 mL) was then added. The solvent was evaporated *in vacuo*, and the oil residue was dissolved in 10% aqueous acetic acid (20 mL). The aqueous layer was washed with ether (3 × 10 mL) and evaporated *in vacuo* (*T*(bath) = 40−50 °C). The white solid residue was triturated with ether and dried *in*

(24) The synthesis of Boc-dmaPhe-OH is described by Bergel, F.; Stock, J. A. *J. Chem. Soc.* **1959**, 90.

(25) Lott, R. S.; Chauhan, V. S.; Stammer, C. H. *J. Chem. Soc., Chem. Commun.* **1979**, 495.

vacuo overnight (T(bath) = 40 °C) to afford a pale yellow solid (690 mg, 91%) which was used in the next step without further purification.

Boc-Ala-Pro-Ala-dmaPhe-Ala-Pro-Ala-OEt was prepared following the general coupling procedure using Boc-Ala-Pro-Ala-OH (2 mmol), H-dmaPhe-Ala-Pro-Ala-OEt (1.6 mmol), HOBT (270 mg, 2 mmol), EDCI (385 mg, 2 mmol), and NMM (0.5 mL) in DMF (30 mL). Purification by column chromatography (90% ethyl acetate/10% MeOH, v/v) and trituration with ether afforded the product as an off-white solid (780 mg, 86%), which was further purified with a second column chromatography using the same solvent mixture to afford a white solid: the ^{1}H NMR spectrum shows about 10% of another isomer (not reported); ^{1}H NMR δ 7.6 (br s, 1H, N*H*), 7.34 (d, 1H, J = 7.1, N*H*), 7.19 (d, 1H, J = 7.1, N*H*), 6.95 (d, 2H, phenyl, J = 8.5, and 1H, N*H*), 6.58 (d, 2H, J = 8.7, phenyl), 5.45 (br s, 1H, N*H*), 4.8 (m, 3H), 4.3−4.6 (m, 3 H), 4.16 (q, 2H, J = 6.0, OCH_2CH$_3$), 3.5−3.8 (m, 1H), 2.7−3.0 (m, 3 H), 2.8−3.0 (m, 2H), 2.95 (s, 6H, −N(CH_3)$_2$), 1.9−2.2 (series of br m, 10 H), 1.44 (s, 9H, C(CH_3)$_2$), 1.4−1.2 (m, 15 H, CHCH_3 and OCH$_2$CH_3) ppm; ^{13}C NMR δ 173.11, 172.78, 172.81, 171.87, 171.78, 171.26, 171.05, 170.68, 155.43 (−*C*(═O)−OEt), 149.59 (phenyl), 130.28 (2C, phenyl), 124.54 (phenyl), 112.92 (2C, phenyl), 79.05 (O−*C*(CH$_3$)$_3$), 61.219, 60.35, 59.92, 54.7, 49.35, 48.50, 48.07, 47.40, 47.24, 46.74, 40.73 (2C, −N(*C*H$_3$)$_2$), 37.68, 28.38 (3C, C(*C*H$_3$)$_3$), 25.21, 25.06, 18.51, 18.36, 18.11, 17.96, 17.90, 14.14 ppm; CI-MS *m*/*z* 576 (100), 562 (4), 520 (8), 503 (3), 330 (11), 286 (6); HRMS calcd for $C_{40}H_{63}N_8O_{10}$ 815.4667, found 815.4665.

Boc-Ala-Pro-Ala-dmaPhe-Ala-Pro-Ala-OH was prepared following the general procedure for ester hydrolysis from Boc-Ala-Pro-Ala-dmaPhe-Ala-Pro-Ala-OEt (123 mg, 0.15 mmol) and NaOH (8 mg in 5 mL of H_2O) in ethanol (40 mL). The peptide solution was cooled with an ice bath before the addition of NaOH, allowed to warm to room temperature over 5 h, and then stirred for 2 h at room temperature and neutralized to pH 6−7 with 5% NaHSO$_4$(aq). The white solid obtained was used in the next step without further purification.

H-Ala-Pro-Ala-dmaPhe-Ala-Pro-Ala-OEt. The deprotected tetrapeptide was prepared following the procedure used for H-dmaPhe-Ala-Pro-Ala-OEt from Boc-Ala-Pro-Ala-dmaPhe-Ala-Pro-Ala-OEt (125 mg, 0.15 mmol), and Me$_3$SiI (34 μL, 0.2 mmol) in CH$_3$CN (10 mL) followed by MeOH (0.5 mL). The reaction flask was protected from light. The off-white solid obtained was dried for 6 h in high vacuum and immediately used in the next step without further purification.

