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Chemically Modulating the Photophysics of the GFP Chromophore

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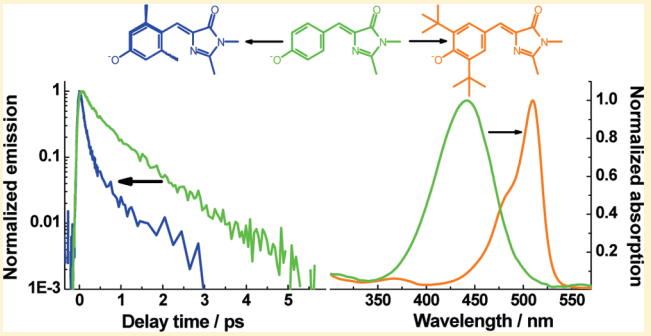
Supporting Information

ABSTRACT: There is growing interest in engineering the properties of fluorescent proteins through modifications to the chromophore structure utilizing mutagenesis with either natural or unnatural amino acids. This entails an understanding of the photophysical and photochemical properties of the modified chromophore. In this work, a range of GFP chromophores with different alkyl substituents are synthesized and their electronic spectra, pH dependence, and ultrafast fluorescence decay kinetics are investigated. The weakly electron donating character of the alkyl substituents leads to dramatic red shifts in the electronic spectra of the anions, which are accompanied by increased fluorescence decay times. This high sensitivity of electronic structure to substitution is also characteristic of some fluorescent proteins. The solvent viscosity dependence of the decay kinetics are investigated, and found to be consistent with a bimodal radiationless relaxation coordinate. Some substituents are shown to distort the planar structure of the chromophore, which results in a blue shift in the electronic spectra and a strong enhancement of the radiationless decay. The significance of these data for the rational design of novel fluorescent proteins is discussed.



INTRODUCTION

The green fluorescent protein is established as a key tool in life sciences.[1] The relative ease with which GFP can be cloned and expressed, combined with the intense green fluorescence from its uniquely covalently bound fluorophore, permits 3D spatial resolution of specifically labeled proteins in living organisms.[2,3] From this original protein, a family of chromoproteins has been developed either through discovery or mutagenesis or, more recently, exploitation of non-natural amino acids.[4−10] These proteins share the same basic β-barrel structure as GFP with only minor modifications in the chromophore and its environment, but nevertheless exhibit an enormous variety of photophysical properties which are revolutionizing bioimaging.

Niwa and co-workers first identified the GFP chromophore and synthesized its close analogue *p*-hydroxybenzilideneimidazolinone (**HBDI**, Figure 1).[11,12] Because of the wide importance of GFP fluorescence, the spectroscopy and photophysics of **HBDI** have attracted considerable attention.[13,14] Niwa et al.[12] correlated the electronic spectra of the chromophore with that of the protein, while others[15−17] used Raman spectroscopy to confirm the charge state of the chromophore in the protein. One unexpected feature of **HBDI** photophysics is that its fluorescence in fluid solvents is extremely weak—a quantum yield of 2×10^{-4} in water at 293 K—compared with about 0.8 in

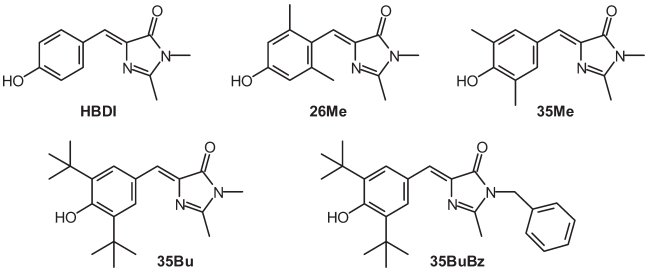


Figure 1. Structures of the alkyl derivatives of **HBDI** studied in this work.

the protein. The mechanism of radiationless decay has been established as an internal conversion, promoted by motion along a volume conserving and nearly barrierless coordinate.[18,19] Efficient radiationless decay is observed in the neutral, anionic and cationic charge states of **HBDI**, with slightly different rates.[18,19] The nature of the coordinate leading to radiationless decay has been the subject of a number of quite detailed theoretical calculations.[20−30] Single bond rotations about both bridging bonds have been considered.[20,23,24] Under different sets of conditions,

