

Fluorescent Signaling Based on Control of Excited State Dynamics. Biarylacetylene Fluorescent Chemosensors

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We recently described a new approach to the development of fluorescent chemosensors based on a signal transduction pathway in which metal binding induces conformational restriction of the fluorophore, resulting in enhanced fluorescence.^{1–3} In our first report, we showed that metal ion binding could restrict the excited-state rotation of a biaryl chromophore, suppressing intersystem crossing and leading to increased emission.^{3a} We have now applied the restriction of excited-state dynamics to suppression of the other fundamental nonradiative decay pathway, internal conversion, in biarylacetylenes.⁴ This indicates that both nonradiative decay pathways are subject to conformational control, and that this signaling pathway should be generally accessible in simple flexible fluorophores. This, in turn, has implications for fluorosensor design, in that the majority of previous approaches require the sacrifice of either architectural simplicity or broad ligand scope;⁵ the approach described here does not appear to suffer from such limitations.

Diphenylacetylene (DPA) is among the simplest fluorescent compounds with appreciable conformational flexibility.⁶ Complementing our previous work on biphenyl derivatives, we have studied the properties of DPA derivatives **1–5** (Figure 1).^{7,8} Compounds **1** and **2** were chosen to evaluate the influence of covalent conformational restriction. Compounds **3–5** combine the biarylacetylene fluorophore with simple crown ether metal binding domains.

The effect of conformational restriction in these DPA derivatives is revealed by comparison of **1** and **2**: the emission of **2** is greater than that of **1** under identical conditions ($I_2/I_1 \approx 4$, Figure 2).^{9–11} The absorbance spectra indicate that restricted rotation in **2** leads to little change in extinction coefficient ($\epsilon_2/\epsilon_1 \approx 1$ at λ_{exc}),¹² and that enhanced emission does not result from enhanced absorption. The properties of **1** and **2** do not change upon addition of a large excess of trifluoroacetic acid, which argues against the involvement of electron or charge transfer. Other processes must thus be involved to account for the “missing” 4-fold enhancement.¹³

In conjunction with quantum yields, the fluorescence lifetimes of DPA, **1**, and **2** shed light on the origin of fluorescence enhancement in **2**: the calculated rate constants indicate that reduction in the rate of nonradiative decay entirely accounts for the observed 4-fold fluorescence increase (Table 1).¹¹ The temperature dependence of the emissive lifetimes (–40 to 23 °C) reveals that, as previously established for DPA,¹⁴ nonradiative decay in **1** and **2** is an activated process,¹⁵ and that the activation barrier is higher in **2** than in **1** or DPA. The parallels between the absorption, emission, and photophysical parameters suggests that the excited state processes are qualitatively the same in all three compounds. This in turn allows inference to be made as to the origin of reduced nonradiative decay in **2**.

DPA is an anomalous fluorophore, wherein excitation occurs to and emission occurs from S_2 . The dominant nonradiative decay pathway in DPA is activated $S_2 \rightarrow S_1$ internal conversion (IC)

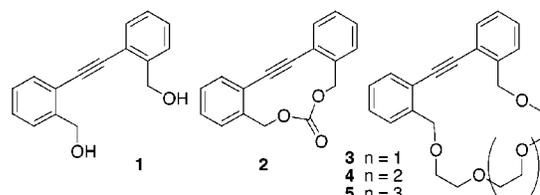


Figure 1. Biarylacetylene fluorophores.

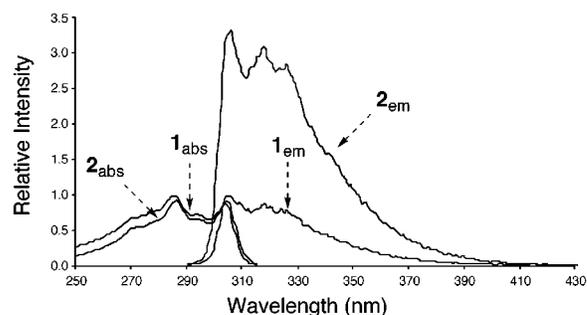


Figure 2. Relative absorbance and emission spectra of **1** and **2**.

Table 1. Photophysical Parameters for DPA, **1**, and **2**^{16,17}

	ϕ	τ (ps)	k_r (s^{-1})	E_a (cm^{-1})	k_{nr} (s^{-1})
DPA	4.0×10^{-3}	10	3.9×10^8	610	9.5×10^{10}
1	9.0×10^{-3}	20	4.6×10^8	980	5.1×10^{10}
2	36×10^{-3}	87	4.0×10^8	1100	1.2×10^{10}

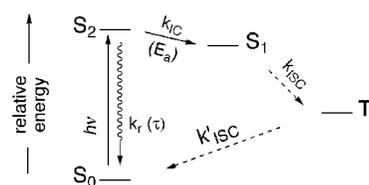


Figure 3. Excitation, emission, and nonradiative decay in DPA, **1** and **2**.