Boc-Ala-Pro-Ala-pyrAla-OMe was prepared following the general coupling procedure from Boc-Ala-Pro-Ala-OH (570 mg, 1.6 mmol), H-pyrAla-OMe (490 mg, mmol),[4a,26] HOBT (230 mg, 1.7 mmol), EDCI (326 mg, 1.7 mmol), and NMM (0.3 mL) in DMF (20 mL). Purification by column chromatography (CH_2Cl_2 followed by 95% ethyl acetate/5% MeOH, v/v) and trituration with ether afforded the peptide as an off-white solid (560 mg, 53%): the ^{1}H NMR spectrum shows about 20% of another isomer (not reported); ^{1}H NMR δ 8.36 (d, 1H, J = 9.3, pyrene), 8.2−8.0 (m, 7 H, pyrene), 7.85 (d, 1H, J = 7.8), 7.03 (d, 1H, J = 7.3, N*H*), 6.85 (d, 1H, J = 7.6, N*H*), 5.26 (d, 1H, J = 7.6, N*H*), 5.08 (dd, 1H, J_1 = 7.6, J_2 = 6.5), 4.5−4.2 (series of m, 2H), 3.9−3.8 (m, 3 H), 3.86 (s, 3 H, OCH_3), 3.7−3.5 (series of m, 3 H), 1.95 (m, 2 H), 1.85 (m, 2H), 1.43 (s, 9H, C(CH_3)$_2$), 1.27 (d, 3H, J = 7.0, CHCH_3), 1.24 (d, 3H, J = 6.8, CHCH_3) ppm; ^{13}C NMR δ 173.11, 171.96, 171.74, 170.93, 155.20 (−*C*(═O)−OEt), 131.32, 130.79, 130.61, 130.06, 129.58, 127.91 (2C), 127.42, 127.21, 126.00, 125.92, 125.10 (2C), 124.70, 123.09, 79.77 (O−*C*(CH$_3$)$_3$), 65.84, 63.20, 59.77, 53.72, 52.35, 48.91, 48.00, 47.11, 42.58, 37.51, 35.31, 28.36 (3C, C(*C*H$_3$)$_3$), 27.07, 25.06, 18.33, 17.50, 15.27 ppm; CI-MS *m*/*z* 644 (28), 643 (10), 543 (8), 414 (8), 386 (45), 372(19), 332 (100), 286 (12); HRMS calcd for $C_{36}H_{43}N_4O_7$ 643.3131, found 643.3120.

Boc-Ala-Pro-Ala-pyrAla-OH. The acid was prepared following the general procedure for ester hydrolysis from Boc-Ala-Pro-Ala-pyrAla-OMe (0.44 g, 0.68 mmol) and NaOH (30 mg in 5 mL of H_2O) in ethanol (20 mL), but by adding the NaOH to the solution cooled with an ice bath. The solution was allowed to warm to room temperature, stirred overnight at room temperature, and then acidified to pH 5 with 5% NaHSO$_4$(aq). The solvent was evaporated, and the white solid obtained was dried *in vacuo* overnight, in the dark, to afford the acid as a white solid, which was used in the next step without further purification.