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both coordinates can lead over low or zero energy barriers to conical intersections between S_0 and S_1, promoting rapid internal conversion (although neither coordinate is strictly volume conserving, as suggested by experiment).[13,18] An alternative volume conserving synchronous rotation about both bonds has been considered (called a "hula-twist")[23,31−33] but this was found to have a higher energy barrier, which is not consistent with ultrafast decay.[23,24] A number of calculations suggest that the medium can play an important role in determining the radiationless coordinate.[20,27,34] A plausible reason for this is that the excited state structural reorganization is accompanied to a significant degree by intramolecular charge transfer.[35]

In this work, we explore the sensitivity of **HBDI** photophysics to substitution of alkyl groups on the phenyl ring (Figure 1). It is shown that the identity and position of the substituent may lead to dramatic spectral shifts to the red or the blue accompanied by either acceleration or suppression of radiationless decay, relative to **HBDI**. Such effects allow us to probe in more detail the nature of the electronic structure and ultrafast excited state decay of **HBDI**. These experiments will lead to a better understanding of the protein chromophore interactions which result in the suppression of radiationless decay and large spectral shifts. This systematic study complements some earlier studies of substituent effects, which have focused on the location and methylation of the OH group,[36−38] and a recent study which reported the effect of loading the **HBDI** chromophore with substituted phenyl substituents.[39]

Additional importance attaches to understanding the effect of substituents on **HBDI** photophysics because the diverse properties of FPs arise from proteins in which the chromophore or its environment is only slightly modified relative to **HBDI**. Some FPs have spectra which are dramatically shifted compared to wt GFP, while others are non-fluorescent and yet others can be reversibly converted between fluorescent and non fluorescent forms.[40,41] Some newly discovered examples of FPs exhibit excited state photochemical reactions in which the cross section for the photochemistry depends strongly on the charge state of the original chromophore.[42] This second generation of FPs has a wide range of applications ranging from optical highlighting in living cells[43] to ultrahigh resolution sub-Rayleigh-limit bioimaging;[44−46] however, the underlying photophysics remain poorly understood. Very recently, FPs have been created using non-natural amino acids to form the chromophore.[6,7] This exciting development opens up an enormous range of opportunities for the manipulation of GFP photophysics, but model studies such as those described here will be essential in predicting the outcome of the very challenging procedures employed in creating these new proteins.

EXPERIMENTAL SECTION

The new alkyl derivatives of the **HBDI** chromophore (Figure 1) were synthesized and purified according to the recently published general procedure for GFP synthetic chromophores.[47] Synthesis was accomplished through the production of aromatic Schiff bases by combining the corresponding aromatic aldehydes with primary amines. Resulting Schiff bases (1 equiv) were combined with the imidate (1.1 equiv) to yield imidazolinones in excellent yields. Purification was completed by washing products with cold Et_2O to yield pure product. Characterization data for all compounds are available in the Supporting Information.

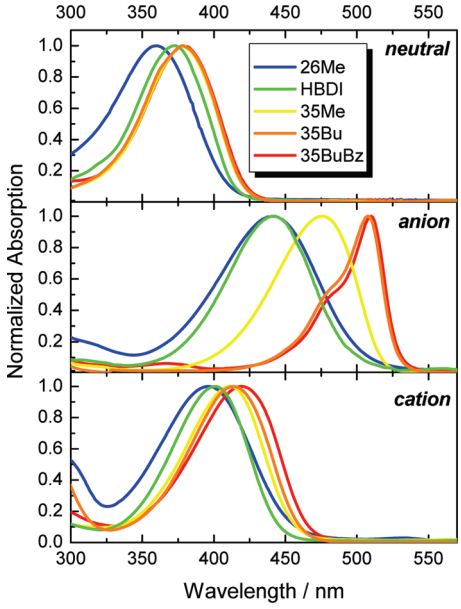

Figure 2. Absorption spectra of the alkyl **HBDIs** in neutral, basic, and acidic ethanol.