followed by rapid activationless $S_1 \rightarrow T_1$ intersystem crossing (ISC). Activated nonradiative decay in **1** and **2** may thus be ascribed to $S_2 \rightarrow S_1$ IC (Figure 3), and it is therefore this process that is modulated by conformational restriction. The increased activation energy for $S_2 \rightarrow S_1$ IC in **2** relative to **1** is consistent with what little is known about the structure of these excited states in DPA. It has previously been determined that the central bond of S_2 DPA retains its ground-state triple bond character while that of S_1 DPA is much more like a double bond in nature.¹⁸ While it has not been possible to distinguish between bent and cumulene-like structures for S_1 DPA, the restricted conformation of **2** could easily alter the energetics of the change in bond order in either scenario. We anticipate that further spectroscopic and computational study of **1** and **2** will clarify this issue.

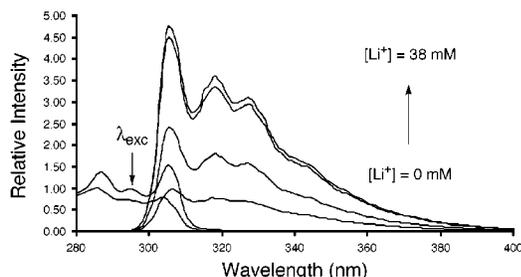


Figure 4. Titration of **3** with LiClO_4 in CH_3CN .

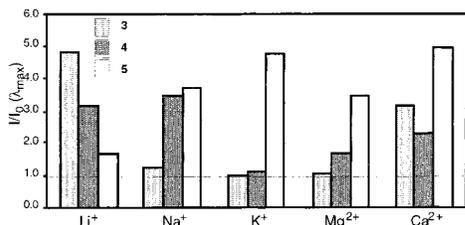


Figure 5. Fluorescence response of **3–5** to added metal cations.

Confirmation that conclusions drawn from **1** and **2** may be applied to noncovalent binding events is provided by the UV and fluorescence spectra from the titration of **3** with Li^+ (Figure 4). The addition of excess Li^+ leads to a small increase in extinction coefficient (ϵ/ϵ_0 at $\lambda_{\text{exc}} \leq 1.3$) and much larger enhancement of fluorescence emission ($I/I_0 \approx 5$ at λ_{max}). As with **1** and **2**, the addition of excess trifluoroacetic acid has no effect on the absorption or emission spectra, again excluding the involvement of electron or charge-transfer processes.¹³ This, combined with the structural and spectroscopic similarities between **2** and **3**, led us to conclude that the observed fluorescence enhancement is the result of metal-binding induced conformational restriction.

The scope of this effect is illustrated by the fluorescence response of **3–5** to the addition of metal cations (Figure 5).⁹ Of the three crowns, **3** is the most discriminating, with a strong preference for Li^+ and Ca^{2+} , while **4** and **5** exhibit less selective binding. Crowns **3** and **5** provide similar response maxima, ≈ 5 -fold fluorescence enhancement.¹⁹ As in our previous work,³ there is a correlation between metal binding and fluorescence enhancement: in each case where NMR titration indicates metal complexation, fluorescence enhancement is observed. As also observed previously,^{3,20} there is no clear correlation between cavity size and binding profile, underscoring the complexity of even these seemingly simple recognition phenomena.

In conclusion, we have shown that the control of excited dynamics can modulate both of the fundamental nonradiative decay pathways, ISC and IC, in simple fluorophores. Now characterized in biaryls,^{3a} biarylpyridines,^{3b} and biarylacetylenes, this approach should be generally applicable to the development of polyaryl fluorescent chemosensors. Ongoing work includes further spectroscopic and computational study of **1–5**, as well as the development of longer wavelength analogues.

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Supporting Information Available: Synthetic procedures, tabulated spectral data, and UV and fluorescence spectra for **1–5**; UV and

fluorescence spectra for metal titrations; and details of photophysical measurements for **1** and **2** (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (12) Force field calculations (SYBYL parameters, Spartan interface; Wavefunction, Inc. Irvine, CA) indicate that the dihedral angle between the phenyl rings of **2** is $\approx 18^\circ$; this is apparently a good approximation of the average ground state conformation of **1**.
- (13) The addition of excess acid is a reliable probe for cases where heteroatom lone pairs are involved in electron or charge-transfer processes. See ref 1 for numerous examples.
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- (15) An activated nonradiative decay process should follow $k_{\text{nr}} = A \exp(-E_a/RT)$. Energetic parameters were thus obtained by plotting $1/\tau$ vs $1/TK$, providing a linear plot with slope E_a and intercept $\ln A$. Data and error estimates are provided in the Supporting Information.
- (16) As $\tau = 1/(k_f + k_{\text{nr}})$ and $\phi = k_f/(k_f + k_{\text{nr}})$, $k_f = \phi/\tau$.
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