Boc-Ala-Pro-Ala-pyrAla-Ala-Pro-Ala-OEt was prepared following the general coupling procedure from H-Ala-Pro-Ala-OEt (1 mmol), Boc-Ala-Pro-Ala-pyrAla-OH (0.68 mmol), HOBT (115 mg, 1 mmol), EDCI (160 mg, 1 mmol), NMM (30 μL), and DMF (10 mL). Purification by two consecutive column chromatography runs (ethyl acetate, followed by 80% ethyl acetate/20% MeOH, v/v) and trituration with ether afforded the peptide as a white solid (410 mg, 67%): ^{1}H NMR δ 8.27 (d, 1H, J = 9.0, pyrene), 8.15 (d, 1H, J = 7.5, pyrene), 8.12 (d, 1H, J = 7.5, pyrene), 7.95−8.05 (m, 5 H, pyrene), 7.8 (d, 1H, J = 7.7, pyrene), 7.14 (d, 1H, J = 6, N*H*), 6.54 (br s, 1H, N*H*), 5.25 (d, 1H, J = 9.0, N*H*), 4.40−4.30 (m, 4H), 4.14 (q, 2H, J = 7.5, OCH_2-CH$_3$), 3.9 (m, 1 H), 3.6 (m, 2H), 3.47 (dd, 2H, J_1 = J_2 = 7), 3.40 (m, 1 H), 3.30 (m, 1H), 2.0−1.85 (br m, 9H), 1.43 (s, 9H, C(CH_3)$_2$), 1.42 (br s, 3H, CH_3), 1.3−1.2 (m, 16H, CHCH_3 and −CH_2−) ppm; ^{13}C NMR δ 173.031, 172.72, 171.82, 171.25, 170.84, 170.62, 169.96, 155.31 (−*C*(═O)−OEt), 131.31,130.97, 130.73, 130.44, 129.43, 128.03, 127.95, 127.64, 127.48, 126.92, 125.94, 125.11, 124.96, 124.81, 124.77, 123.24, 79.95 (O−*C*(CH$_3$)$_3$), 65.85, 61.25, 60.32, 59.46, 54.69, 49.44, 48.63, 47.99, 47.24, 46.93, 35.84, 28.58 (3C, O−C(*C*H$_3$)$_3$), 27.55, 25.19, 24.78, 18.04, 17.99 (2C), 17.67, 15.27, 14.11 ppm; CI-MS *m*/*z* 896 (100), 796 (18), 670(1), 626 (7); HRMS calcd for $C_{48}H_{62}N_7O_{10}$ 896.4558, found 896.4558.

Boc-Ala-Pro-Ala-pyrAla-Ala-Pro-Ala-OH. The acid was prepared following the general procedure for ester hydrolysis from Boc-Ala-Pro-Ala-pyrAla-Ala-Pro-Ala-OMe (140 mg, 0.15 mmol) and NaOH (8 mg in 10 mL of H_2O) in ethanol (20 mL). The peptide solution was cooled with an ice bath before adding the NaOH solution. The solution was allowed to warm to room temperature over 4 h, stirred overnight at room temperature, and then acidified to pH 5 with 5% NaHSO$_4$(aq). The solvent was evaporated, and the white solid obtained was dried *in vacuo* overnight to afford the acid as a white solid, which was used in the next step without further purification.

H-Ala-Pro-Ala-pyrAla-Ala-Pro-Ala-OEt. The deprotected peptide was prepared following the procedure used for Boc-dmaPhe-Ala-Pro-Ala-OEt from Boc-Ala-Pro-Ala-pyrAla-Ala-Pro-Ala-OEt (145 mg, 0.15 mmol) and Me$_3$SiI (40 μL, 0.28 mmol) in CH$_3$CN (10 mL) followed by MeOH (0.5 mL).