Absorption spectra were measured in aqueous solutions as a function of pH, measured with a calibrated pH meter. pK_a values were determined from the Henderson−Hasselbalch equation:

$$pH = pK_a - \log[(A - A_0)/(A_f - A)]$$

Where A is the absorbance at a chosen wavelength, A_0 is the absorbance of the pure neutral species and A_f is the absorbance of the final fully deprotonated or protonated sample. The intercept of a plot of pH against $\log[(A - A_0)/(A_f - A)]$ yields the pK_a with an accuracy better than 0.5 units. Three separate wavelengths were chosen for each determination and the results were averaged.

The apparatus for fluorescence up-conversion measurements has been described in detail elsewhere.[48] The laser excitation wavelength was either 405 or 415 nm for blue or red-shifted absorption bands, respectively. Time resolution is ultimately limited by the cross correlation of the excitation pulse and the 810 or 830 nm up-converting pulses. This was measured by up-converting Raman scattering of the excitation pulse from the solvent, thus ensuring the same isotropic spatial distribution as the fluorescence. By minimizing dispersive optical elements and carefully balancing group velocity dispersion, a time resolution of better than 50 fs is attainable.[48] In the present experiments, additional glass filters were employed to remove scattered light, which degraded the time resolution to 70 fs. Wavelength selection was accomplished by tuning the sum frequency crystal and selecting the appropriate up-converted wavelength. For up-conversion measurements, samples were contained in a 1-mm path length cell and the concentration was 100 μM.

RESULTS AND DISCUSSION

Electronic Spectroscopy. Figure 2 shows the electronic absorption spectra for the derivatives shown in Figure 1 in neutral, acidic, or basic ethanol. For the neutral forms, the most striking effect of alkyl substitution is found for 2,6-disubstitution with methyl groups (**26Me**). This causes a large blue shift in

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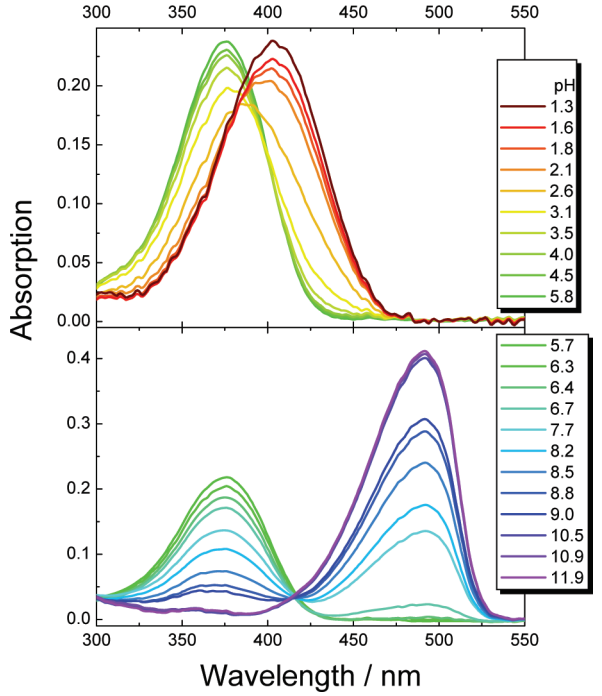

Figure 3. Absorption spectra of **35BuBz** in water as a function of pH.

absorption accompanied by a halving of the extinction coefficient relative to **HBDI** (Table 1). The effect of Me or *t*-Bu disubstitutions in the 3,5-positions (**35Me**, **35Bu**) is a 6-nm red shift with no major change in the oscillator strength.

Neutral derivatives can be converted to cationic (ring N-protonated) or anionic (phenolic hydroxyl deprotonated) forms by additions of acid or base, respectively. The two pK_a values for each of the four derivatives are presented in Table 1. An example of a pH-dependent measurement is shown in Figure 3 for **35Bu**. Here, as in every other case, a well-defined isosbestic point is apparent, which suggests only two charge forms are in equilibrium in the stated pH range. Within the experimental error there is no evidence for a dramatic change in pK_a being induced by the different substitutions, although the neutral−anion pK_a for **26Me** is somewhat higher than the others. This near constant pK_a contrasts with the behavior for substituted phenols where 2,6-dimethylphenol has a higher pK_a than phenol itself. This serves to show the importance of delocalization in the chromophore.

On generation of the cation, the spectrum for each derivative shifts to the red, as was already observed for **HBDI** itself.[37] For **HBDI** in aqueous solution, the shift is 23 nm, whereas for **35Bu** and **35BuBz** marginally larger shifts of 25 and 27 nm are found (here and below the spectral shift stated refers to the maxima of the electronic spectra). Similar shifts are found in acidified ethanol (Table 1). Again 2,6-disubstitution leaves the cation blue-shifted from the **HBDI** cation, and its anomalously low oscillator strength is retained.

Addition of base to generate the anionic form of the chromophore (which is the state responsible for the emission in wtGFP) changes this picture remarkably (e.g., for ethanol solvent, Figure 2). As is already established, deprotonation of **HBDI** results in a 55 nm red shift relative to the neutral form. The effect of substitution of alkyl groups at the 3,5 positions causes a further red shift in anion absorption relative to the **HBDI** anion. In

Table 1. Absorption Maxima, Extinction Coefficient, ε and pK_a for HBDI and Its Alkyl Derivatives

		HBDI	26Me	35Me	35Bu
pK_a	neut/an	7.8	8.8	7.5	8.3
	cat/neut	2.7	3.2	3.0	3.2
water λ_{max}/nm	anion	425	411	450	492
$\varepsilon/10^3 M^{-1}cm^{-1}$		32.4	14.6	38.8	46.7
	neutral	370	348	376	376
		23.9	10.7	25.9	22.9
	cation	393	384	401	403
		24.8	10.2	29.7	23.1
ethanol λ_{max}/nm	anion	442	442	476	508
	neutral	372	360	380	379
	cation	401	396	411	418
pentanol λ_{max}/nm	anion	441	445	477	511
	neutral	378	366	382	380

ethanol solvent **35Me** is red-shifted by a further 34 nm while **35Bu** has an additional 66 nm red shift; the overall neutral to anion red shift in this derivative is thus 129 nm! Very similar but slightly smaller effects (116 nm) are seen in aqueous solution (Table 1). Evidently, the red shift scales with the inductive electron donating ability of the substituent. This result is also consistent with the blue shift of the **26Me** (although in that case, structural factors also contribute, as discussed further below).

It has frequently been noted that **HBDI** in solution absorbs substantially to the blue of the identical chromophore in the protein, and this "protein red-shift" is larger for the anionic than for the neutral form.[13] It is interesting to note that even with only the weakly electron donating methyl 3,5-disubstitution on **HBDI**, the **35Me** spectrum already matches the protein red-shift for **HBDI**, suggesting that small changes in the chromophore electronic structure stabilized by the protein matrix can give rise to the shifted absorption observed. The more strongly electron donating *t*-Bu substitution shifts the **HBDI** spectrum further than in wtGFP; moreover, the *t*-Bu-induced red shift is accompanied by a large enhancement in the oscillator strength (Table 1). Significantly, the red shift observed for the neutral form is much smaller than that for the anionic from (Table 1) which suggests the possibility of engineering proteins in their neutral ground state such that, if they undergo the excited state proton transfer reaction characteristic of wt GFP,[49,50] then they will exhibit a giant Stokes shift; such large Stokes shifts have important applications in bioimaging.[51]

The final point of note in Table 1 is the solvent dependence. The solvent dependence of the absorption wavelength of **HBDI** (and its methoxy derivatives) has already been studied by some of us.[37] It was shown that the spectral shifts involve a complex balance of H-bonding, non-specific effects, and solvent acidity and basicity, with the largest solvent effects being observed for anionic forms. The present results are consistent with this picture. The limited data in Table 1 show larger solvent shifts for the anionic forms, with water having the most blue-shifted absorption, presumably reflecting the stronger H-bonding interactions. The shifts for the neutral forms are smaller, with water again the most blue-shifted but pentanol the most red-shifted, illustrating that factors other than H-bond interactions are important.