Boc-Ala-Pro-Ala-pyrAla-(Ala-Pro-Ala)$_2$-Ala-dmaPhe-Ala-Pro-Ala-OEt (3). The peptide **3** was prepared following the general coupling procedure from H-Ala-Pro-Ala-dmaPhe-Ala-Pro-Ala-OEt (0.15 mmol), Boc-Ala-Pro-Ala-pyrAla-Ala-Pro-Ala-OH (0.15 mmol), HOBT (30 mg, 0.2 mmol), EDCI (40 mg, 0.2 mmol), and NMM (110 μL) in DMF (10 mL). Purification by a short silica gel column (ethyl acetate followed by increasing amounts of EtOH and then pure EtOH) and trituration with ether afforded **3** as a white solid (30 mg, 12%): ^{1}H NMR δ 8.36 (d, 1H, J = 9.8, pyrene), 8.15 (d, 1H, J = 8.4, pyrene), 8.12 (d, 1H, J = 8.4, pyrene), 7.9−8.1 (m, 6 H, pyrene), 7.8 (br s, 1H, N*H*), 7.4−7.6 (series of br m, 6 H, N*H*), 7.17 (d, 2H, J = 8.7, phenyl), 6.54 (d, 2H, J = 8.7, phenyl), 5.29 (br s, 1H, N*H*), 4.90 (m, 1H), 4.0−4.55 (m, 13 H and q, 2H, J = 7.0, OCH_2CH$_3$), 3.5−3.9 (m, 10 H), 3.2 (br m, 1H), 2.7−3.0 (br m, 2H), 2.69 (s, 6H, −N(CH_3)$_2$), 1.6−2.4 (br m, 16 H), 1.57 (d, 3H, J = 8, CHCH_3), 1.52 (d, 3H, J = 7, CHCH_3), 1.49 (s, 9H, tBu, C(CH_3)$_2$), 1.44 (d, 3H, J = 3, CHCH_3), 1.40 (d, 3H, J = 7, CHCH_3), 1.38 (d, 3H, J = 7, CHCH_3), 1.34 (d, 3H, J = 7, CHCH_3), 1.29 (d, 3H, J = 6, CHCH_3), 1.24 (t, 3H, J = 7, OCH$_2$CH_3), 1.16 (d, 3H, J = 7, CHCH_3) ppm; ^{13}C NMR δ 173.39, 173.43, 173.28, 173.03, 172.83, 172.74, 172.62, 172.57 (2C), 172.77 (2C), 171.67, 171.63 (2C), 156.08 (−*C*(═O)−OEt), 149.34 (phenyl), 131.47, 131.24, 130.71, 130.40, 130.26 (2C, phenyl), 129.91, 129.01, 127.78, 127.35, 127.07, 126.03, 125.24, 125.08, 124.82, 124.32 (phenyl), 122.82, 112.73 (2C, phenyl), 80.96 (O−*C*(CH$_3$)$_2$), 65.85, 63.15, 62.77, 62.65, 61.11, 60.17, 58.44, 55.32, 54.51, 53.68, 52.44, 51.27, 50.71, 49.06, 48.24, 48.01, 47.79, 47.74, 47.26, 40.69 (2C, −N(*C*H$_3$)$_2$), 28.76, 28.38 (3C, C(*C*H$_3$)$_2$), 27.76, 26.15, 26.10, 25.97, 25.21, 18.45, 17.95, 17.75, 17.47, 16.95, 16.57, 16.46, 16.19, 15.27, 15.14, 14.15 ppm; FAB-MS *m*/*z* 1586 (88), 1565 (90), 1564 (100), 1563 (75), 1562 (70), 1487 (35), 1487 (50), 1250 (40); HRMS calcd for $C_{81}H_{110}N_{15}O_{17}$ 1564.8204, found 1564.8209.

Boc-Ala-Pro-Ala-dmaPhe-(Ala-Pro-Ala)$_2$-pyrAla-Ala-Pro-Ala-

(26) The synthesis of H-pyrAla-OMe was adapted from (a) Schmidt, U.; Lieberknecht, A.; Wild, J. *Synthesis* **1988**, 159. (b) Schmidt, U.; Lieberknecht, A.; Wild, J. *Synthesis* **1984**, 53. (c) Burk, M. J; Feaster, J. E.; Nugent, W. A.; Harlow, R. L. *J. Am. Chem. Soc.* **1993**, *115*, 10125.

OEt (4) was prepared following the general coupling procedure from H-Ala-Pro-Ala-pyrAla-Ala-Pro-Ala-OEt (0.15 mmol), Boc-Ala-Pro-Ala-dmaPhe-Ala-Pro-Ala-OH (0.15 mmol), HOBT (30 mg, 0.2 mmol), EDCI (40 mg, 0.2 mmol), and NMM (110 μL) in DMF (10 mL). Purification by a short silica gel column (ethyl acetate followed by increasing amounts of EtOH and then pure EtOH) and trituration with ether afforded **4** as a white solid (64 mg, 27%): ^{1}H NMR δ 8.44 (d, 1H, J = 9.8, pyrene), 8.2−7.9 (m, 8H, pyrene), 7.8 (br s, 1H, N*H*), 7.4−7.6 (series of br m, 8 H, N*H*), 7.02 (d, 2H, J = 8.0, phenyl), 6.54 (d, 2H, phenyl, J = 8.0), 5.1 (br s, 1H, N*H*), 4.90 (m, 1H), 4.0−4.55 (m, 13 H, and q, 2H, J = 7.0, OCH_2CH$_3$), 3.5−3.9 (m, 10 H), 3.2 (br m, 1H), 2.8−3.0 (m, 2H), 2.82 (s, 6H, −N(CH_3)$_2$), 1.6−2.4 (br m, 16 H), 1.61 (d, 3H, J = 8.0, CHCH_3), 1.54 (d, 3H, J = 7.9, CHCH_3), 1.51 (d, 3H, J = 8, CHCH_3), 1.46 (s, 9H, C(CH_3)$_2$), 1.40 (d, 3H, J = 7, CHCH_3), 1.32 (d, 3H, J = 7, CHCH_3), 1.27 (d, 3H, J = 7, CHCH_3), 1.27 (d, 3H, J = 6, CHCH_3), 1.24 (t, 3H, J = 6.6, OCH$_2$CH_3), 1.17 (d, 3H, J = 7, CHCH_3) ppm; ^{13}C NMR δ 174.47, 173.40, 173.23 (2C), 173.12, 172.97, 172.75 (2C), 172.47, 172.22, 171.70, 171.60, 171.53 (2C), 155.91 (−*C*(═O)−OEt), 149.55 (phenyl), 132.29, 131.19, 130.83, 130.19 (2C, phenyl), 129.84, 129.75, 129.64 (2C), 128.92, 127.49, 126.61, 125.63, 125.00, 124.93, 124.86, 124.72, 124.40 (phenyl), 123.38, 112.59 (2C, phenyl), 80.59 (O−*C*(CH$_3$)$_2$), 65.79, 62.72, 62.58, 61.19, 61.08, 60.03, 54.69, 51.14, 50.59, 49.00, 48.16, 48.03, 47.76, 47.62, 47.19, 40.66, 40.57 (2C, −N(*C*H$_3$)$_2$), 36.19, 35.38, 29.63, 28.73, 28.61, 28.30 (3C, C(*C*H$_3$)$_2$), 27.83, 26.02, 25.78, 25.12, 17.93, 17.80, 17.68, 17.54, 16.85, 16.53, 16.36, 15.21, 15.06, 14.08 ppm; FAB-MS *m*/*z* 1586 (100), 1564 (83), 1563 (60), 1562 (50), 1561 (65), 1487 (35), 1485 (20), 1483 (20), 1466 (25), 1464 (25), 1251 (18); HRMS calcd for $C_{81}H_{110}N_{15}O_{17}$ 1564.8204, found 1564.8229.