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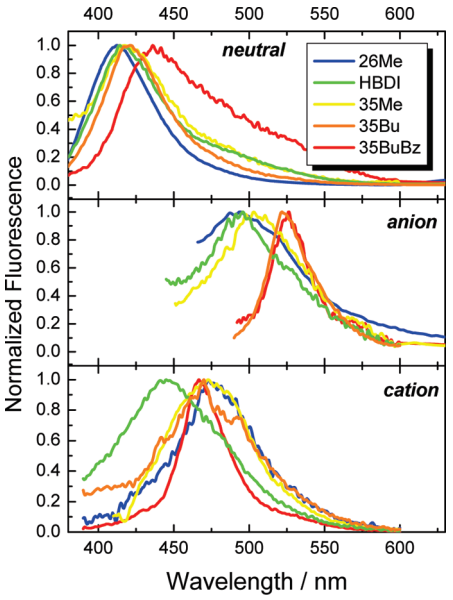

Figure 4. Emission spectra of the alkyl **HBDIs** in neutral, basic, and acidic ethanol. Excitation was at the absorption maximum. Solvent Raman scattering has been subtracted.

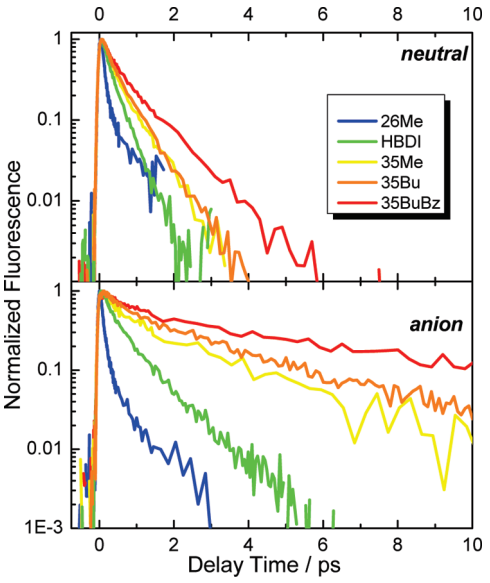

Figure 5. Fluorescence decay curves of alkyl **HBDIs** in neutral and basic ethanol. Neutral: $\lambda_{ex/em}$ 405/480 nm. Basic: λ_{ex} 415 nm, and the λ_{em} were the corresponding emission maxima from Figure 2b.

The steady-state emission measured at room temperature for the neutral, cationic, and anionic forms of the **HBDI** and its derivatives are shown in Figure 4. In all cases, the fluorescence emission is extremely weak and obscured by Raman scattering, which has been subtracted to create the spectra. This causes some uncertainty in the exact spectral profiles, but the general trends are reproducible. The emission spectra associated with the neutral chromophores follow the trend seen in absorption, with blue-shifted absorption spectra corresponding with blue-shifted emission. The exception is **35BuBz** which has a larger Stoke shift and a broader spectrum than **35Bu**, suggesting a role for the phenyl substituent in the excited state, even though it is not conjugated to the π-electron system. The emission from the anionic forms exhibits more variation, with the red-shifted **35Bu** and **35BuBz** anions showing a good mirror image relationship with their respective absorption spectra and no large Stokes shift, even in the moderately polar ethanol solvent. This suggests that there is no large-scale change in either the dipole moment or the nuclear structure between the absorbing and emitting states of these derivatives. In contrast, both **HBDI** and **26Me** anions have broader emission (and absorption) spectra than **35Bu**, and also exhibit a larger Stokes shift. This is assigned to a fast intramolecular structural relaxation in the excited state, since a time-resolved shift in the fluorescence is not observed (see below). As was observed for the neutral chromophore the variation in the emission spectra of the cations largely reflects that seen in the absorption spectra. The exception in this case is **26Me**, which shows a larger Stokes shift than **HBDI** for example. The origin of this is unclear, but protonation is at the N atom, where steric crowding has led to a non-planar form of the chromophore.

Excited State Decay Times. The excited state decay kinetics for the derivatives in Figure 1 are shown in Figure 5a,b for the neutral and anionic forms, respectively. These data were measured at the peak intensity of the emission spectra. None of these data sets were well fit by a single exponential function, but a sum of two exponentially decaying components was usually adequate. The biexponential fitting parameters and the calculated average lifetimes are reported in Table 2. Additional measurements on the red and blue sides of the emission did not reveal any wavelength dependence of the decay constants, as was previously found for **HBDI**.[52] For example, the mean decay times as a function of wavelength for neutral **35Bu** in pentanol were 0.61, 0.57, and 0.61 ps at 460, 480, and 500 nm respectively; the superimposable decay kinetics are shown in the Supporting Information (Figure S1) indicating that the weight of the components is also independent of wavelength. In no case was a rise time detected on the red side of the emission, indicating that there is no significant shift in the spectrum to lower energy with time following excitation, as might be expected for an excited state of increased dipole moment stabilized by solvation dynamics,[53] or from the formation of new species, such as by excited state proton transfer.[49] In all cases, the decays are fast (ranging between 0.1 and 5 ps). Such a fast decay was already demonstrated for the parent **HBDI** and ascribed to a near barrierless excited state reorganization resulting in approach to a conical intersection between excited and ground state potential energy surfaces followed by internal conversion to repopulate the ground electronic state.[18,19,54] Such nonexponential population dynamics may arise from a distribution of conformations in the ground state or intrinsically nonexponential torsional relaxation on the upper surface reflecting solvent friction effects.[55]

The most striking result in Table 2 is the extraordinarily fast excited state decay of **26Me**. The decay is dominated by a sub 100 fs lifetime with a minor (<10%) component of ~1 ps, resulting in a mean lifetime for the anion which is five times shorter than that for the parent **HBDI** anion. The decay time associated with the anion is actually shorter than for the neutral form, which is unique among the derivatives studied here. We ascribe the anomalous behavior of **26Me** to the existence of a non-planar ground state structure. DFT calculations show that steric crowding forces the phenyl and imidazole rings to adopt a non-coplanar geometry (Figure 6) which is in contrast to the planar geometry of **HBDI**. Such a distortion of the ground state structure is expected to reduce the π-electron delocalization and may also reduce the charge transfer character of the transition,

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Table 2. Lifetime Data for HBDI and Its Alkyl Derivatives[a]

	A_1	τ_1 /ps	A_2	τ_2/ps	$\langle\tau\rangle$ /ps
neutral					
HBDI	0.54	0.18	0.46	0.41	0.29
26Me	0.96	0.09	0.04	1.36	0.15
35BuBz	0.51	0.25	0.49	0.94	0.58
35Me	0.51	0.22	0.49	0.61	0.39
35Bu	0.53	0.27	0.47	0.66	0.45
anionic					
HBDI	0.55	0.25	0.45	0.87	0.53
26Me	0.91	0.07	0.09	0.49	0.11
35BuBz	0.51	1.12	0.49	7.40	4.18
35Me	0.57	0.44	0.43	2.71	1.41
35Bu	0.44	0.59	0.56	2.97	1.94
35BuBz					
solvent (viscosity/cP)					
ethanol (1.1)	0.51	0.25	0.49	0.94	0.58
decanol (10.9)	0.56	0.32	0.54	1.66	0.90
ethylene glycol (16.1)	0.59	0.34	0.41	2.53	1.24
35Bu					
solvent (polarity/ε_r)					
toluene (2.4)	0.54	0.40	0.46	1.53	0.92
acetonitrile (37.5)	0.57	0.44	0.43	0.90	0.64
methanol (33.4)	0.49	0.22	0.51	0.59	0.41

[a] Data for neutral and anionic forms are reported in ethanol solvent while the dependence on viscosity (for **35BuBz**) and on polarity (for **35Bu**) in their neutral forms are shown.

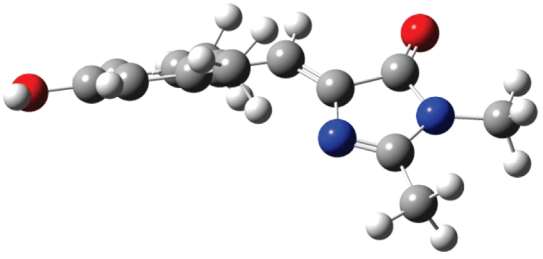

Figure 6. DFT Calculated gas phase structure for **26Me**.

both of which are consistent with the blue shift and reduced oscillator strength of the electronic transition in **26Me** noted above. These speculations could be tested by detailed quantum mechanical calculations of the lowest allowed electronic transition, such as have already been reported for **HBDI** itself.