Conclusions

The described experiments were designed to ascertain whether the observed differences in photoinduced electron transfer rates in peptides **1** and **2** are indeed caused by a helix dipole effect. We have obtained the cancellation of the dipole by denaturation of the helix in peptides **1** and **2** using hydrogen bonding solvents and by introduction of helix-breaking proline residues in peptides **3** and **4**. Identical electron transfer rates were observed for peptides **1** and **2** in 2,2,2-trifluoroethanol, a low dielectric constant solvent which denatures the peptides. In hydrogen bonding solvents with high dielectric constant, the observed identical electron transfer rates in **1** and **2** may be an effect of the perturbation of the local electric field by helix unfolding and/or of the decrease of the electric field in the high dielectric medium.

Helix unfolding could also be accomplished by replacement of Aib by proline in the peptide sequence. The proline-containing peptides **3** and **4** adopt different average conformations depending on the solvent, and small differences between **3** and **4** in the rates of intramolecular electron transfer are better ascribed to a conformational effect rather than to the dipole effect. Electron transfer rates in protic solvents were found to be identical. In all peptides studied, the decrease in differences in photoinduced electron transfer rates may be related to the alteration of a helix electrostatic field generated by the helix macroscopic dipole, corroborating the original proposal that the observed differences in rates between **1** and **2** may be attributed to the orientation of the charged ion pair with or against that field.

Nevertheless, it is important to recognize that the perturbations of the helical structure introduced either by using protic solvents or by changing the nature of the backbone do not unequivocally exclude the possibility that identical rates are the effect of a variety of factors other than a simple cancellation of the local electric field. First, the rates of electron transfer may change as the relative positions of the chromophores are randomized by the denaturation process, creating conformational populations that differ from those present in the helical peptides. Second, the electron transfer may also proceed through the backbone,[6] and it is possible that it is completely altered by the denaturation, which could cancel favorable conformational relationships that promote significant superexchange with the backbone. Therefore, our results emphasize the importance of studying conformationally rigid model peptides, where the relative orientation of the chromophores and the distance between them are well-defined and fixed, if the effect of the helix dipole on electron transfer rates[21] is to be probed unambiguously.

Acknowledgment. This work was supported by the Office of Basic Energy Sciences, U.S. Department of Energy. We are grateful to Professor J. K. Whitesell for helpful suggestions about the synthesis of oligopeptides substituted with conformationally restricted chromophores and to Dr. Donald O'Connor (Center for Fast Kinetic Research) for assistance in the time-resolved fluorescence measurements. We also thank Professor Dennis Dougherty for suggesting the use of a guanidinum salt to denaturate peptides **1** and **2**.

JA963269K