A number of calculations of **HBDI** dynamics on the excited state potential surface suggest that internal conversion occurs at a conical intersection reached by internal reorganization including a 90° twist about a single bridging bond.[20,35] To achieve a full twist in **26Me** in <100 fs however appears difficult, even in a fluid solvent like ethanol, unless the potential energy surface is very strongly downhill. An explanation for the anomalously fast decay in **26Me** is that the conical intersection (or at least a sufficiently close approach of the ground and excited states to promote

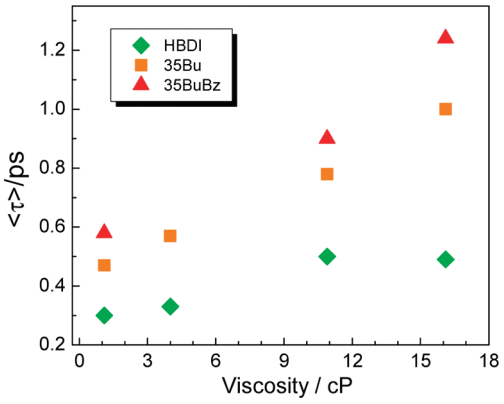

Figure 7. Viscosity dependence of the average fluorescence decay times for three derivatives.

ultrafast internal conversion) is in fact reached before a full twist occurs. In this way, the Franck−Condon transition from the twisted ground state of **26Me** may place the excited state in a position already adjacent to the conical intersection, thus promoting ultrafast decay.

The accelerated excited state decay in non-planar **26Me** is interesting in the context of recent observations of non-planar chromophores in fluorescent proteins isolated from coral.[56−58] In some cases, non-coplanar chromophores have been reported to be non-fluorescent (although this property is not shared by all nonplanar chromophores[56]). This result correlates with the ultrafast lifetime observed here for **26Me**. However, in proteins the noncoplanar forms are more often associated with the trans protonated form of the chromophore and exhibit red-shifted electronic spectra, so the relevance of the current observations is unclear; DFT calculations of the trans isomer of **26Me** show it to be both less stable than the cis form, and also to be twisted out of plane. Nevertheless, the present data provide further support for a coupling between twisting in the ground state and radiationless decay.

Comparing the mean decay times of the remaining derivatives, it is evident that there is a correlation between a longer excited state lifetime and both the red shift in absorption and the increasing loading of the rings with substituents. The same trend is observed in both charge forms, but the increased lifetime is more apparent in the anionic chromophores. From this result, the longer lifetime could result either from intrinsic changes induced in the electronic structure by the inductive electron donating substituents or from increased friction opposing motion of the larger substituents along the isomerization coordinate. The latter would arise if the coordinate required extensive motion of the phenyl or (for **35BuBz**) imidazolinone rings through the solvent.

To separate the electronic from the friction effects, we investigated the neutral forms of **35Bu** and **35BuBz** as a function of viscosity in a series of similar solvents. The mean decay times are plotted in Figure 7. Comparison of **HBDI** and **35Bu** shows slightly increased viscosity dependence in the latter. Specifically, a fit of the decay data in a series of solvents to the function $\tau \propto \eta^{a}$ yields an exponent α = 0.18 for **HBDI** and 0.23 for **35Bu**. However, the dependence on viscosity remains much weaker than observed for other excited state reactions, which involve large scale structural changes.[59,60] Thus, there is certainly *some* viscosity dependence and the dependence becomes larger when the imidazole ring is additionally loaded with a toluene

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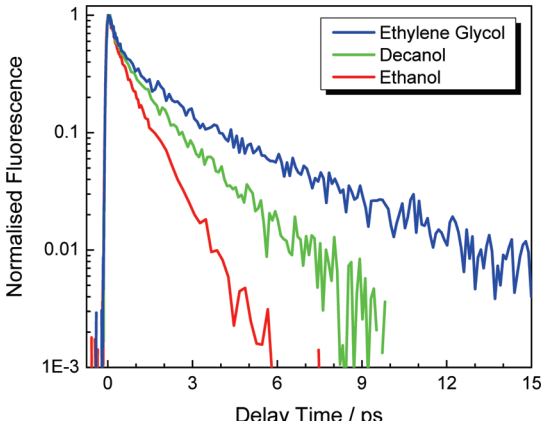

Figure 8. Fluorescence decay curves of **35BuBz** in various alcohols $\lambda_{ex/em}$ 400/480 nm.

substituent. These data suggest a coordinate leading to the conical intersection which involves a degree of reorganization of the initially planar structure of the chromophore, but not one that requires the displacement of large volumes of solvent. Similar conclusions have been reached for **HBDI** itself.[13] Indeed, the broad (if weak) emission spectrum of **HBDI** can be reproduced by convolution of additional vibrational modes with the emission spectrum of wtGFP itself,[61] which suggests that the conical intersection is only slightly displaced from the resting geometry. This result is in turn consistent with the observations of **26Me**, where the twisted ground state geometry appears to place the Franck−Condon state close to the conical intersection.

However, the detailed decay kinetics of the most viscosity sensitive **35BuBz** derivative suggests a somewhat more complex picture than is provided by the mean lifetime data (Table 2, Figure 8). The subpicosecond kinetics are rather insensitive to the medium viscosity, increasing by less that 40% for a 16-fold increase in viscosity while, in contrast, the slower component increases almost 3-fold. This result suggests that the dynamics on the excited state potential surface may be bimodal. An initial volume conserving (weakly friction dependent) step either quenches the excited state or removes it from the Franck−Condon region such that it cannot contribute, or contributes little, to the fluorescence decay. This initial step is followed by a slower process involving a more viscosity dependent (and therefore probably larger scale) intramolecular reorganization. Such bimodal dynamics have been mentioned previously in experiments and calculations of minimum energy pathways on the excited state surface of **HBDI** and feature in a number of models of excited state relaxation.[19,24,62,63] If the fast step quenches fluorescence but leaves the molecule on the excited state surface, then one would predict a ground state recovery that is slower than the excited state decay.[64] This might be observable in high time resolution transient absorption measurements. Such experiments are planned.

Finally, we address the solvent dependence of the fluorescence decay time, focusing on the neutral form of **35Bu** in polar protic, polar, and nonpolar solvents (Table 2). In all solvents studied, the decay is ultrafast but, as was previously noted for **HBDI**, the decay is slower in nonpolar solvents. A remarkable solvent effect on the quantum yield of $Z \rightarrow E$ isomerization of **HBDI** has been reported, where the yield is enhanced ~5-fold when the solvent is changed from protic methanol to aprotic acetonitrile.[65] In contrast, the fluorescence decay time increases by only a factor of ~1.5 (Table 2). This suggests that the competition between isomerization and internal conversion does not occur directly in the fluorescent state. Possibly the partitioning takes place in a dark state populated on the ultrafast time scale or on the hot ground state potential surface in competition with vibrational relaxation.

CONCLUSIONS

The electronic spectra and ultrafast excited state decay of a range of newly synthesized alkyl substituted derivatives of the GFP chromophore have been presented. The **26Me** substitution distorts the planar structure of the chromophore, blue shifts the spectrum, and leads to strong excited state quenching. In contrast, the electronic spectra are red-shifted by **35Me** and **35Bu** substitution, with the anion of the latter exhibiting an extreme red shift compared with **HBDI**. The large red shift is accompanied by an increase in the fluorescence lifetime. Detailed analysis of the viscosity dependence suggests a bimodal excited state decay pathway.

The results discussed here provide further insight into the tight coupling between fast internal conversion and twisting of the chromophore along the reaction coordinate. This suggests the possibility of custom engineering FPs using unnatural amino acids to generate FPs with optical and photochemical properties for use in addressing specific mechanistic issues in molecular biology. In particular, the formation of chromophores with very large Stokes shifts is one tantalizing possibility. This will require the study of a wider range of derivatives accompanied by detailed quantum mechanical calculations of ground and excited state structure. Such work is currently in progress.

ASSOCIATED CONTENT

Supporting Information. Data regarding the characterization of materials used in synthesis and a figure of the wavelength dependent decay kinetics. This material is available free of charge via the Internet at http://pubs.acs.org.

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